

10X Genomics
Publications

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北京易研科技有限公司

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Targeting CD38 with Daratumumab in Refractory Systemic Lupus Erythematosus

New England Journal of Medicine (IF: 72.1), 2020-9, Falk Hiepe et al. DOI: 10.1056/NEJMoa2023325

PubMed: 32937047

Single Cell Immune Profiling, Human, Immunology.

Abstract

Daratumumab, a human monoclonal antibody that targets CD38, depletes plasma cells and is approved for the treatment of multiple myeloma. Long-lived plasma cells are implicated in the pathogenesis of systemic lupus erythematosus because they secrete autoantibodies, but they are unresponsive to standard immunosuppression. We describe the use of daratumumab that induced substantial clinical responses in two patients with life-threatening lupus, with the clinical responses sustained by maintenance therapy with belimumab, an antibody to B-cell activating factor. Significant depletion of long-lived plasma cells, reduction of interferon type I activity, and down-regulation of T-cell transcripts associated with chronic inflammation were documented. (Supported by the Deutsche Forschungsgemeinschaft and others.).

A dominant autoinflammatory disease caused by non-cleavable variants of RIPK1

Nature (IF: 46.49), 2019-12, Junying Yuan et al. DOI: 10.1038/s41586-019-1830 PubMed: 31827280

Single Cell Gene Expression, Human, Immunology.

Abstract

Activation of RIPK1 controls TNF-mediated apoptosis, necroptosis and inflammatory pathways. Cleavage of human and mouse RIPK1 after residues D324 and D325, respectively, by caspase-8 separates the RIPK1 kinase domain from the intermediate and death domains. The D325A mutation in mouse RIPK1 leads to embryonic lethality during mouse development. However, the functional importance of blocking caspase-8-mediated cleavage of RIPK1 on RIPK1 activation in humans is unknown. Here we identify two families with variants in RIPK1 (D324V and D324H) that lead to distinct symptoms of recurrent fevers and lymphadenopathy in an autosomal-dominant manner. Impaired cleavage of RIPK1 D324 variants by caspase-8 sensitized patients' peripheral blood mononuclear cells to RIPK1 activation, apoptosis and necroptosis induced by TNF. The patients showed strong RIPK1-dependent activation of inflammatory signalling pathways and overproduction of inflammatory cytokines and chemokines compared with unaffected controls. Furthermore, we show that expression of the RIPK1 mutants D325V or D325H in mouse embryonic fibroblasts confers not only increased sensitivity to RIPK1 activation-mediated apoptosis and necroptosis, but also induction of pro-inflammatory cytokines such as IL-6 and TNF. By contrast, patient-derived fibroblasts showed reduced expression of RIPK1 and downregulated production of reactive oxygen species, resulting in resistance to necroptosis and ferroptosis. Together, these data suggest that human non-cleavable RIPK1 variants promote activation of RIPK1, and lead to an autoinflammatory disease characterized by hypersensitivity to apoptosis and necroptosis and increased inflammatory response in peripheral blood mononuclear cells, as well as a compensatory mechanism to protect against several pro-death stimuli in fibroblasts.

Resolving the fibrotic niche of human liver cirrhosis at single-cell level

Nature (IF: 46.49), 2019-10, S A Teichmann et al. DOI: 10.1038/s41586-019-1631-3 PubMed: 31597160

Single Cell Gene Expression, Human & Mouse, Cell Atlas, Immunology.

Abstract

Liver cirrhosis is a major cause of death worldwide and is characterized by extensive fibrosis. There are currently no effective antifibrotic therapies available. To obtain a better understanding of the cellular and molecular mechanisms involved in disease pathogenesis and enable the discovery of therapeutic targets, here we profile the transcriptomes of more than 100,000 single human cells, yielding molecular definitions for non-parenchymal cell types that are found in healthy and cirrhotic human liver. We identify a scar-associated TREM2CD9 subpopulation of macrophages, which expands in liver fibrosis, differentiates from circulating monocytes and is pro-fibrogenic. We also define ACKR1 and PLVAP endothelial cells that expand in cirrhosis, are topographically restricted to the fibrotic niche and enhance the transmigration of leucocytes. Multi-lineage modelling of ligand and receptor interactions between the scar-associated macrophages, endothelial cells and PDGFR α collagen-producing mesenchymal cells reveals intra-scar activity of several pro-fibrogenic pathways including TNFRSF12A, PDGFR and NOTCH signalling. Our work dissects unanticipated aspects of the cellular and molecular basis of human organ fibrosis at a single-cell level, and provides a conceptual framework for the discovery of rational therapeutic targets in liver cirrhosis.

Genetic predisposition to mosaic Y chromosome loss in blood

Nature (IF: 46.49), 2019-11, Lars A Forsberg et al. DOI: 10.1038/s41586-019-1765-3 PubMed: 31748747

Single Cell Gene Expression, Human, Genetic Health, Population Genetics.

Abstract

Mosaic loss of chromosome Y (LOY) in circulating white blood cells is the most common form of clonal mosaicism, yet our knowledge of the causes and consequences of this is limited. Here, using a computational approach, we estimate that 20% of the male population represented in the UK Biobank study ($n = 205,011$) has detectable LOY. We identify 156 autosomal genetic determinants of LOY, which we replicate in 757,114 men of European and Japanese ancestry. These loci highlight genes that are involved in cell-cycle regulation and cancer susceptibility, as well as somatic drivers of tumour growth and targets of cancer therapy. We demonstrate that genetic susceptibility to LOY is associated with non-haematological effects on health in both men and women, which supports the hypothesis that clonal haematopoiesis is a biomarker of genomic instability in other tissues. Single-cell RNA sequencing identifies dysregulated expression of autosomal genes in leukocytes with LOY and provides insights into why clonal expansion of these cells may occur. Collectively, these data highlight the value of studying clonal mosaicism to uncover fundamental mechanisms that underlie cancer and other ageing-related diseases.

Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike

Nature (IF: 46.49), 2020-8, Lawrence Shapiro et al. DOI: 10.1038/s41586-020-2571-7 PubMed: 32698192

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic continues, with devastating consequences for human lives and the global economy. The discovery and development of virus-neutralizing monoclonal antibodies could be one approach to treat or prevent infection by this coronavirus. Here we report the isolation of sixty-one SARS-CoV-2-neutralizing monoclonal antibodies from five patients infected with SARS-CoV-2 and admitted to hospital with severe coronavirus disease 2019 (COVID-19). Among these are nineteen antibodies that potently neutralized authentic SARS-CoV-2 in vitro, nine of which exhibited very high potency, with 50% virus-inhibitory concentrations of 0.7 to 9 ng ml. Epitope mapping showed that this collection of nineteen antibodies was about equally divided between those directed against the receptor-binding domain (RBD) and those directed against the N-terminal domain (NTD), indicating that both of these regions at the top of the viral spike are immunogenic. In addition, two other powerful neutralizing antibodies recognized quaternary epitopes that overlap with the domains at the top of the spike. Cryo-electron microscopy reconstructions of one antibody that targets the RBD, a second that targets the NTD, and a third that bridges two separate RBDs showed that the antibodies recognize the closed, 'all RBD-down' conformation of the spike. Several of these monoclonal antibodies are promising candidates for clinical development as potential therapeutic and/or prophylactic agents against SARS-CoV-2.

Somatic mutations and cell identity linked by Genotyping of Transcriptomes

Nature (IF: 46.49), 2019-7, Peter Smibert et al. DOI: 10.1038/s41586-019-1367-0 PubMed: 31270458

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Assay Method, Cancer Research.

Abstract

Defining the transcriptomic identity of malignant cells is challenging in the absence of surface markers that distinguish cancer clones from one another, or from admixed non-neoplastic cells. To address this challenge, here we developed Genotyping of Transcriptomes (GoT), a method to integrate genotyping with high-throughput droplet-based single-cell RNA sequencing. We apply GoT to profile 38,290 CD34⁺ cells from patients with CALR-mutated myeloproliferative neoplasms to study how somatic mutations corrupt the complex process of human haematopoiesis. High-resolution mapping of malignant versus normal haematopoietic progenitors revealed an increasing fitness advantage with myeloid differentiation of cells with mutated CALR. We identified the unfolded protein response as a predominant outcome of CALR mutations, with a considerable dependency on cell identity, as well as upregulation of the NF- κ B pathway specifically in uncommitted stem cells. We further extended the GoT toolkit to genotype multiple targets and loci that are distant from transcript ends. Together, these findings reveal that the transcriptional output of somatic mutations in myeloproliferative neoplasms is dependent on the native cell identity.

Human germinal centres engage memory and naive B cells after influenza vaccination

Nature (IF: 46.49), 2020-10, Andrew B Ward et al. DOI: 10.1038/s41586-020-2711-0 PubMed: 32866963

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

Influenza viruses remain a major public health threat. Seasonal influenza vaccination in humans primarily stimulates pre-existing memory B cells, which differentiate into a transient wave of circulating antibody-secreting plasmablasts. This recall response contributes to original antigenic sin the selective increase of antibody species elicited by previous exposures to influenza virus antigens. It remains unclear whether such vaccination can also induce germinal centre reactions in the draining lymph nodes, where diversification and maturation of recruited B cells can occur. Here we used ultrasound-guided fine needle aspiration to serially sample the draining lymph nodes and investigate the dynamics and specificity of germinal centre B cell responses after influenza vaccination in humans. Germinal centre B cells that bind to influenza vaccine could be detected as early as one week after vaccination. In three out of eight participants, we detected vaccine-binding germinal centre B cells up to nine weeks after vaccination. Between 12% and 88% of the responding germinal centre B cell clones overlapped with B cells detected among early circulating plasmablasts. These shared B cell clones had high frequencies of somatic hypermutation and encoded broadly cross-reactive monoclonal antibodies. By contrast, vaccine-induced B cell clones detected only in the germinal centre compartment exhibited significantly lower frequencies of somatic hypermutation and predominantly encoded strain-specific monoclonal antibodies, which suggests a naive B cell origin. Some of these strain-specific monoclonal antibodies recognized epitopes that were not targeted by the early plasmablast response. Thus, influenza virus vaccination in humans can elicit a germinal centre reaction that recruits B cell clones that can target new epitopes, thereby broadening the spectrum of vaccine-induced protective antibodies.

CRISPR-engineered T cells in patients with refractory cancer

Science (IF: 44.37), 2020-2, Simon F Lacey et al. DOI: 10.1126/science.aba7365 PubMed: 32029687

Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

CRISPR-Cas9 gene editing provides a powerful tool to enhance the natural ability of human T cells to fight cancer. We report a first-in-human phase 1 clinical trial to test the safety and feasibility of multiplex CRISPR-Cas9 editing to engineer T cells in three patients with refractory cancer. Two genes encoding the endogenous T cell receptor (TCR) chains, TCR α () and TCR β (), were deleted in T cells to reduce TCR mispairing and to enhance the expression of a synthetic, cancer-specific TCR transgene (NY-ESO-1). Removal of a third gene encoding programmed cell death protein 1 (PD-1;), was performed to improve antitumor immunity. Adoptive transfer of engineered T cells into patients resulted in durable engraftment with edits at all three genomic loci. Although chromosomal translocations were detected, the frequency decreased over time. Modified T cells persisted for up to 9 months, suggesting that immunogenicity is minimal under these conditions and demonstrating the feasibility of CRISPR gene editing for cancer immunotherapy.

Single-cell reconstruction of the early maternal–fetal interface in humans

Nature (IF: 46.49), 2018-11, Ashley MoffettSarah A Teichmann DOI: 10.1038/s41586-018-0698-6

PubMed: 30429548

Single Cell Gene Expression, Human, Cell Atlas, Reproductive Biology.

Abstract

During early human pregnancy the uterine mucosa transforms into the decidua, into which the fetal placenta implants and where placental trophoblast cells intermingle and communicate with maternal cells. Trophoblast-decidual interactions underlie common diseases of pregnancy, including pre-eclampsia and stillbirth. Here we profile the transcriptomes of about 70,000 single cells from first-trimester placentas with matched maternal blood and decidual cells. The cellular composition of human decidua reveals subsets of perivascular and stromal cells that are located in distinct decidual layers. There are three major subsets of decidual natural killer cells that have distinctive immunomodulatory and chemokine profiles. We develop a repository of ligand-receptor complexes and a statistical tool to predict the cell-type specificity of cell-cell communication via these molecular interactions. Our data identify many regulatory interactions that prevent harmful innate or adaptive immune responses in this environment. Our single-cell atlas of the maternal-fetal interface reveals the cellular organization of the decidua and placenta, and the interactions that are critical for placentation and reproductive success.

Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans

Science (IF: 44.37), 2020-9, Malik Peiris et al. DOI: 10.1126/science.abc6261 PubMed: 32788292

Single Cell Gene Expression, Human, Infectious Disease.

Abstract

Coronavirus disease 2019 (COVID-19) represents a global crisis, yet major knowledge gaps remain about human immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We analyzed immune responses in 76 COVID-19 patients and 69 healthy individuals from Hong Kong and Atlanta, Georgia, United States. In the peripheral blood mononuclear cells (PBMCs) of COVID-19 patients, we observed reduced expression of human leukocyte antigen class DR (HLA-DR) and proinflammatory cytokines by myeloid cells as well as impaired mammalian target of rapamycin (mTOR) signaling and interferon- α (IFN- α) production by plasmacytoid dendritic cells. By contrast, we detected enhanced plasma levels of inflammatory mediators-including EN-RAGE, TNFSF14, and oncostatin M-which correlated with disease severity and increased bacterial products in plasma. Single-cell transcriptomics revealed a lack of type I IFNs, reduced HLA-DR in the myeloid cells of patients with severe COVID-19, and transient expression of IFN-stimulated genes. This was consistent with bulk PBMC transcriptomics and transient, low IFN- α levels in plasma during infection. These results reveal mechanisms and potential therapeutic targets for COVID-19.

Single-cell multiomic analysis identifies regulatory programs in mixed-phenotype acute leukemia

Nature Biotechnology (IF: 42.3), 2019-12, Ravindra Majeti et al. DOI: 10.1038/s41587-019-0332-7

PubMed: 31792411

Single Cell Gene Expression & Single Cell ATAC, Human, Developmental Biology, Immunology.

Abstract

Identifying the causes of human diseases requires deconvolution of abnormal molecular phenotypes spanning DNA accessibility, gene expression and protein abundance. We present a single-cell framework that integrates highly multiplexed protein quantification, transcriptome profiling and analysis of chromatin accessibility. Using this approach, we establish a normal epigenetic baseline for healthy blood development, which we then use to deconvolve aberrant molecular features within blood from patients with mixed-phenotype acute leukemia. Despite widespread epigenetic heterogeneity within the patient cohort, we observe common malignant signatures across patients as well as patient-specific regulatory features that are shared across phenotypic compartments of individual patients. Integrative analysis of transcriptomic and chromatin-accessibility maps identified 91,601 putative peak-to-gene linkages and transcription factors that regulate leukemia-specific genes, such as RUNX1-linked regulatory elements proximal to the marker gene CD69. These results demonstrate how integrative, multiomic analysis of single cells within the framework of normal development can reveal both distinct and shared molecular mechanisms of disease from patient samples.

Multiplexed quantification of proteins and transcripts in single cells

Nature Biotechnology (IF: 42.3), 2017-10, Svetlana SadekovaJoel A Klappenbach DOI: 10.1038/nbt.3973

PubMed: 28854175

Single Cell Gene Expression, Human, Assay Method, Single Cell Multiomics Method.

Abstract

We present a tool to measure gene and protein expression levels in single cells with DNA-labeled antibodies and droplet microfluidics. Using the RNA expression and protein sequencing assay (REAP-seq), we quantified proteins with 82 barcoded antibodies and >20,000 genes in a single workflow. We used REAP-seq to assess the costimulatory effects of a CD27 agonist on human CD8 lymphocytes and to identify and characterize an unknown cell type.

Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19

Cell (IF: 38.62), 2020-12, Jason D Goldman et al. DOI: 10.1016/j.cell.2020.10.037 PubMed: 33171100

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

We present an integrated analysis of the clinical measurements, immune cells, and plasma multi-omics of 139 COVID-19 patients representing all levels of disease severity, from serial blood draws collected during the first week of infection following diagnosis. We identify a major shift between mild and moderate disease, at which point elevated inflammatory signaling is accompanied by the loss of specific classes of metabolites and metabolic processes. Within this stressed plasma environment at moderate disease, multiple unusual immune cell phenotypes emerge and amplify with increasing disease severity. We condensed over 120,000 immune features into a single axis to capture how different immune cell classes coordinate in response to SARS-CoV-2. This immune-response axis independently aligns with the major plasma composition changes, with clinical metrics of blood clotting, and with the sharp transition between mild and moderate disease. This study suggests that moderate disease may provide the most effective setting for therapeutic intervention.

Imbalance of Regulatory and Cytotoxic SARS-CoV-2-Reactive CD4+ T Cells in COVID-19

Cell (IF: 38.62), 2020-11, Christian H Ottensmeier et al. DOI: 10.1016/j.cell.2020.10.001 PubMed: 33096020

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

The contribution of CD4T cells to protective or pathogenic immune responses to SARS-CoV-2 infection remains unknown. Here, we present single-cell transcriptomic analysis of >100,000 viral antigen-reactive CD4T cells from 40 COVID-19 patients. In hospitalized patients compared to non-hospitalized patients, we found increased proportions of cytotoxic follicular helper cells and cytotoxic T helper (T) cells (CD4-CTLs) responding to SARS-CoV-2 and reduced proportion of SARS-CoV-2-reactive regulatory T cells (T). Importantly, in hospitalized COVID-19 patients, a strong cytotoxic T response was observed early in the illness, which correlated negatively with antibody levels to SARS-CoV-2 spike protein. Polyfunctional T1 and T17 cell subsets were underrepresented in the repertoire of SARS-CoV-2-reactive CD4T cells compared to influenza-reactive CD4T cells. Together, our analyses provide insights into the gene expression patterns of SARS-CoV-2-reactive CD4T cells in distinct disease severities.

Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19

Cell (IF: 38.62), 2020-9, Michaela Fontenay et al. DOI: 10.1016/j.cell.2020.08.002 PubMed: 32810439

Single Cell Gene Expression, Human, Infectious Disease.

Abstract

Blood myeloid cells are known to be dysregulated in coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2. It is unknown whether the innate myeloid response differs with disease severity and whether markers of innate immunity discriminate high-risk patients. Thus, we performed high-dimensional flow cytometry and single-cell RNA sequencing of COVID-19 patient peripheral blood cells and detected disappearance of non-classical CD14CD16 monocytes, accumulation of HLA-DR classical monocytes (Human Leukocyte Antigen - DR isotype), and release of massive amounts of calprotectin (S100A8/S100A9) in severe cases. Immature CD10CD101CXCR4 neutrophils with an immunosuppressive profile accumulated in the blood and lungs, suggesting emergency myelopoiesis. Finally, we show that calprotectin plasma level and a routine flow cytometry assay detecting decreased frequencies of non-classical monocytes could discriminate patients who develop a severe form of COVID-19, suggesting a predictive value that deserves prospective evaluation.

Intratumoral CD4⁺ T Cells Mediate Anti-tumor Cytotoxicity in Human Bladder Cancer

Cell (IF: 38.62), 2020-6, Chun Jimmie Ye et al. DOI: 10.1016/j.cell.2020.05.017 PubMed: 32497499

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Responses to anti-PD-1 immunotherapy occur but are infrequent in bladder cancer. The specific T cells that mediate tumor rejection are unknown. T cells from human bladder tumors and non-malignant tissue were assessed with single-cell RNA and paired T cell receptor (TCR) sequencing of 30,604 T cells from 7 patients. We find that the states and repertoires of CD8⁺ T cells are not distinct in tumors compared with non-malignant tissues. In contrast, single-cell analysis of CD4⁺ T cells demonstrates several tumor-specific states, including multiple distinct states of regulatory T cells. Surprisingly, we also find multiple cytotoxic CD4⁺ T cell states that are clonally expanded. These CD4⁺ T cells can kill autologous tumors in an MHC class II-dependent fashion and are suppressed by regulatory T cells. Further, a gene signature of cytotoxic CD4⁺ T cells in tumors predicts a clinical response in 244 metastatic bladder cancer patients treated with anti-PD-L1.

Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells

Cell (IF: 38.62), 2020-7, Ronghua Jin et al. DOI: 10.1016/j.cell.2020.05.025 PubMed: 32425270

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

The COVID-19 pandemic urgently needs therapeutic and prophylactic interventions. Here, we report the rapid identification of SARS-CoV-2-neutralizing antibodies by high-throughput single-cell RNA and VDJ sequencing of antigen-enriched B cells from 60 convalescent patients. From 8,558 antigen-binding IgG1 clonotypes, 14 potent neutralizing antibodies were identified, with the most potent one, BD-368-2, exhibiting an IC₅₀ of 1.2 and 15 ng/mL against pseudotyped and authentic SARS-CoV-2, respectively. BD-368-2 also displayed strong therapeutic and prophylactic efficacy in SARS-CoV-2-infected hACE2-transgenic mice. Additionally, the 3.8 Å cryo-EM structure of a neutralizing antibody in complex with the spike-ectodomain trimer revealed the antibody's epitope overlaps with the ACE2 binding site. Moreover, we demonstrated that SARS-CoV-2-neutralizing antibodies could be directly selected based on similarities of their predicted CDR3 structures to those of SARS-CoV-neutralizing antibodies. Altogether, we showed that human neutralizing antibodies could be efficiently discovered by high-throughput single B cell sequencing in response to pandemic infectious diseases.

Pooled Knockin Targeting for Genome Engineering of Cellular Immunotherapies

Cell (IF: 38.62), 2020-4, Eric Shifrut et al. DOI: 10.1016/j.cell.2020.03.039 PubMed: 32302591

Single Cell Gene Expression, Human, Assay Method.

Abstract

Adoptive transfer of genetically modified immune cells holds great promise for cancer immunotherapy. CRISPR knockin targeting can improve cell therapies, but more high-throughput methods are needed to test which knockin gene constructs most potently enhance primary cell functions in vivo. We developed a widely adaptable technology to barcode and track targeted integrations of large non-viral DNA templates and applied it to perform pooled knockin screens in primary human T cells. Pooled knockin of dozens of unique barcoded templates into the T cell receptor (TCR)-locus revealed gene constructs that enhanced fitness in vitro and in vivo. We further developed pooled knockin sequencing (PoKI-seq), combining single-cell transcriptome analysis and pooled knockin screening to measure cell abundance and cell state ex vivo and in vivo. This platform nominated a novel transforming growth factor β (TGF- β) R2-41BB chimeric receptor that improved solid tumor clearance. Pooled knockin screening enables parallelized re-writing of endogenous genetic sequences to accelerate discovery of knockin programs for cell therapies.

Genome-wide CRISPR Screens in Primary Human T Cells Reveal Key Regulators of Immune Function

Cell (IF: 38.62), 2018-12, Alan Ashworth Alexander Marson DOI: 10.1016/j.cell.2018.10.024 PubMed: 30449619

Single Cell Gene Expression, Human, Assay Method, Immunology.

Abstract

Human T cells are central effectors of immunity and cancer immunotherapy. CRISPR-based functional studies in T cells could prioritize novel targets for drug development and improve the design of genetically reprogrammed cell-based therapies. However, large-scale CRISPR screens have been challenging in primary human cells. We developed a new method, single guide RNA (sgRNA) lentiviral infection with Cas9 protein electroporation (SLICE), to identify regulators of stimulation responses in primary human T cells. Genome-wide loss-of-function screens identified essential T cell receptor signaling components and genes that negatively tune proliferation following stimulation. Targeted ablation of individual candidate genes characterized hits and identified perturbations that enhanced cancer cell killing. SLICE coupled with single-cell RNA sequencing (RNA-seq) revealed signature stimulation-response gene programs altered by key genetic perturbations. SLICE genome-wide screening was also adaptable to identify mediators of immunosuppression, revealing genes controlling responses to adenosine signaling. The SLICE platform enables unbiased discovery and characterization of functional gene targets in primary cells.

Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis

Nature Medicine (IF: 36.23), 2020-11, Gianpietro Dotti et al. DOI: 10.1038/s41591-020-1074-2 PubMed: 33046868

Single Cell Immune Profiling, Human, Cancer Research.

Abstract

V α 24-invariant natural killer T (NKT) cells have shown potent anti-tumor properties in murine tumor models and have been linked to favorable outcomes in patients with cancer. However, low numbers of these cells in humans have hindered their clinical applications. Here we report interim results from all three patients enrolled on dose level 1 in a phase 1 dose-escalation trial of autologous NKT cells engineered to co-express a GD2-specific chimeric antigen receptor (CAR) with interleukin-15 in children with relapsed or resistant neuroblastoma (NCT03294954). Primary and secondary objectives were to assess safety and anti-tumor responses, respectively, with immune response evaluation as an additional objective. We ex vivo expanded highly pure NKT cells (mean $\pm$ s.d., 94.7 $\pm$ 3.8%) and treated patients with 3×10^6 CAR-NKT cells per square meter of body surface area after lymphodepleting conditioning with cyclophosphamide/fludarabine (Cy/Flu). Cy/Flu conditioning was the probable cause for grade 3-4 hematologic adverse events, as they occurred before CAR-NKT cell infusion, and no dose-limiting toxicities were observed. CAR-NKT cells expanded in vivo, localized to tumors and, in one patient, induced an objective response with regression of bone metastatic lesions. These initial results suggest that CAR-NKT cells can be expanded to clinical scale and safely applied to treat patients with cancer.

High-Throughput Mapping of B Cell Receptor Sequences to Antigen Specificity

Cell (IF: 38.62), 2019-12, Lynn Morris et al. DOI: 10.1016/j.cell.2019.11.003 PubMed: 31787378

Single Cell Immune Profiling, Human, Immunology.

Abstract

B cell receptor (BCR) sequencing is a powerful tool for interrogating immune responses to infection and vaccination, but it provides limited information about the antigen specificity of the sequenced BCRs. Here, we present LIBRA-seq (linking B cell receptor to antigen specificity through sequencing), a technology for high-throughput mapping of paired heavy- and light-chain BCR sequences to their cognate antigen specificities. B cells are mixed with a panel of DNA-barcoded antigens so that both the antigen barcode(s) and BCR sequence are recovered via single-cell next-generation sequencing. Using LIBRA-seq, we mapped the antigen specificity of thousands of B cells from two HIV-infected subjects. The predicted specificities were confirmed for a number of HIV- and influenza-specific antibodies, including known and novel broadly neutralizing antibodies. LIBRA-seq will be an integral tool for antibody discovery and vaccine development efforts against a wide range of antigen targets.

Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas

Nature Medicine (IF: 36.23), 2020-12, Linghua Wang et al. DOI: 10.1038/s41591-020-1061-7 PubMed: 33020644

Single Cell Immune Profiling, Human, Cancer Research.

Abstract

Autologous chimeric antigen receptor (CAR) T cell therapies targeting CD19 have high efficacy in large B cell lymphomas (LBCLs), but long-term remissions are observed in less than half of patients, and treatment-associated adverse events, such as immune effector cell-associated neurotoxicity syndrome (ICANS), are a clinical challenge. We performed single-cell RNA sequencing with capture-based cell identification on autologous axicabtagene ciloleucel (axi-cel) anti-CD19 CAR T cell infusion products to identify transcriptomic features associated with efficacy and toxicity in 24 patients with LBCL. Patients who achieved a complete response by positron emission tomography/computed tomography at their 3-month follow-up had three-fold higher frequencies of CD8 T cells expressing memory signatures than patients with partial response or progressive disease. Molecular response measured by cell-free DNA sequencing at day 7 after infusion was significantly associated with clinical response ($P = 0.008$), and a signature of CD8 T cell exhaustion was associated ($q = 2.8 \times 10^{-5}$) with a poor molecular response. Furthermore, a rare cell population with monocyte-like transcriptional features was associated ($P = 0.0002$) with high-grade ICANS. Our results suggest that heterogeneity in the cellular and molecular features of CAR T cell infusion products contributes to variation in efficacy and toxicity after axi-cel therapy in LBCL, and that day 7 molecular response might serve as an early predictor of CAR T cell efficacy.

Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus

Nature Medicine (IF: 36.23), 2020-4, Susan Moir et al. DOI: 10.1038/s41591-020-0769-8 PubMed: 32094927
Single Cell Gene Expression, Human, Immunology.

Abstract

Responses to vaccination and to diseases vary widely across individuals, which may be partly due to baseline immune variations. Identifying such baseline predictors of immune responses and their biological basis is of broad interest, given their potential importance for cancer immunotherapy, disease outcomes, vaccination and infection responses. Here we uncover baseline blood transcriptional signatures predictive of antibody responses to both influenza and yellow fever vaccinations in healthy subjects. These same signatures evaluated at clinical quiescence are correlated with disease activity in patients with systemic lupus erythematosus with plasmablast-associated flares. CITE-seq profiling of 82 surface proteins and transcriptomes of 53,201 single cells from healthy high and low influenza vaccination responders revealed that our signatures reflect the extent of activation in a plasmacytoid dendritic cell-type I IFN-T/B lymphocyte network. Our findings raise the prospect that modulating such immune baseline states may improve vaccine responsiveness and mitigate undesirable autoimmune disease activity.

An immune-cell signature of bacterial sepsis

Nature Medicine (IF: 36.23), 2020-3, Marcia B Goldberg et al. DOI: 10.1038/s41591-020-0752-4
PubMed: 32066974
Single Cell Gene Expression, Human, Immunology.

Abstract

Dysregulation of the immune response to bacterial infection can lead to sepsis, a condition with high mortality. Multiple whole-blood gene-expression studies have defined sepsis-associated molecular signatures, but have not resolved changes in transcriptional states of specific cell types. Here, we used single-cell RNA-sequencing to profile the blood of people with sepsis (n = 29) across three clinical cohorts with corresponding controls (n = 36). We profiled total peripheral blood mononuclear cells (PBMCs, 106,545 cells) and dendritic cells (19,806 cells) across all subjects and, on the basis of clustering of their gene-expression profiles, defined 16 immune-cell states. We identified a unique CD14monocyte state that is expanded in people with sepsis and validated its power in distinguishing these individuals from controls using public transcriptomic data from subjects with different disease etiologies and from multiple geographic locations (18 cohorts, n = 1,467 subjects). We identified a panel of surface markers for isolation and quantification of the monocyte state and characterized its epigenomic and functional phenotypes, and propose a model for its induction from human bone marrow. This study demonstrates the utility of single-cell genomics in discovering disease-associated cytologic signatures and provides insight into the cellular basis of immune dysregulation in bacterial sepsis.

Peripheral CD8+ T cell characteristics associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma

Nature Medicine (IF: 36.23), 2020-2, Miranda Payne et al. DOI: 10.1038/s41591-019-0734-6 PubMed: 32042196
Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Immune checkpoint blockade (ICB) of PD-1 and CTLA-4 to treat metastatic melanoma (MM) has variable therapeutic benefit. To explore this in peripheral samples, we characterized CD8T cell gene expression across a cohort of patients with MM receiving anti-PD-1 alone (sICB) or in combination with anti-CTLA-4 (cICB). Whereas CD8 transcriptional responses to sICB and cICB involve a shared gene set, the magnitude of cICB response is over fourfold greater, with preferential induction of mitosis- and interferon-related genes. Early samples from patients with durable clinical benefit demonstrated overexpression of T cell receptor-encoding genes. By mapping T cell receptor clonality, we find that responding patients have more large clones (those occupying $>0.5\%$ of repertoire) post-treatment than non-responding patients or controls, and this correlates with effector memory T cell percentage. Single-cell RNA-sequencing of eight post-treatment samples demonstrates that large clones overexpress genes implicated in cytotoxicity and characteristic of effector memory T cells, including CCL4, GNLY and NKG7. The 6-month clinical response to ICB in patients with MM is associated with the large CD8T cell clone count 21 d after treatment and agnostic to clonal specificity, suggesting that post-ICB peripheral CD8 clonality can provide information regarding long-term treatment response and, potentially, facilitate treatment stratification.

Fast interpolation-based t-SNE for improved visualization of single-cell RNA-seq data

Nature Methods (IF: 36.15), 2019-2, Stefan Steinerberger et al. DOI: 10.1038/s41592-018-0308-4
PubMed: 30742040

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

t-distributed stochastic neighbor embedding (t-SNE) is widely used for visualizing single-cell RNA-sequencing (scRNA-seq) data, but it scales poorly to large datasets. We dramatically accelerate t-SNE, obviating the need for data downsampling, and hence allowing visualization of rare cell populations. Furthermore, we implement a heatmap-style visualization for scRNA-seq based on one-dimensional t-SNE for simultaneously visualizing the expression patterns of thousands of genes. Software is available at <https://github.com/KlugerLab/Flt-SNE> and <https://github.com/KlugerLab/t-SNE-Heatmaps>.

Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report

Nature Medicine (IF: 36.23), 2020-1, Alexandra F Freeman et al. DOI: 10.1038/s41591-019-0733-7

PubMed: 31959990

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immunology.

Abstract

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) is a potentially fatal multiorgan inflammatory disease associated with herpesvirus reactivation and subsequent onset of autoimmune diseases. Pathophysiology remains elusive and therapeutic options are limited. Cases refractory to corticosteroid therapy pose a clinical challenge and approximately 30% of patients with DiHS/DRESS develop complications, including infections and inflammatory and autoimmune diseases. Progress in single-cell RNA sequencing (scRNA-seq) provides an opportunity to dissect human disease pathophysiology at unprecedented resolutions, particularly in diseases lacking animal models, such as DiHS/DRESS. We performed scRNA-seq on skin and blood from a patient with refractory DiHS/DRESS, identifying the JAK-STAT signaling pathway as a potential target. We further showed that central memory CD4T cells were enriched with DNA from human herpesvirus 6b. Intervention via tofacitinib enabled disease control and tapering of other immunosuppressive agents. Tofacitinib, as well as antiviral agents, suppressed culprit-induced T cell proliferation in vitro, further supporting the roles of the JAK-STAT pathway and herpesviruses in mediating the adverse drug reaction. Thus, scRNA-seq analyses guided successful therapeutic intervention in the patient with refractory DiHS/DRESS. scRNA-seq may improve our understanding of complicated human disease pathophysiology and provide an alternative approach in personalized medicine.

Multiplexed detection of proteins, transcriptomes, clonotypes and CRISPR perturbations in single cells

Nature Methods (IF: 36.15), 2019-4, Sergei B KoralovPeter Smibert DOI: 10.1038/s41592-019-0392-0

PubMed: 31011186

Single Cell Immune Profiling, Human, Cancer Research, Single Cell Multiomics Method.

Abstract

Multimodal single-cell assays provide high-resolution snapshots of complex cell populations, but are mostly limited to transcriptome plus an additional modality. Here, we describe expanded CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing (ECCITE-seq) for the high-throughput characterization of at least five modalities of information from each single cell. We demonstrate application of ECCITE-seq to multimodal CRISPR screens with robust direct single-guide RNA capture and to clonotype-aware multimodal phenotyping of cancer samples.

Single-cell immune landscape of human atherosclerotic plaques

Nature Medicine (IF: 36.23), 2019-10, Miriam Merad et al. DOI: 10.1038/s41591-019-0590-4 PubMed: 31591603
Single Cell Gene Expression, Human, Immunology, Single Cell Multiomics Method.

Abstract

Atherosclerosis is driven by multifaceted contributions of the immune system within the circulation and at vascular focal sites. However, specific characteristics of dysregulated immune cells within atherosclerotic lesions that lead to clinical events such as ischemic stroke or myocardial infarction are poorly understood. Here, using single-cell proteomic and transcriptomic analyses, we uncovered distinct features of both T cells and macrophages in carotid artery plaques of patients with clinically symptomatic disease (recent stroke or transient ischemic attack) compared to asymptomatic disease (no recent stroke). Plaques from symptomatic patients were characterized by a distinct subset of CD4T cells and by T cells that were activated and differentiated. Moreover, some T cell subsets in these plaques presented markers of T cell exhaustion. Additionally, macrophages from these plaques contained alternatively activated phenotypes, including subsets associated with plaque vulnerability. In plaques from asymptomatic patients, T cells and macrophages were activated and displayed evidence of interleukin-1 β signaling. The identification of specific features of innate and adaptive immune cells in plaques that are associated with cerebrovascular events may enable the design of more precisely tailored cardiovascular immunotherapies.

A test metric for assessing single-cell RNA-seq batch correction

Nature Methods (IF: 36.15), 2018-12, Sarah A Teichmann Fabian J Theis DOI: 10.1038/s41592-018-0254-1
PubMed: 30573817
Single Cell Gene Expression, Human, Computational Method.

Abstract

Single-cell transcriptomics is a versatile tool for exploring heterogeneous cell populations, but as with all genomics experiments, batch effects can hamper data integration and interpretation. The success of batch-effect correction is often evaluated by visual inspection of low-dimensional embeddings, which are inherently imprecise. Here we present a user-friendly, robust and sensitive k-nearest-neighbor batch-effect test (kBET; <https://github.com/theislab/kBET>) for quantification of batch effects. We used kBET to assess commonly used batch-regression and normalization approaches, and to quantify the extent to which they remove batch effects while preserving biological variability. We also demonstrate the application of kBET to data from peripheral blood mononuclear cells (PBMCs) from healthy donors to distinguish cell-type-specific inter-individual variability from changes in relative proportions of cell populations. This has important implications for future data-integration efforts, central to projects such as the Human Cell Atlas.

Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia

Nature Medicine (IF: 36.23), 2018-11, Clayton SmithCraig T Jordan DOI: 10.1038/s41591-018-0233-1

PubMed: 30420752

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Leukemia stem cells (LSCs) drive the initiation and perpetuation of AML, are quantifiably associated with worse clinical outcomes, and often persist after conventional chemotherapy resulting in relapse. In this report, we show that treatment of older patients with AML with the B cell lymphoma 2 (BCL-2) inhibitor venetoclax in combination with azacitidine results in deep and durable remissions and is superior to conventional treatments. We hypothesized that these promising clinical results were due to targeting LSCs. Analysis of LSCs from patients undergoing treatment with venetoclax + azacitidine showed disruption of the tricarboxylic acid (TCA) cycle manifested by decreased α -ketoglutarate and increased succinate levels, suggesting inhibition of electron transport chain complex II. In vitro modeling confirmed inhibition of complex II via reduced glutathionylation of succinate dehydrogenase. These metabolic perturbations suppress oxidative phosphorylation (OXPHOS), which efficiently and selectively targets LSCs. Our findings show for the first time that a therapeutic intervention can eradicate LSCs in patients with AML by disrupting the metabolic machinery driving energy metabolism, resulting in promising clinical activity in a patient population with historically poor outcomes.

Visualization and analysis of single-cell RNA-seq data by kernel-based similarity learning

Nature Methods (IF: 36.15), 2017-4, Daniele RamazzottiSerafim Batzoglou DOI: 10.1038/nmeth.4207 PubMed: 28263960

Single Cell Gene Expression, Human, Computational Method.

Abstract

We present single-cell interpretation via multikernel learning (SIMLR), an analytic framework and software which learns a similarity measure from single-cell RNA-seq data in order to perform dimension reduction, clustering and visualization. On seven published data sets, we benchmark SIMLR against state-of-the-art methods. We show that SIMLR is scalable and greatly enhances clustering performance while improving the visualization and interpretability of single-cell sequencing data.

Extensive Remodeling of the Immune Microenvironment in B Cell Acute Lymphoblastic Leukemia

Cancer Cell (IF: 30.24), 2020-6, William L Carroll et al. DOI: 10.1016/j.ccell.2020.04.015 PubMed: 32470390
Single Cell Gene Expression, Human, Cancer Research.

Abstract

A subset of B cell acute lymphoblastic leukemia (B-ALL) patients will relapse and succumb to therapy-resistant disease. The bone marrow microenvironment may support B-ALL progression and treatment evasion. Utilizing single-cell approaches, we demonstrate B-ALL bone marrow immune microenvironment remodeling upon disease initiation and subsequent re-emergence during conventional chemotherapy. We uncover a role for non-classical monocytes in B-ALL survival, and demonstrate monocyte abundance at B-ALL diagnosis is predictive of pediatric and adult B-ALL patient survival. We show that human B-ALL blasts alter a vascularized microenvironment promoting monocytic differentiation, while depleting leukemia-associated monocytes in B-ALL animal models prolongs disease remission in vivo. Our profiling of the B-ALL immune microenvironment identifies extrinsic regulators of B-ALL survival supporting new immune-based therapeutic approaches for high-risk B-ALL treatment.

Impaired Death Receptor Signaling in Leukemia Causes Antigen-Independent Resistance by Inducing CAR T-cell Dysfunction

Cancer Discovery (IF: 28.3), 2020-4, Saar Gill et al. DOI: 10.1158/2159-8290.CD-19-0813 PubMed: 32001516
Single Cell Immune Profiling, Human, Functional Genomics, Immuno-oncology.

Abstract

Primary resistance to CD19-directed chimeric antigen receptor T-cell therapy (CAR19) occurs in 10% to 20% of patients with acute lymphoblastic leukemia (ALL); however, the mechanisms of this resistance remain elusive. Using a genome-wide loss-of-function screen, we identified that impaired death receptor signaling in ALL led to rapidly progressive disease despite CAR19 treatment. This was mediated by an inherent resistance to T-cell cytotoxicity that permitted antigen persistence and was subsequently magnified by the induction of CAR T-cell functional impairment. These findings were validated using samples from two CAR T-cell clinical trials in ALL, where we found that reduced expression of death receptor genes was associated with worse overall survival and reduced T-cell fitness. Our findings suggest that inherent dysregulation of death receptor signaling in ALL directly leads to CAR T-cell failure by impairing T-cell cytotoxicity and promoting progressive CAR T-cell dysfunction. SIGNIFICANCE: Resistance to CAR19 is a significant barrier to efficacy in the treatment of B-cell malignancies. This work demonstrates that impaired death receptor signaling in tumor cells causes failed CAR19 cytotoxicity and drives CAR19 dysfunction, identifying a novel mechanism of antigen-independent resistance to CAR therapy..

Clonally Expanded T Cells Reveal Immunogenicity of Rhabdoid Tumors

Cancer Cell (IF: 30.24), 2019-12, Eliane Piaggio et al. DOI: 10.1016/j.ccell.2019.10.008 PubMed: 31708437

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Rhabdoid tumors (RTs) are genomically simple pediatric cancers driven by the biallelic inactivation of SMARCB1, leading to SWI/SNF chromatin remodeler complex deficiency. Comprehensive evaluation of the immune infiltrates of human and mice RTs, including immunohistochemistry, bulk RNA sequencing and DNA methylation profiling studies showed a high rate of tumors infiltrated by T and myeloid cells. Single-cell RNA (scRNA) and T cell receptor sequencing highlighted the heterogeneity of these cells and revealed therapeutically targetable exhausted effector and clonally expanded tissue resident memory CD8T subpopulations, likely representing tumor-specific cells. Checkpoint blockade therapy in an experimental RT model induced the regression of established tumors and durable immune responses. Finally, we show that one mechanism mediating RTs immunogenicity involves SMARCB1-dependent re-expression of endogenous retroviruses and interferon-signaling activation.

Unbiased Screens Show CD8+ T Cells of COVID-19 Patients Recognize Shared Epitopes in SARS-CoV-2 that Largely Reside outside the Spike Protein

Immunity (IF: 25.73), 2020-11, Shrikanta Chattopadhyay et al. DOI: 10.1016/j.immuni.2020.10.006

PubMed: 33128877

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

Developing effective strategies to prevent or treat coronavirus disease 2019 (COVID-19) requires understanding the natural immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We used an unbiased, genome-wide screening technology to determine the precise peptide sequences in SARS-CoV-2 that are recognized by the memory CD8T cells of COVID-19 patients. In total, we identified 3-8 epitopes for each of the 6 most prevalent human leukocyte antigen (HLA) types. These epitopes were broadly shared across patients and located in regions of the virus that are not subject to mutational variation. Notably, only 3 of the 29 shared epitopes were located in the spike protein, whereas most epitopes were located in ORF1ab or the nucleocapsid protein. We also found that CD8T cells generally do not cross-react with epitopes in the four seasonal coronaviruses that cause the common cold. Overall, these findings can inform development of next-generation vaccines that better recapitulate natural CD8T cell immunity to SARS-CoV-2.

Comprehensive Profiling of an Aging Immune System Reveals Clonal GZMK⁺ CD8⁺ T Cells as Conserved Hallmark of Inflammaging

Immunity (IF: 25.73), 2020-11, Marco Colonna et al. DOI: 10.1016/j.immuni.2020.11.005 PubMed: 33271118
Single Cell Immune Profiling & Single Cell ATAC, Human & Mouse, Immunology.

Abstract

Systematic understanding of immune aging on a whole-body scale is currently lacking. We characterized age-associated alterations in immune cells across multiple mouse organs using single-cell RNA and antigen receptor sequencing and flow cytometry-based validation. We defined organ-specific and common immune alterations and identified a subpopulation of age-associated granzyme K (GZMK)-expressing CD8T (Taa) cells that are distinct from T effector memory (Tem) cells. Taa cells were highly clonal, had specific epigenetic and transcriptional signatures, developed in response to an aged host environment, and expressed markers of exhaustion and tissue homing. Activated Taa cells were the primary source of GZMK, which enhanced inflammatory functions of non-immune cells. In humans, proportions of the circulating GZMKCD8T cell population that shares transcriptional and epigenetic signatures with mouse Taa cells increased during healthy aging. These results identify GZMKTaa cells as a potential target to address age-associated dysfunctions of the immune system.

Transcriptional and Functional Analysis of CD1c⁺ Human Dendritic Cells Identifies a CD163⁺ Subset Priming CD8⁺ CD103⁺ T Cells

Immunity (IF: 25.73), 2020-8, Julie Helft et al. DOI: 10.1016/j.immuni.2020.06.002 PubMed: 32610077
Single Cell Gene Expression, Human & Mouse, Cancer Research, Immunology.

Abstract

Dendritic cells (DCs) are antigen-presenting cells controlling T cell activation. In humans, the diversity, ontogeny, and functional capabilities of DC subsets are not fully understood. Here, we identified circulating CD88CD1cCD163DCs (called DC3s) as immediate precursors of inflammatory CD88CD14CD1cCD163FcεR1DCs. DC3s develop via a specific pathway activated by GM-CSF, independent of cDC-restricted (CDP) and monocyte-restricted (cMoP) progenitors. Like classical DCs but unlike monocytes, DC3s drove activation of naive T cells. In vitro, DC3s displayed a distinctive ability to prime CD8T cells expressing a tissue homing signature and the epithelial homing α-E integrin (CD103) through transforming growth factor β (TGF-β) signaling. In vivo, DC3s infiltrated luminal breast cancer primary tumors, and DC3 infiltration correlated positively with CD8CD103CD69tissue-resident memory T cells. Together, these findings define DC3s as a lineage of inflammatory DCs endowed with a strong potential to regulate tumor immunity.

Complex Autoinflammatory Syndrome Unveils Fundamental Principles of JAK1 Kinase Transcriptional and Biochemical Function

Immunity (IF: 25.73), 2020-9, Bruce D Gelb et al. DOI: 10.1016/j.immuni.2020.07.006 PubMed: 32750333
Single Cell Gene Expression, Human, Genetic Health, Immunology.

Abstract

Autoinflammatory disease can result from monogenic errors of immunity. We describe a patient with early-onset multi-organ immune dysregulation resulting from a mosaic, gain-of-function mutation (S703I) in JAK1, encoding a kinase essential for signaling downstream of >25 cytokines. By custom single-cell RNA sequencing, we examine mosaicism with single-cell resolution. We find that JAK1 transcription was predominantly restricted to a single allele across different cells, introducing the concept of a mutational “transcriptotype” that differs from the genotype. Functionally, the mutation increases JAK1 activity and transactivates partnering JAKs, independent of its catalytic domain. S703I JAK1 is not only hypermorphic for cytokine signaling but also neomorphic, as it enables signaling cascades not canonically mediated by JAK1. Given these results, the patient was treated with tofacitinib, a JAK inhibitor, leading to the rapid resolution of clinical disease. These findings offer a platform for personalized medicine with the concurrent discovery of fundamental biological principles.

Immune Landscape of Viral- and Carcinogen-Driven Head and Neck Cancer

Immunity (IF: 25.73), 2020-1, Robert L Ferris et al. DOI: 10.1016/j.immuni.2019.11.014 PubMed: 31924475
Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Head and neck squamous cell carcinoma (HNSCC) arises through exposure to environmental carcinogens or malignant transformation by human papillomavirus (HPV). Here, we assessed the transcriptional profiles of 131,224 single cells from peripheral and intra-tumoral immune populations from patients with HPV and HPV-HNSCC and healthy donors. Immune cells within tumors of HPV and HPV-HNSCC displayed a spectrum of transcriptional signatures, with helper CD4⁺T cells and B cells being relatively divergent and CD8⁺ T cells and CD4⁺ regulatory T cells being relatively similar. Transcriptional results were contextualized through multispectral immunofluorescence analyses and evaluating putative cell-cell communication based on spatial proximity. These analyses defined a gene expression signature associated with CD4⁺T follicular helper cells that is associated with longer progression-free survival in HNSCC patients. The datasets and analytical approaches herein provide a resource for the further study of the impact of immune cells on viral- and carcinogen-induced cancers.

The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages

Immunity (IF: 25.73), 2019-2, Holm H Uhlig Fiona Powrie DOI: 10.1016/j.immuni.2018.12.018 PubMed: 30683619
Single Cell Gene Expression, Human, Immunology.

Abstract

Host microbial cross-talk is essential to maintain intestinal homeostasis. However, maladaptation of this response through microbial dysbiosis or defective host defense toward invasive intestinal bacteria can result in chronic inflammation. We have shown that macrophages differentiated in the presence of the bacterial metabolite butyrate display enhanced antimicrobial activity. Butyrate-induced antimicrobial activity was associated with a shift in macrophage metabolism, a reduction in mTOR kinase activity, increased LC3-associated host defense and antimicrobial peptide production in the absence of an increased inflammatory cytokine response. Butyrate drove this monocyte to macrophage differentiation program through histone deacetylase 3 (HDAC3) inhibition. Administration of butyrate induced antimicrobial activity in intestinal macrophages in vivo and increased resistance to enteropathogens. Our data suggest that (1) increased intestinal butyrate might represent a strategy to bolster host defense without tissue damaging inflammation and (2) that pharmacological HDAC3 inhibition might drive selective macrophage functions toward antimicrobial host defense.

High-Dimensional Single-Cell Analysis Identifies Organ-Specific Signatures and Conserved NK Cell Subsets in Humans and Mice

Immunity (IF: 25.73), 2018-11, Emilie Narni-Mancinelli Eric Vivier DOI: 10.1016/j.immuni.2018.09.009
PubMed: 30413361
Single Cell Gene Expression, Human, Immunology.

Abstract

Natural killer (NK) cells are innate lymphoid cells (ILCs) involved in antimicrobial and antitumoral responses. Several NK cell subsets have been reported in humans and mice, but their heterogeneity across organs and species remains poorly characterized. We assessed the diversity of human and mouse NK cells by single-cell RNA sequencing on thousands of individual cells isolated from spleen and blood. Unbiased transcriptional clustering revealed two distinct signatures differentiating between splenic and blood NK cells. This analysis at single-cell resolution identified three subpopulations in mouse spleen and four in human spleen, and two subsets each in mouse and human blood. A comparison of transcriptomic profiles within and between species highlighted the similarity of the two major subsets, NK1 and NK2, across organs and species. This unbiased approach provides insight into the biology of NK cells and establishes a rationale for the translation of mouse studies to human physiology and disease.

Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages

Immunity (IF: 25.73), 2017-9, Sebastian Amigorena/Elodie Segura DOI: 10.1016/j.immuni.2017.08.016 PubMed: 28930664

Single Cell Gene Expression, Human, Immunology.

Abstract

After entering tissues, monocytes differentiate into cells that share functional features with either macrophages or dendritic cells (DCs). How monocyte fate is directed toward monocyte-derived macrophages (mo-Macs) or monocyte-derived DCs (mo-DCs) and which transcription factors control these differentiation pathways remains unknown. Using an in vitro culture model yielding human mo-DCs and mo-Macs closely resembling those found in vivo in ascites, we show that IRF4 and MAFB were critical regulators of monocyte differentiation into mo-DCs and mo-Macs, respectively. Activation of the aryl hydrocarbon receptor (AHR) promoted mo-DC differentiation through the induction of BLIMP-1, while impairing differentiation into mo-Macs. Ahr deficiency also impaired the in vivo differentiation of mouse mo-DCs. Finally, AHR activation correlated with mo-DC infiltration in leprosy lesions. These results establish that mo-DCs and mo-Macs are controlled by distinct transcription factors and show that AHR acts as a molecular switch for monocyte fate specification in response to micro-environmental factors.

Two subsets of stem-like CD8⁺ memory T cell progenitors with distinct fate commitments in humans

Nature Immunology (IF: 22.3), 2020-12, Monica Casucci et al. DOI: 10.1038/s41590-020-0791-5 PubMed: 33046887

Single Cell Gene Expression, Human, Developmental Biology, Immunology.

Abstract

T cell memory relies on the generation of antigen-specific progenitors with stem-like properties. However, the identity of these progenitors has remained unclear, precluding a full understanding of the differentiation trajectories that underpin the heterogeneity of antigen-experienced T cells. We used a systematic approach guided by single-cell RNA-sequencing data to map the organizational structure of the human CD8⁺ memory T cell pool under physiological conditions. We identified two previously unrecognized subsets of clonally, epigenetically, functionally, phenotypically and transcriptionally distinct stem-like CD8⁺ memory T cells. Progenitors lacking the inhibitory receptors programmed death-1 (PD-1) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) were committed to a functional lineage, whereas progenitors expressing PD-1 and TIGIT were committed to a dysfunctional, exhausted-like lineage. Collectively, these data reveal the existence of parallel differentiation programs in the human CD8⁺ memory T cell pool, with potentially broad implications for the development of immunotherapies and vaccines.

Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19

Nature Immunology (IF: 22.3), 2020-12, F Eun-Hyung Lee et al. DOI: 10.1038/s41590-020-00814

PubMed: 33028979

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

A wide spectrum of clinical manifestations has become a hallmark of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) COVID-19 pandemic, although the immunological underpinnings of diverse disease outcomes remain to be defined. We performed detailed characterization of B cell responses through high-dimensional flow cytometry to reveal substantial heterogeneity in both effector and immature populations. More notably, critically ill patients displayed hallmarks of extrafollicular B cell activation and shared B cell repertoire features previously described in autoimmune settings. Extrafollicular activation correlated strongly with large antibody-secreting cell expansion and early production of high concentrations of SARS-CoV-2-specific neutralizing antibodies. Yet, these patients had severe disease with elevated inflammatory biomarkers, multiorgan failure and death. Overall, these findings strongly suggest a pathogenic role for immune activation in subsets of patients with COVID-19. Our study provides further evidence that targeted immunomodulatory therapy may be beneficial in specific patient subpopulations and can be informed by careful immune profiling.

Functional CRISPR dissection of gene networks controlling human regulatory T cell identity

Nature Immunology (IF: 22.3), 2020-11, Chun Jimmie Ye et al. DOI: 10.1038/s41590-020-0784-4

PubMed: 32989329

Single Cell Gene Expression, Human, Functional Genomics, Immunology.

Abstract

Human regulatory T (T) cells are essential for immune homeostasis. The transcription factor FOXP3 maintains Tcell identity, yet the complete set of key transcription factors that control Tcell gene expression remains unknown. Here, we used pooled and arrayed Cas9 ribonucleoprotein screens to identify transcription factors that regulate critical proteins in primary human Tcells under basal and proinflammatory conditions. We then generated 54,424 single-cell transcriptomes from Tcells subjected to genetic perturbations and cytokine stimulation, which revealed distinct gene networks individually regulated by FOXP3 and PRDM1, in addition to a network coregulated by FOXO1 and IRF4. We also discovered that HIVP2, to our knowledge not previously implicated in Tcell function, coregulates another gene network with SATB1 and is important for Tcell-mediated immunosuppression. By integrating CRISPR screens and single-cell RNA-sequencing profiling, we have uncovered transcriptional regulators and downstream gene networks in human Tcells that could be targeted for immunotherapies.

Single-cell landscape of immunological responses in patients with COVID-19

Nature Immunology (IF: 22.3), 2020-9, Fan Bai et al. DOI: 10.1038/s41590-020-0762 PubMed: 32788748

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

In coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the relationship between disease severity and the host immune response is not fully understood. Here we performed single-cell RNA sequencing in peripheral blood samples of 5 healthy donors and 13 patients with COVID-19, including moderate, severe and convalescent cases. Through determining the transcriptional profiles of immune cells, coupled with assembled T cell receptor and B cell receptor sequences, we analyzed the functional properties of immune cells. Most cell types in patients with COVID-19 showed a strong interferon- α response and an overall acute inflammatory response. Moreover, intensive expansion of highly cytotoxic effector T cell subsets, such as CD4effector-GNLY (granulysin), CD8effector-GNLY and NKT CD160, was associated with convalescence in moderate patients. In severe patients, the immune landscape featured a deranged interferon response, profound immune exhaustion with skewed T cell receptor repertoire and broad T cell expansion. These findings illustrate the dynamic nature of immune responses during disease progression.

Mapping systemic lupus erythematosus heterogeneity at the single-cell level

Nature Immunology (IF: 22.3), 2020-9, Virginia Pascual et al. DOI: 10.1038/s41590-020-0743-0

PubMed: 32747814

Single Cell Gene Expression, Human, Immunology.

Abstract

Patients with systemic lupus erythematosus (SLE) display a complex blood transcriptome whose cellular origin is poorly resolved. Using single-cell RNA sequencing, we profiled ~276,000 peripheral blood mononuclear cells from 33 children with SLE with different degrees of disease activity and 11 matched controls. Increased expression of interferon-stimulated genes (ISGs) distinguished cells from children with SLE from healthy control cells. The high ISG expression signature (ISG) derived from a small number of transcriptionally defined subpopulations within major cell types, including monocytes, CD4and CD8T cells, natural killer cells, conventional and plasmacytoid dendritic cells, B cells and especially plasma cells. Expansion of unique subpopulations enriched in ISGs and/or in monogenic lupus-associated genes classified patients with the highest disease activity. Profiling of ~82,000 single peripheral blood mononuclear cells from adults with SLE confirmed the expansion of similar subpopulations in patients with the highest disease activity. This study lays the groundwork for resolving the origin of the SLE transcriptional signatures and the disease heterogeneity towards precision medicine applications.

Sestrins induce natural killer function in senescent-like CD8+ T cells

Nature Immunology (IF: 22.3), 2020-6, Jacques Banchereau et al. DOI: 10.1038/s41590-020-0643-3

PubMed: 32231301

Single Cell Gene Expression, Human, Immunology.

Abstract

Aging is associated with remodeling of the immune system to enable the maintenance of life-long immunity. In the CD8T cell compartment, aging results in the expansion of highly differentiated cells that exhibit characteristics of cellular senescence. Here we found that CD27CD28CD8T cells lost the signaling activity of the T cell antigen receptor (TCR) and expressed a protein complex containing the agonistic natural killer (NK) receptor NKG2D and the NK adaptor molecule DAP12, which promoted cytotoxicity against cells that expressed NKG2D ligands. Immunoprecipitation and imaging cytometry indicated that the NKG2D-DAP12 complex was associated with sestrin 2. The genetic inhibition of sestrin 2 resulted in decreased expression of NKG2D and DAP12 and restored TCR signaling in senescent-like CD27CD28CD8T cells. Therefore, during aging, sestrins induce the reprogramming of non-proliferative senescent-like CD27CD28CD8T cells to acquire a broad-spectrum, innate-like killing activity.

Beta cell-specific CD8+ T cells maintain stem cell memory-associated epigenetic programs during type 1 diabetes

Nature Immunology (IF: 22.3), 2020-5, Gerald T Nepom et al. DOI: 10.1038/s41590-020-0633-5

PubMed: 32231298

Single Cell ATAC, Human, Immunology.

Abstract

The pool of beta cell-specific CD8T cells in type 1 diabetes (T1D) sustains an autoreactive potential despite having access to a constant source of antigen. To investigate the long-lived nature of these cells, we established a DNA methylation-based T cell 'multipotency index' and found that beta cell-specific CD8T cells retained a stem-like epigenetic multipotency score. Single-cell assay for transposase-accessible chromatin using sequencing confirmed the coexistence of naive and effector-associated epigenetic programs in individual beta cell-specific CD8T cells. Assessment of beta cell-specific CD8T cell anatomical distribution and the establishment of stem-associated epigenetic programs revealed that self-reactive CD8T cells isolated from murine lymphoid tissue retained developmentally plastic phenotypic and epigenetic profiles relative to the same cells isolated from the pancreas. Collectively, these data provide new insight into the longevity of beta cell-specific CD8T cell responses and document the use of this methylation-based multipotency index for investigating human and mouse CD8T cell differentiation.

Human retinoic acid–regulated CD161+ regulatory T cells support wound repair in intestinal mucosa

Nature Immunology (IF: 22.3), 2018-11, Giovanna Lombardi Behdad Afzali DOI: 10.1038/s41590-018-0230-PubMed: 30397350

Single Cell Gene Expression, Human, Immunology.

Abstract

Repair of tissue damaged during inflammatory processes is key to the return of local homeostasis and restoration of epithelial integrity. Here we describe CD161regulatory T (T) cells as a distinct, highly suppressive population of Tcells that mediate wound healing. These Tcells were enriched in intestinal lamina propria, particularly in Crohn's disease. CD161Tcells had an all-trans retinoic acid (ATRA)-regulated gene signature, and CD161 expression on Tcells was induced by ATRA, which directly regulated the CD161 gene. CD161 was co-stimulatory, and ligation with the T cell antigen receptor induced cytokines that accelerated the wound healing of intestinal epithelial cells. We identified a transcription-factor network, including BACH2, ROR γ t, FOSL2, AP-1 and RUNX1, that controlled expression of the wound-healing program, and found a CD161Tcell signature in Crohn's disease mucosa associated with reduced inflammation. These findings identify CD161Tcells as a population involved in controlling the balance between inflammation and epithelial barrier healing in the gut.

Human germinal center transcriptional programs are de-synchronized in B cell lymphoma

Nature Immunology (IF: 22.3), 2018-8, Lionel Spinelli Bertrand Nadel DOI: 10.1038/s41590-018-0181-4 PubMed: 30104629

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Most adult B cell lymphomas originate from germinal center (GC) B cells, but it is unclear to what extent B cells in overt lymphoma retain the functional dynamics of GC B cells or are blocked at a particular stage of the GC reaction. Here we used integrative single-cell analysis of phenotype, gene expression and variable-region sequence of the immunoglobulin heavy-chain locus to track the characteristic human GC B cell program in follicular lymphoma B cells. By modeling the cyclic continuum of GC B cell transitional states, we identified characteristic patterns of synchronously expressed gene clusters. GC-specific gene-expression synchrony was lost in single lymphoma B cells. However, distinct follicular lymphoma-specific cell states co-existed within single patient biopsies. Our data show that lymphoma B cells are not blocked in a GC B cell state but might adopt new dynamic modes of functional diversity, which opens the possibility of novel definitions of lymphoma identity.

Cell Hashing with barcoded antibodies enables multiplexing and doublet detection for single cell genomics

Genome Biology (IF: 19.04), 2018-12, Peter Smibert et al. DOI: 10.1186/s13059-018-1603-1 PubMed: 30567574
Single Cell Gene Expression, Human & Mouse, Assay Method.

Abstract

Despite rapid developments in single cell sequencing, sample-specific batch effects, detection of cell multiplets, and experimental costs remain outstanding challenges. Here, we introduce Cell Hashing, where oligo-tagged antibodies against ubiquitously expressed surface proteins uniquely label cells from distinct samples, which can be subsequently pooled. By sequencing these tags alongside the cellular transcriptome, we can assign each cell to its original sample, robustly identify cross-sample multiplets, and super-load; commercial droplet-based systems for significant cost reduction. We validate our approach using a complementary genetic approach and demonstrate how hashing can generalize the benefits of single cell multiplexing to diverse samples and experimental designs.

Natural display of nuclear-encoded RNA on the cell surface and its impact on cell interaction

Genome Biology (IF: 19.04), 2020-9, Liangfang Zhang et al. DOI: 10.1186/s13059-020-02145-6

PubMed: 32907628

Single Cell Gene Expression, Human, Assay Method.

Abstract

Compared to proteins, glycans, and lipids, much less is known about RNAs on the cell surface. We develop a series of technologies to test for any nuclear-encoded RNAs that are stably attached to the cell surface and exposed to the extracellular space, hereafter called membrane-associated extracellular RNAs (maxRNAs). We develop a technique called Surface-seq to selectively sequence maxRNAs and validate two Surface-seq identified maxRNAs by RNA fluorescence in situ hybridization. To test for cell-type specificity of maxRNA, we use antisense oligos to hybridize to single-stranded transcripts exposed on the surface of human peripheral blood mononuclear cells (PBMCs). Combining this strategy with imaging flow cytometry, single-cell RNA sequencing, and maxRNA sequencing, we identify monocytes as the major type of maxRNA+ PBMCs and prioritize 11 candidate maxRNAs for functional tests. Extracellular application of antisense oligos of FNDC3B and CTSS transcripts inhibits monocyte adhesion to vascular endothelial cells. Collectively, these data highlight maxRNAs as functional components of the cell surface, suggesting an expanded role for RNA in cell-cell and cell-environment interactions.

GMM-Demux: sample demultiplexing, multiplet detection, experiment planning, and novel cell-type verification in single cell sequencing

Genome Biology (IF: 19.04), 2020-7, Kong Chen et al. DOI: 10.1186/s13059-020-02084-2 PubMed: 32731885
Single Cell Gene Expression, Human, Computational Method, Method Comparison.

Abstract

Identifying and removing multiplets are essential to improving the scalability and the reliability of single cell RNA sequencing (scRNA-seq). Multiplets create artificial cell types in the dataset. We propose a Gaussian mixture model-based multiplet identification method, GMM-Demux. GMM-Demux accurately identifies and removes multiplets through sample barcoding, including cell hashing and MULTI-seq. GMM-Demux uses a droplet formation model to authenticate putative cell types discovered from a scRNA-seq dataset. We generate two in-house cell-hashing datasets and compared GMM-Demux against three state-of-the-art sample barcoding classifiers. We show that GMM-Demux is stable and highly accurate and recognizes 9 multiplet-induced fake cell types in a PBMC dataset.

dropEst: pipeline for accurate estimation of molecular counts in droplet-based single-cell RNA-seq experiments

Genome Biology (IF: 19.04), 2018-12, Maria G SamsonovaPeter V Kharchenko DOI: 10.1186/s13059-018-1449-6
PubMed: 29921301
Single Cell Gene Expression, Human, Computational Method.

Abstract

Recent single-cell RNA-seq protocols based on droplet microfluidics use massively multiplexed barcoding to enable simultaneous measurements of transcriptomes for thousands of individual cells. The increasing complexity of such data creates challenges for subsequent computational processing and troubleshooting of these experiments, with few software options currently available. Here, we describe a flexible pipeline for processing droplet-based transcriptome data that implements barcode corrections, classification of cell quality, and diagnostic information about the droplet libraries. We introduce advanced methods for correcting composition bias and sequencing errors affecting cellular and molecular barcodes to provide more accurate estimates of molecular counts in individual cells.

Sampling time-dependent artifacts in single-cell genomics studies

Genome Biology (IF: 19.04), 2020-5, José Ignacio Martín-Subero et al. DOI: 10.1186/s13059-020-02032-0
PubMed: 32393363

Single Cell ATAC, Human, Computational Method.

Abstract

Robust protocols and automation now enable large-scale single-cell RNA and ATAC sequencing experiments and their application on biobank and clinical cohorts. However, technical biases introduced during sample acquisition can hinder solid, reproducible results, and a systematic benchmarking is required before entering large-scale data production. Here, we report the existence and extent of gene expression and chromatin accessibility artifacts introduced during sampling and identify experimental and computational solutions for their prevention.

The human tissue-resident CCR5+ T cell compartment maintains protective and functional properties during inflammation

Science Translational Medicine (IF: 18.56), 2019-12, Douglas R Dixon et al. DOI: 10.1126/scitranslmed.aaw8718
PubMed: 31801887

Single Cell Gene Expression, Human, Immunology.

Abstract

CCR5 is thought to play a central role in orchestrating migration of cells in response to inflammation. CCR5 antagonists can reduce inflammatory disease processes, which has led to an increased interest in using CCR5 antagonists in a wide range of inflammation-driven diseases. Paradoxically, these antagonists appear to function without negatively affecting host immunity at barrier sites. We reasoned that the resolution to this paradox may lie in the CCR5⁺ T cell populations that permanently reside in tissues. We used a single-cell analysis approach to examine the human CCR5⁺ T cell compartment in the blood, healthy, and inflamed mucosal tissues to resolve these seemingly contradictory observations. We found that 65% of the CD4⁺ tissue-resident memory T (TRM) cell compartment expressed CCR5. These CCR5⁺ T cells were enriched in and near the epithelial layer and not only limited to T1-type cells but also contained a large T17-producing and a stable regulatory T cell population. The CCR5⁺ T compartment was stably maintained even in inflamed tissues including the preservation of T17 and regulatory T cell populations. Further, using tissues from the CHARM-03 clinical trial, we found that CCR5⁺ T are preserved in human mucosal tissue during treatment with the CCR5 antagonist Maraviroc. Our data suggest that the human CCR5⁺ T compartment is functionally and spatially equipped to maintain barrier immunity even in the absence of CCR5-mediated, de novo T cell recruitment from the periphery.

Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics

Nature Biomedical Engineering (IF: 18.95), 2020-11, Larissa B Thackray et al. DOI: 10.1038/s41551-020-0594
PubMed: 32747832

Single Cell Immune Profiling, Human, Assay Method, Infectious Disease.

Abstract

The emergence and re-emergence of highly virulent viral pathogens with the potential to cause a pandemic creates an urgent need for the accelerated discovery of antiviral therapeutics. Antiviral human monoclonal antibodies (mAbs) are promising candidates for the prevention and treatment of severe viral diseases, but their long development timeframes limit their rapid deployment and use. Here, we report the development of an integrated sequence of technologies, including single-cell mRNA-sequence analysis, bioinformatics, synthetic biology and high-throughput functional analysis, that enables the rapid discovery of highly potent antiviral human mAbs, the activity of which we validated in vivo. In a 78-d study modelling the deployment of a rapid response to an outbreak, we isolated more than 100 human mAbs that are specific to Zika virus, assessed their function, identified that 29 of these mAbs have broadly neutralizing activity, and verified the therapeutic potency of the lead candidates in mice and non-human primate models of infection through the delivery of an antibody-encoding mRNA formulation and of the respective IgG antibody. The pipeline provides a roadmap for rapid antibody-discovery programmes against viral pathogens of global concern.

De novo prediction of cancer-associated T cell receptors for noninvasive cancer detection

Science Translational Medicine (IF: 18.56), 2020-8, Jayanthi Lea et al. DOI: 10.1126/scitranslmed.aaz3738
PubMed: 32817363

Single Cell Immune Profiling, Human, Cancer Research, Computational Method.

Abstract

The adaptive immune system recognizes tumor antigens at an early stage to eradicate cancer cells. This process is accompanied by systemic proliferation of the tumor antigen-specific T lymphocytes. While detection of asymptomatic early-stage cancers is challenging due to small tumor size and limited somatic alterations, tracking peripheral T cell repertoire changes may provide an attractive solution to cancer diagnosis. Here, we developed a deep learning method called DeepCAT to enable de novo prediction of cancer-associated T cell receptors (TCRs). We validated DeepCAT using cancer-specific or non-cancer TCRs obtained from multiple major histocompatibility complex I (MHC-I) multimer-sorting experiments and demonstrated its prediction power for TCRs specific to cancer antigens. We blindly applied DeepCAT to distinguish over 250 patients with cancer from over 600 healthy individuals using blood TCR sequences and observed high prediction accuracy, with area under the curve (AUC) ≥ 0.95 for multiple early-stage cancers. This work sets the stage for using the peripheral blood TCR repertoire for noninvasive cancer detection.

Comparative Analysis of Droplet-Based Ultra-High-Throughput Single-Cell RNA-Seq Systems

Molecular Cell (IF: 16.13), 2019-1, Yanyi et al. DOI: 10.1016/j.molcel.2018.10.020

PubMed: 30472192

Single Cell Gene Expression, Human, Method Comparison.

Abstract

Since its establishment in 2009, single-cell RNA sequencing (RNA-seq) has been a major driver behind progress in biomedical research. In developmental biology and stem cell studies, the ability to profile single cells confers particular benefits. Although most studies still focus on individual tissues or organs, the recent development of ultra-high-throughput single-cell RNA-seq has demonstrated potential power in characterizing more complex systems or even the entire body. However, although multiple ultra-high-throughput single-cell RNA-seq systems have attracted attention, no systematic comparison of these systems has been performed. Here, with the same cell line and bioinformatics pipeline, we developed directly comparable datasets for each of three widely used droplet-based ultra-high-throughput single-cell RNA-seq systems, inDrop, Drop-seq, and 10X Genomics Chromium. Although each system is capable of profiling single-cell transcriptomes, their detailed comparison revealed the distinguishing features and suitable applications for each system.

Single-Cell Analyses Reveal Megakaryocyte-Biased Hematopoiesis in Myelofibrosis and Identify Mutant Clone-Specific Targets

Molecular Cell (IF: 16.13), 2020-5, Supat Thongjuea et al. DOI: 10.1016/j.molcel.2020.04.008 PubMed: 32386542

Single Cell Gene Expression, Human, Immunology.

Abstract

Myelofibrosis is a severe myeloproliferative neoplasm characterized by increased numbers of abnormal bone marrow megakaryocytes that induce fibrosis, destroying the hematopoietic microenvironment. To determine the cellular and molecular basis for aberrant megakaryopoiesis in myelofibrosis, we performed single-cell transcriptome profiling of 135,929 CD34lineagehematopoietic stem and progenitor cells (HSPCs), single-cell proteomics, genomics, and functional assays. We identified a bias toward megakaryocyte differentiation apparent from early multipotent stem cells in myelofibrosis and associated aberrant molecular signatures. A sub-fraction of myelofibrosis megakaryocyte progenitors (MkPs) are transcriptionally similar to healthy-donor MkPs, but the majority are disease specific, with distinct populations expressing fibrosis- and proliferation-associated genes. Mutant-clone HSPCs have increased expression of megakaryocyte-associated genes compared to wild-type HSPCs, and we provide early validation of G6B as a potential immunotherapy target. Our study paves the way for selective targeting of the myelofibrosis clone and illustrates the power of single-cell multi-omics to discover tumor-specific therapeutic targets and mediators of tissue fibrosis.

CD3 bispecific antibody–induced cytokine release is dispensable for cytotoxic T cell activity

Science Translational Medicine (IF: 18.56), 2019-9, Melissa R Junttila et al. DOI: 10.1126/scitranslmed.aax8861
PubMed: 31484792

Single Cell Gene Expression, Human, Functional Genomics, Immuno-oncology.

Abstract

T cell-retargeting therapies have transformed the therapeutic landscape of oncology. Regardless of the modality, T cell activating therapies are commonly accompanied by systemic cytokine release, which can progress to deadly cytokine release syndrome (CRS). Because of incomplete mechanistic understanding of the relationship between T cell activation and systemic cytokine release, optimal toxicity management that retains full therapeutic potential remains unclear. Here, we report the cell type-specific cellular mechanisms that link CD3 bispecific antibody-mediated killing to toxic cytokine release. The immunologic cascade is initiated by T cell triggering, whereas monocytes and macrophages are the primary source of systemic toxic cytokine release. We demonstrate that T cell-generated tumor necrosis factor- α (TNF- α) is the primary mechanism mediating monocyte activation and systemic cytokine release after CD3 bispecific treatment. Prevention of TNF- α release is sufficient to impair systemic release of monocyte cytokines without affecting antitumor efficacy. Systemic cytokine release is only observed upon initial exposure to CD3 bispecific antibody not subsequent doses, indicating a biological distinction between doses. Despite impaired cytokine release after second exposure, T cell cytotoxicity remained unaffected, demonstrating that cytolytic activity of T cells can be achieved in the absence of cytokine release. The mechanistic uncoupling of toxic cytokines and T cell cytolytic activity in the context of CD3 bispecifics provides a biological rationale to clinically explore preventative treatment approaches to mitigate toxicity.

Integrated genomic analysis reveals mutated ELF3 as a potential gallbladder cancer vaccine candidate

Nature Communications (IF: 13.61), 2020-8, Jin-Young Jang et al. DOI: 10.1038/s41467-020-17880-4
PubMed: 32839463

Single Cell Immune Profiling, Human, Cancer Research, Genetic Health.

Abstract

Gallbladder cancer (GBC) is an aggressive gastrointestinal malignancy with no approved targeted therapy. Here, we analyze exomes ($n = 160$), transcriptomes ($n = 115$), and low pass whole genomes ($n = 146$) from 167 gallbladder cancers (GBCs) from patients in Korea, India and Chile. In addition, we also sequence samples from 39 GBC high-risk patients and detect evidence of early cancer-related genomic lesions. Among the several significantly mutated genes not previously linked to GBC are ETS domain genes ELF3 and EHF, CTNNB1, APC, NSD1, KAT8, STK11 and NFE2L2. A majority of ELF3 alterations are frame-shift mutations that result in several cancer-specific neoantigens that activate T-cells indicating that they are cancer vaccine candidates. In addition, we identify recurrent alterations in KEAP1/NFE2L2 and WNT pathway in GBC. Taken together, these define multiple targetable therapeutic interventions opportunities for GBC treatment and management.

Tumor intrinsic and extrinsic determinants of response to blinatumomab in adults with B-ALL

Blood (IF: 14.82), 2020-9, Charles G Mullighan et al. DOI: 10.1182/blood.2020006287 PubMed: 32881995

Single Cell Immune Profiling, Human, Cancer Research, Genetic Health.

Abstract

Blinatumomab, a bispecific antibody that directs CD3⁺ T cells to CD19⁺ tumor cells shows variable efficacy in B-progenitor acute lymphoblastic leukemia (B-ALL). To determine tumor-intrinsic and extrinsic determinants of response, we studied 44 adults with relapsed or refractory B-ALL (including two MRD positive) treated with blinatumomab using bulk tumor and single-cell sequencing. The overall response rate in patients with hematological disease was 55%, with a high response rate CRLF2-rearranged Ph-like ALL (12/16, 75%). Pretreatment samples of responders exhibited a tumor-intrinsic transcriptomic signature of heightened immune response. Multiple mechanisms resulted in loss of CD19 expression, including CD19 mutations, CD19 mutant allele-specific expression, low CD19 RNA expression and mutations in CD19 signaling complex member CD81. Low hypodiploid patients were prone to CD19 negative relapse due to aneuploidy-mediated loss of the non-mutated CD19 allele. Increased expression of a CD19 isoform with intra-exonic splicing of exon 2, CD19 ex2part, at baseline or during therapy was associated with treatment failure. These analyses demonstrate both tumor intrinsic and extrinsic factors influence blinatumomab response. We show that CD19 mutations are commonly detected in CD19-negative relapse during blinatumomab treatment. Identification of the CD19 ex2part splice variant represents a new biomarker predictive of failure to blinatumomab therapy.

High-parametric evaluation of human invariant natural killer T cells to delineate heterogeneity in allo- and autoimmunity

Blood (IF: 14.82), 2020-3, Holden T Maecker et al. DOI: 10.1182/blood.2019001903 PubMed: 31935280

Single Cell Gene Expression, Human, Immunology.

Abstract

Human invariant natural killer T (iNKT) cells are a rare innate-like lymphocyte population that recognizes glycolipids presented on CD1d. Studies in mice have shown that these cells are heterogeneous and are capable of enacting diverse functions, and the composition of iNKT cell subsets can alter disease outcomes. In contrast, far less is known about how heterogeneity in human iNKT cells relates to disease. To address this, we used a high-dimensional, data-driven approach to devise a framework for parsing human iNKT heterogeneity. Our data revealed novel and previously described iNKT cell phenotypes with distinct functions. In particular, we found 2 phenotypes of interest: (1) a population with T helper 1 function that was increased with iNKT activation characterized by HLA-II+CD161⁻ expression, and (2) a population with enhanced cytotoxic function characterized by CD4-CD94⁺ expression. These populations correlate with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation and with new onset type 1 diabetes, respectively. Our study identifies human iNKT cell phenotypes associated with human disease that could aid in the development of biomarkers or therapeutics targeting iNKT cells.

Single-cell transcriptomics identifies multiple pathways underlying antitumor function of TCR- and CD8 $\alpha\beta$ -engineered human CD4+ T cells

Science Advances (IF: 14.09), 2020-7, Gfeller, David, Gfeller et al. DOI: 10.1126/sciadv.aaz7809 PubMed: 32937466
Single Cell Gene Expression, Human, Immuno-oncology.

Abstract

Transgenic coexpression of a class I-restricted tumor antigen-specific T cell receptor (TCR) and CD8 $\alpha\beta$ (TCR8) redirects antigen specificity of CD4+ T cells. Reinforcement of biophysical properties and early TCR signaling explain how redirected CD4+ T cells recognize target cells, but the transcriptional basis for their acquired antitumor function remains elusive. We, therefore, interrogated redirected human CD4+ and CD8+ T cells by single-cell RNA sequencing and characterized them experimentally in bulk and single-cell assays and a mouse xenograft model. TCR8 expression enhanced CD8+ T cell function and preserved less differentiated CD4+ and CD8+ T cells after tumor challenge. TCR8+CD4+ T cells were most potent by activating multiple transcriptional programs associated with enhanced antitumor function. We found sustained activation of cytotoxicity, costimulation, oxidative phosphorylation- and proliferation-related genes, and simultaneously reduced differentiation and exhaustion. Our study identifies molecular features of TCR8 expression that can guide the development of enhanced immunotherapies.

An accurate and robust imputation method scImpute for single-cell RNA-seq data

Nature Communications (IF: 13.61), 2018-12, Wei Vivian Li et al. DOI: 10.1038/s41467-018-03405-7
PubMed: 29520097

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

The emerging single-cell RNA sequencing (scRNA-seq) technologies enable the investigation of transcriptomic landscapes at the single-cell resolution. ScRNA-seq data analysis is complicated by excess zero counts, the so-called dropouts due to low amounts of mRNA sequenced within individual cells. We introduce scImpute, a statistical method to accurately and robustly impute the dropouts in scRNA-seq data. scImpute automatically identifies likely dropouts, and only perform imputation on these values without introducing new biases to the rest data. scImpute also detects outlier cells and excludes them from imputation. Evaluation based on both simulated and real human and mouse scRNA-seq data suggests that scImpute is an effective tool to recover transcriptome dynamics masked by dropouts. scImpute is shown to identify likely dropouts, enhance the clustering of cell subpopulations, improve the accuracy of differential expression analysis, and aid the study of gene expression dynamics.

Single-cell analysis of two severe COVID-19 patients reveals a monocyte-associated and tocilizumab-responding cytokine storm

Nature Communications (IF: 13.61), 2020-8, Jun Lin et al. DOI: 10.1038/s41467-020-17834 PubMed: 32764665
Single Cell Gene Expression, Human, Infectious Disease.

Abstract

Several studies show that the immunosuppressive drugs targeting the interleukin-6 (IL-6) receptor, including tocilizumab, ameliorate lethal inflammatory responses in COVID-19 patients infected with SARS-CoV-2. Here, by employing single-cell analysis of the immune cell composition of two severe-stage COVID-19 patients prior to and following tocilizumab-induced remission, we identify a monocyte subpopulation that contributes to the inflammatory cytokine storms. Furthermore, although tocilizumab treatment attenuates the inflammation, immune cells, including plasma B cells and CD8T cells, still exhibit robust humoral and cellular antiviral immune responses. Thus, in addition to providing a high-dimensional dataset on the immune cell distribution at multiple stages of the COVID-19, our work also provides insights into the therapeutic effects of tocilizumab, and identifies potential target cell populations for treating COVID-19-related cytokine storms.

Somatic mTOR mutation in clonally expanded T lymphocytes associated with chronic graft versus host disease

Nature Communications (IF: 13.61), 2020-5, Mikko A I Keränen et al. DOI: 10.1038/s41467-020-16115 PubMed: 32382059
Single Cell Immune Profiling, Human, Immunology.

Abstract

Graft versus host disease (GvHD) is the main complication of allogeneic hematopoietic stem cell transplantation (HSCT). Here we report studies of a patient with chronic GvHD (cGvHD) carrying persistent CD4T cell clonal expansion harboring somatic mTOR, NFKB2, and TLR2 mutations. In the screening cohort (n = 134), we detect the mTOR P2229R kinase domain mutation in two additional cGvHD patients, but not in healthy or HSCT patients without cGvHD. Functional analyses of the mTOR mutation indicate a gain-of-function alteration and activation of both mTORC1 and mTORC2 signaling pathways, leading to increased cell proliferation and decreased apoptosis. Single-cell RNA sequencing and real-time impedance measurements support increased cytotoxicity of mutated CD4T cells. High throughput drug-sensitivity testing suggests that mutations induce resistance to mTOR inhibitors, but increase sensitivity for HSP90 inhibitors. Our findings imply that somatic mutations may contribute to aberrant T cell proliferations and persistent immune activation in cGvHD, thereby paving the way for targeted therapies.

Single-cell transcriptomics identifies an effectorness gradient shaping the response of CD4⁺ T cells to cytokines

Nature Communications (IF: 13.61), 2020-4, Jyoti S Choudhary et al. DOI: 10.1038/s41467-020-15543

PubMed: 32286271

Single Cell Gene Expression, Human, Immunology.

Abstract

Naïve CD4⁺T cells coordinate the immune response by acquiring an effector phenotype in response to cytokines. However, the cytokine responses in memory T cells remain largely understudied. Here we use quantitative proteomics, bulk RNA-seq, and single-cell RNA-seq of over 40,000 human naïve and memory CD4⁺T cells to show that responses to cytokines differ substantially between these cell types. Memory T cells are unable to differentiate into the Th2 phenotype, and acquire a Th17-like phenotype in response to iTreg polarization. Single-cell analyses show that T cells constitute a transcriptional continuum that progresses from naïve to central and effector memory T cells, forming an effectorness gradient accompanied by an increase in the expression of chemokines and cytokines. Finally, we show that T cell activation and cytokine responses are influenced by the effectorness gradient. Our results illustrate the heterogeneity of T cell responses, furthering our understanding of inflammation.

Endothelialization of arterial vascular grafts by circulating monocytes

Nature Communications (IF: 13.61), 2020-4, Daniel D Swartz et al. DOI: 10.1038/s41467-020-15361-2

PubMed: 32238801

Single Cell Gene Expression, Human, Cell Biology.

Abstract

Recently our group demonstrated that acellular tissue engineered vessels (A-TEVs) comprised of small intestinal submucosa (SIS) immobilized with heparin and vascular endothelial growth factor (VEGF) could be implanted into the arterial system of a pre-clinical ovine animal model, where they endothelialized within one month and remained patent. Here we report that immobilized VEGF captures blood circulating monocytes (MC) with high specificity under a range of shear stresses. Adherent MC differentiate into a mixed endothelial (EC) and macrophage (M ϕ) phenotype and further develop into mature EC that align in the direction of flow and produce nitric oxide under high shear stress. In-vivo, newly recruited cells on the vascular lumen express MC markers and at later times they co-express MC and EC-specific proteins and maintain graft patency. This novel finding indicates that the highly prevalent circulating MC contribute directly to the endothelialization of acellular vascular grafts under the right chemical and biomechanical cues.

Inference and effects of barcode multiplets in droplet-based single-cell assays

Nature Communications (IF: 13.61), 2020-2, Fabiana M Duarte et al. DOI: 10.1038/s41467-020-14667-5

PubMed: 32054859

Single Cell ATAC, Human, Method Comparison.

Abstract

A widespread assumption for single-cell analyses specifies that one cell's nucleic acids are predominantly captured by one oligonucleotide barcode. Here, we show that ~13-21% of cell barcodes from the 10x Chromium scATAC-seq assay may have been derived from a droplet with more than one oligonucleotide sequence, which we call barcode multiplets. We demonstrate that barcode multiplets can be derived from at least two different sources. First, we confirm that approximately 4% of droplets from the 10x platform may contain multiple beads. Additionally, we find that approximately 5% of beads may contain detectable levels of multiple oligonucleotide barcodes. We show that this artifact can confound single-cell analyses, including the interpretation of clonal diversity and proliferation of intra-tumor lymphocytes. Overall, our work provides a conceptual and computational framework to identify and assess the impacts of barcode multiplets in single-cell data.

Chromatin mapping and single-cell immune profiling define the temporal dynamics of ibrutinib response in CLL

Nature Communications (IF: 13.61), 2020-1, Christian Schmidl et al. DOI: 10.1038/s41467-019-14081-6

PubMed: 31996669

Single Cell Gene Expression, Human, Cancer Research.

Abstract

The Bruton tyrosine kinase (BTK) inhibitor ibrutinib provides effective treatment for patients with chronic lymphocytic leukemia (CLL), despite extensive heterogeneity in this disease. To define the underlining regulatory dynamics, we analyze high-resolution time courses of ibrutinib treatment in patients with CLL, combining immune-phenotyping, single-cell transcriptome profiling, and chromatin mapping. We identify a consistent regulatory program starting with a sharp decrease of NF- κ B binding in CLL cells, which is followed by reduced activity of lineage-defining transcription factors, erosion of CLL cell identity, and acquisition of a quiescence-like gene signature. We observe patient-to-patient variation in the speed of execution of this program, which we exploit to predict patient-specific dynamics in the response to ibrutinib based on the pre-treatment patient samples. In aggregate, our study describes time-dependent cellular, molecular, and regulatory effects for therapeutic inhibition of B cell receptor signaling in CLL, and it establishes a broadly applicable method for epigenome/transcriptome-based treatment monitoring.

Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis

Nature Communications (IF: 13.61), 2020-1, Nir Yosef et al. DOI: 10.1038/s41467-019-14118 PubMed: 31937773
Single Cell Gene Expression, Human, Immunology, Neuroscience.

Abstract

Cerebrospinal fluid (CSF) protects the central nervous system (CNS) and analyzing CSF aids the diagnosis of CNS diseases, but our understanding of CSF leukocytes remains superficial. Here, using single cell transcriptomics, we identify a specific location-associated composition and transcriptome of CSF leukocytes. Multiple sclerosis (MS) - an autoimmune disease of the CNS - increases transcriptional diversity in blood, but increases cell type diversity in CSF including a higher abundance of cytotoxic phenotype T helper cells. An analytical approach, named cell set enrichment analysis (CSEA) identifies a cluster-independent increase of follicular (TFH) cells potentially driving the known expansion of B lineage cells in the CSF in MS. In mice, TFH cells accordingly promote B cell infiltration into the CNS and the severity of MS animal models. Immune mechanisms in MS are thus highly compartmentalized and indicate ongoing local T/B cell interaction.

Clonal kinetics and single-cell transcriptional profiling of CAR-T cells in patients undergoing CD19 CAR-T immunotherapy

Nature Communications (IF: 13.61), 2020-1, Peter S Linsley et al. DOI: 10.1038/s41467-019-13880-1
PubMed: 31924795

Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Chimeric antigen receptor (CAR) T-cell therapy has produced remarkable anti-tumor responses in patients with B-cell malignancies. However, clonal kinetics and transcriptional programs that regulate the fate of CAR-T cells after infusion remain poorly understood. Here we perform TCRB sequencing, integration site analysis, and single-cell RNA sequencing (scRNA-seq) to profile CD8CAR-T cells from infusion products (IPs) and blood of patients undergoing CD19 CAR-T immunotherapy. TCRB sequencing shows that clonal diversity of CAR-T cells is highest in the IPs and declines following infusion. We observe clones that display distinct patterns of clonal kinetics, making variable contributions to the CAR-T cell pool after infusion. Although integration site does not appear to be a key driver of clonal kinetics, scRNA-seq demonstrates that clones that expand after infusion mainly originate from infused clusters with higher expression of cytotoxicity and proliferation genes. Thus, we uncover transcriptional programs associated with CAR-T cell behavior after infusion.

Realistic in silico generation and augmentation of single-cell RNA-seq data using generative adversarial networks

Nature Communications (IF: 13.61), 2020-1, Christian F Krebs et al. DOI: 10.1038/s41467-019-14018

PubMed: 31919373

Single Cell Gene Expression, Human, Computational Method.

Abstract

A fundamental problem in biomedical research is the low number of observations available, mostly due to a lack of available biosamples, prohibitive costs, or ethical reasons. Augmenting few real observations with generated in silico samples could lead to more robust analysis results and a higher reproducibility rate. Here, we propose the use of conditional single-cell generative adversarial neural networks (cscGAN) for the realistic generation of single-cell RNA-seq data. cscGAN learns non-linear gene-gene dependencies from complex, multiple cell type samples and uses this information to generate realistic cells of defined types. Augmenting sparse cell populations with cscGAN generated cells improves downstream analyses such as the detection of marker genes, the robustness and reliability of classifiers, the assessment of novel analysis algorithms, and might reduce the number of animal experiments and costs in consequence. cscGAN outperforms existing methods for single-cell RNA-seq data generation in quality and hold great promise for the realistic generation and augmentation of other biomedical data types.

Estimation of immune cell content in tumour tissue using single-cell RNA-seq data

Nature Communications (IF: 13.61), 2017-12, Birgit Schoeberl Andreas Raue DOI: 10.1038/s41467-017-02289-3

PubMed: 29230012

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

As interactions between the immune system and tumour cells are governed by a complex network of cell-cell interactions, knowing the specific immune cell composition of a solid tumour may be essential to predict a patient's response to immunotherapy. Here, we analyse in depth how to derive the cellular composition of a solid tumour from bulk gene expression data by mathematical deconvolution, using indication-specific and cell type-specific reference gene expression profiles (RGEPs) from tumour-derived single-cell RNA sequencing data. We demonstrate that tumour-derived RGEPs are essential for the successful deconvolution and that RGEPs from peripheral blood are insufficient. We distinguish nine major cell types, as well as three T cell subtypes. Using the tumour-derived RGEPs, we can estimate the content of many tumours associated immune and stromal cell types, their therapeutically relevant ratios, as well as an improved gene expression profile of the malignant cells.

Context-specific regulation of surface and soluble IL7R expression by an autoimmune risk allele

Nature Communications (IF: 13.61), 2019-10, Paul Bowness et al. DOI: 10.1038/s41467-019-12393-1

PubMed: 31594933

Single Cell Gene Expression, Human, Genetic Health, Immunology.

Abstract

IL-7 is a key factor in T cell immunity and common variants at IL7R, encoding its receptor, are associated with autoimmune disease susceptibility. IL7R mRNA is induced in stimulated monocytes, yet a function for IL7R in monocyte biology remains unexplored. Here we characterize genetic regulation of IL7R at the protein level in healthy individuals, and find that monocyte surface and soluble IL7R (sIL7R) are markedly induced by lipopolysaccharide. In monocytes, both surface IL7R and sIL7R expression strongly associate with allelic carriage of rs6897932, a disease-associated IL7R polymorphism. Monocytes produce more sIL7R than CD4⁺ T cells, and the amount is additionally correlated with the expression of DDX39A, encoding a splicing factor. Synovial fluid-derived monocytes from patients with spondyloarthritis are enriched for IL7R cells with a unique transcriptional profile that overlaps with IL-7-induced gene sets. Our data thus suggest a previously unappreciated function for monocytes in IL-7 biology and IL7R-associated diseases.

Heterogeneity of human bone marrow and blood natural killer cells defined by single-cell transcriptome

Nature Communications (IF: 13.61), 2019-9, Monica S Thakar et al. DOI: 10.1038/s41467-019-11947-7

PubMed: 31477722

Single Cell Gene Expression, Human, Immunology.

Abstract

Natural killer (NK) cells are critical to both innate and adaptive immunity. However, the development and heterogeneity of human NK cells are yet to be fully defined. Using single-cell RNA-sequencing technology, here we identify distinct NK populations in human bone marrow and blood, including one population expressing higher levels of immediate early genes indicative of a homeostatic activation. Functionally matured NK cells with high expression of CX3CR1, HAVCR2 (TIM-3), and ZEB2 represents terminally differentiated status with the unique transcriptional profile. Transcriptomic and pseudotime analyses identify a transitional population between CD56^{low} and CD56^{high} NK cells. Finally, a donor with GATA2 mutation exhibits reduced percentage of CD56^{high} NK cells with altered transcriptome and elevated cell death. These data expand our understanding of the heterogeneity and development of human NK cells.

Dissecting the heterogeneity of DENV vaccine-elicited cellular immunity using single-cell RNA sequencing and metabolic profiling

Nature Communications (IF: 13.61), 2019-8, Heather Friberg et al. DOI: 10.1038/s41467-019-11634-7

PubMed: 31413301

Single Cell Immune Profiling, Human, Immunology, Infectious Disease.

Abstract

Generating effective and durable T cell immunity is a critical prerequisite for vaccination against dengue virus (DENV) and other viral diseases. However, understanding the molecular mechanisms of vaccine-elicited T cell immunity remains a critical knowledge gap in vaccinology. In this study, we utilize single-cell RNA sequencing (scRNAseq) and longitudinal TCR clonotype analysis to identify a unique transcriptional signature present in acutely activated and clonally-expanded T cells that become committed to the memory repertoire. This effector/memory-associated transcriptional signature is dominated by a robust metabolic transcriptional program. Based on this transcriptional signature, we are able to define a set of markers that identify the most durable vaccine-reactive memory-precursor CD8T cells. This study illustrates the power of scRNAseq as an analytical tool to assess the molecular mechanisms of host control and vaccine modality in determining the magnitude, diversity and persistence of vaccine-elicited cell-mediated immunity.

Predicting bacterial infection outcomes using single cell RNA-sequencing analysis of human immune cells

Nature Communications (IF: 13.61), 2019-7, Mihai G Netea et al. DOI: 10.1038/s41467-019-11257 PubMed: 31332193

Single Cell Gene Expression, Human, Immunology, Infectious Disease.

Abstract

Complex interactions between different host immune cell types can determine the outcome of pathogen infections. Advances in single cell RNA-sequencing (scRNA-seq) allow probing of these immune interactions, such as cell-type compositions, which are then interpreted by deconvolution algorithms using bulk RNA-seq measurements. However, not all aspects of immune surveillance are represented by current algorithms. Here, using scRNA-seq of human peripheral blood cells infected with Salmonella, we develop a deconvolution algorithm for inferring cell-type specific infection responses from bulk measurements. We apply our dynamic deconvolution algorithm to a cohort of healthy individuals challenged ex vivo with Salmonella, and to three cohorts of tuberculosis patients during different stages of disease. We reveal cell-type specific immune responses associated not only with ex vivo infection phenotype but also with clinical disease stage. We propose that our approach provides a predictive power to identify risk for disease, and human infection outcomes.

Impact of constitutional TET2 haploinsufficiency on molecular and clinical phenotype in humans

Nature Communications (IF: 13.61), 2019-3, Outi KilpivaaraLauri A Aaltonen et al. DOI: 10.1038/s41467-019-09198-7 PubMed: 30890702

Single Cell Gene Expression, Human, Genetic Health.

Abstract

Clonal hematopoiesis driven by somatic heterozygous TET2 loss is linked to malignant degeneration via consequent aberrant DNA methylation, and possibly to cardiovascular disease via increased cytokine and chemokine expression as reported in mice. Here, we discover a germline TET2 mutation in a lymphoma family. We observe neither unusual predisposition to atherosclerosis nor abnormal pro-inflammatory cytokine or chemokine expression. The latter finding is confirmed in cells from three additional unrelated TET2 germline mutation carriers. The TET2 defect elevates blood DNA methylation levels, especially at active enhancers and cell-type specific regulatory regions with binding sequences of master transcription factors involved in hematopoiesis. The regions display reduced methylation relative to all open chromatin regions in four DNMT3A germline mutation carriers, potentially due to TET2-mediated oxidation. Our findings provide insight into the interplay between epigenetic modulators and transcription factor activity in hematological neoplasia, but do not confirm the putative role of TET2 in atherosclerosis.

Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway

Nature Communications (IF: 13.61), 2018-7, Sebastian AmigorenaElodie Segura et al. DOI: 10.1038/s41467-018-04985-0 PubMed: 29967419

Single Cell Gene Expression, Human, Immunology.

Abstract

Presentation of exogenous antigens on MHC-I molecules, termed cross-presentation, is essential for cytotoxic CD8T cell responses. In mice, dendritic cells (DCs) that arise from monocytes (mo-DCs) during inflammation have a key function in these responses by cross-presenting antigens locally in peripheral tissues. Whether human naturally-occurring mo-DCs can cross-present is unknown. Here, we use human mo-DCs and macrophages directly purified from ascites to address this question. Single-cell RNA-seq data show that ascites CD1cDCs contain exclusively monocyte-derived cells. Both ascites mo-DCs and monocyte-derived macrophages cross-present efficiently, but are inefficient for transferring exogenous proteins into their cytosol. Inhibition of cysteine proteases, but not of proteasome, abolishes cross-presentation in these cells. We conclude that human monocyte-derived cells cross-present exclusively using a vacuolar pathway. Finally, only ascites mo-DCs provide co-stimulatory signals to induce effector cytotoxic CD8T cells. Our findings thus provide important insights on how to harness cross-presentation for therapeutic purposes.

Biological process activity transformation of single cell gene expression for cross-species alignment

Nature Communications (IF: 13.61), 2019-10, Ying Yang et al. DOI: 10.1038/s41467-019-12924

PubMed: 31653878

Single Cell Gene Expression, Human, Computational Method.

Abstract

The maintenance and transition of cellular states are controlled by biological processes. Here we present a gene set-based transformation of single cell RNA-Seq data into biological process activities that provides a robust description of cellular states. Moreover, as these activities represent species-independent descriptors, they facilitate the alignment of single cell states across different organisms.

Type I interferons drive the maturation of human DC3s with a distinct costimulatory profile characterized by high GITRL

Science Immunology (IF: 13.41), 2020-11, Maria I Edilova et al. DOI: 10.1126/sciimmunol.abe0347

PubMed: 33188059

Single Cell Gene Expression, Human, Immunology.

Abstract

Human mononuclear phagocytes comprise specialized subsets of dendritic cells (DCs) and monocytes, but how these subsets individually regulate expression of the molecular signals involved in T cell costimulation is incompletely understood. Here, we used multiparameter flow cytometry and CITE-sequencing to investigate the cell type-specific responses of human peripheral blood DC and monocyte subsets to type I interferons (IFN-I), focusing on differential regulation of costimulatory molecules. We report that IFN- β drives the maturation of the recently identified human CD1cCD5DC3 subset into cells with higher GITRL and lower CD86 expression compared with other conventional DC subsets. Transcriptomic analysis confirmed that DC3s have an intermediate phenotype between that of CD1cCD5DC2s and CD14monocytes, characterized by high expression of MHCII, Fc receptors, and components of the phagocyte NADPH oxidase. IFN- β induced a shared core response in human DC and monocyte subsets as well as subset-specific responses, including differential expression of costimulatory molecules. Gene regulatory network analysis suggests that upon IFN- β stimulation NFKB1 drives DC3s to acquire a maturation program shared with DC2s. Accordingly, inhibition of NF- κ B activation prevented the acquisition of a mature phenotype by DC3s upon IFN- β exposure. Collectively, this study provides insight into the cell type-specific response of human DC and monocyte subsets to IFN-I and highlights the distinct costimulatory potential of DC3s.

The myeloid type I interferon response to myocardial infarction begins in bone marrow and is regulated by Nrf2-activated macrophages

Science Immunology (IF: 13.41), 2020-9, Zhenxing Fu et al. DOI: 10.1126/sciimmunol.aaz1974 PubMed: 32978242
Single Cell Gene Expression, Human & Mouse, Immunology.

Abstract

Sterile tissue injury is thought to locally activate innate immune responses via damage-associated molecular patterns (DAMPs). Whether innate immune pathways are remotely activated remains relatively unexplored. Here, by analyzing ~145,000 single-cell transcriptomes at steady state and after myocardial infarction (MI) in mice and humans, we show that the type I interferon (IFN) response, characterized by expression of IFN-stimulated genes (ISGs), begins far from the site of injury, in neutrophil and monocyte progenitors within the bone marrow. In the peripheral blood of patients, we observed defined subsets of ISG-expressing neutrophils and monocytes. In the bone marrow and blood of mice, ISG expression was detected in neutrophils and monocytes and their progenitors, intensified with maturation at steady-state and after MI, and was controlled by Tet2 and Irf3 transcriptional regulators. Within the infarcted heart, ISG-expressing cells were negatively regulated by Nrf2 activation in Ccr2steady-state cardiac macrophages. Our results show that IFN signaling begins in the bone marrow, implicate multiple transcriptional regulators (Tet2, Irf3, and Nrf2) in governing ISG expression, and provide a clinical biomarker (ISG score) for studying IFN signaling in patients.

Heterogeneity and clonal relationships of adaptive immune cells in ulcerative colitis revealed by single-cell analyses

Science Immunology (IF: 13.41), 2020-8, Gene W Yeo et al. DOI: 10.1126/sciimmunol.abb4432 PubMed: 32826341
Single Cell Immune Profiling, Human, Immunology.

Abstract

Inflammatory bowel disease (IBD) encompasses a spectrum of gastrointestinal disorders driven by dysregulated immune responses against gut microbiota. We integrated single-cell RNA and antigen receptor sequencing to elucidate key components, cellular states, and clonal relationships of the peripheral and gastrointestinal mucosal immune systems in health and ulcerative colitis (UC). UC was associated with an increase in IgG1 plasma cells in colonic tissue, increased colonic regulatory T cells characterized by elevated expression of the transcription factor ZEB2, and an enrichment of a $\gamma\delta$ T cell subset in the peripheral blood. Moreover, we observed heterogeneity in CD8 tissue-resident memory T (T) cells in colonic tissue, with four transcriptionally distinct states of differentiation observed across health and disease. In the setting of UC, there was a marked shift of clonally related CD8 T cells toward an inflammatory state, mediated, in part, by increased expression of the T-box transcription factor Eomesodermin. Together, these results provide a detailed atlas of transcriptional changes occurring in adaptive immune cells in the context of UC and suggest a role for CD8 T cells in IBD.

Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19

Science Immunology (IF: 13.41), 2020-7, Inkyung Jung et al. DOI: 10.1126/sciimmunol.abd1554

PubMed: 32651212

Single Cell Gene Expression, Human, Infectious Disease.

Abstract

Although most SARS-CoV-2-infected individuals experience mild coronavirus disease 2019 (COVID-19), some patients suffer from severe COVID-19, which is accompanied by acute respiratory distress syndrome and systemic inflammation. To identify factors driving severe progression of COVID-19, we performed single-cell RNA-seq using peripheral blood mononuclear cells (PBMCs) obtained from healthy donors, patients with mild or severe COVID-19, and patients with severe influenza. Patients with COVID-19 exhibited hyper-inflammatory signatures across all types of cells among PBMCs, particularly up-regulation of the TNF/IL-1 β -driven inflammatory response as compared to severe influenza. In classical monocytes from patients with severe COVID-19, type I IFN response co-existed with the TNF/IL-1 β -driven inflammation, and this was not seen in patients with milder COVID-19. Interestingly, we documented type I IFN-driven inflammatory features in patients with severe influenza as well. Based on this, we propose that the type I IFN response plays a pivotal role in exacerbating inflammation in severe COVID-19.

Adaptive immune responses to SARS-CoV-2 infection in severe versus mild individuals

Signal Transduction and Targeted Therapy (IF: 13.2), 2020-8, Xiaoke Wu et al. DOI: 10.1038/s41392-020-00263

PubMed: 32796814

Single Cell Immune Profiling, Human, Infectious Disease.

Abstract

The global Coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 has affected more than eight million people. There is an urgent need to investigate how the adaptive immunity is established in COVID-19 patients. In this study, we profiled adaptive immune cells of PBMCs from recovered COVID-19 patients with varying disease severity using single-cell RNA and TCR/BCR V(D)J sequencing. The sequencing data revealed SARS-CoV-2-specific shuffling of adaptive immune repertoires and COVID-19-induced remodeling of peripheral lymphocytes. Characterization of variations in the peripheral T and B cells from the COVID-19 patients revealed a positive correlation of humoral immune response and T-cell immune memory with disease severity. Sequencing and functional data revealed SARS-CoV-2-specific T-cell immune memory in the convalescent COVID-19 patients. Furthermore, we also identified novel antigens that are responsive in the convalescent patients. Altogether, our study reveals adaptive immune repertoires underlying pathogenesis and recovery in severe versus mild COVID-19 patients, providing valuable information for potential vaccine and therapeutic development against SARS-CoV-2 infection.

Single-cell transcriptomic analysis of allergen-specific T cells in allergy and asthma

Science Immunology (IF: 13.41), 2020-6, Alessandro Sette et al. DOI: 10.1126/sciimmunol.aba6087

PubMed: 32532832

Single Cell Gene Expression, Human, Immunology.

Abstract

CD4T helper (T) cells and regulatory T (T) cells that respond to common allergens play an important role in driving and dampening airway inflammation in patients with asthma. Until recently, direct, unbiased molecular analysis of allergen-reactive T cells has not been possible. To better understand the diversity of these T cell subsets in allergy and asthma, we analyzed the single-cell transcriptome of ~50,000 house dust mite (HDM) allergen-reactive T cells and T cells from asthmatics with HDM allergy and from three control groups: asthmatics without HDM allergy and nonasthmatics with and without HDM allergy. Our analyses show that HDM allergen-reactive T cells are highly heterogeneous and certain subsets are quantitatively and qualitatively different in individuals with HDM-reactive asthma. The number of interleukin-9 (IL-9)-expressing HDM-reactive T cells is greater in asthmatics with HDM allergy compared with nonasthmatics with HDM allergy, and this IL-9-expressing T subset displays enhanced pathogenic properties. More HDM-reactive T cells expressing the interferon response signature (TIFNR and TIFNR) are present in asthmatics without HDM allergy compared with those with HDM allergy. In cells from these subsets (TIFNR and TIFNR), expression of *IFITM1* was enriched; its product, tumor necrosis factor-related apoptosis-inducing ligand, dampens activation of T cells. These findings suggest that the TIFNR and TIFNR subsets may dampen allergic responses, which may help explain why only some people develop T2 responses to nearly ubiquitous allergens.

Targeting individual cells by barcode in pooled sequence libraries

Nucleic Acids Research (IF: 11.8), 2019-1, Nir Hacohen Paul C Blainey DOI: 10.1093/nar/gky856

PubMed: 30256981

Single Cell Gene Expression, Human, Assay Method.

Abstract

Transcriptional profiling of thousands of single cells in parallel by RNA-seq is now routine. However, due to reliance on pooled library preparation, targeting analysis to particular cells of interest is difficult. Here, we present a multiplexed PCR method for targeted sequencing of select cells from pooled single-cell sequence libraries. We demonstrated this molecular enrichment method on multiple cell types within pooled single-cell RNA-seq libraries produced from primary human blood cells. We show how molecular enrichment can be combined with FACS to efficiently target ultra-rare cell types, such as the recently identified AXL+SIGLEC6+ dendritic cell (AS DC) subset, in order to reduce the required sequencing effort to profile single cells by 100-fold. Our results demonstrate that DNA barcodes identifying cells within pooled sequencing libraries can be used as targets to enrich for specific molecules of interest, for example reads from a set of target cells.

Association of Clonal Hematopoiesis of Indeterminate Potential With Inflammatory Gene Expression in Patients With Severe Degenerative Aortic Valve Stenosis or Chronic Postischemic Heart Failure

JAMA cardiology (IF: 12.28), 2020-10, Stefanie Dimmeler et al. DOI: 10.1001/jamacardio.2020.2468

PubMed: 32639511

Single Cell Gene Expression, Human, Genetic Health ,Immunology.

Abstract

Cytokine release syndrome is a complication of coronavirus disease 2019. Clinically, advanced age and cardiovascular comorbidities are the most important risk factors. To determine whether clonal hematopoiesis of indeterminate potential (CHIP), an age-associated condition with excess cardiovascular risk defined as the presence of an expanded, mutated somatic blood cell clone in persons without other hematological abnormalities, may be associated with an inflammatory gene expression sensitizing monocytes to aggravated immune responses. This hypothesis-generating diagnostic study examined a cohort of patients with severe degenerative aortic valve stenosis or chronic postinfarction heart failure, as well as age-matched healthy control participants. Single-cell RNA sequencing and analyses of circulating peripheral monocytes was done between 2017 and 2019 to assess the transcriptome of circulating monocytes. Severe degenerative aortic valve stenosis or chronic postinfarction heart failure. CHIP-driver sequence variations in monocytes with a proinflammatory signature of genes involved in cytokine release syndrome. The study included 8 patients with severe degenerative aortic valve stenosis, 6 with chronic postinfarction heart failure, and 3 healthy control participants. Their mean age was 75.7 (range, 54-89) years, and 6 were women. Mean CHIP-driver gene variant allele frequency was 4.2% (range, 2.5%-6.9%) for DNMT3A and 14.3% (range, 2.6%-37.4%) for TET2. Participants with DNMT3A or TET2 CHIP-driver sequence variations displayed increased expression of interleukin 1 β (no CHIP-driver sequence variations, 1.6217 normalized Unique Molecular Identifiers [nUMI]; DNMT3A, 5.3956 nUMI; P < .001; TET2, 10.8216 nUMI; P < .001), the interleukin 6 receptor (no CHIP-driver sequence variations, 0.5386 nUMI; DNMT3A, 0.9162 nUMI; P < .001; TET2, 0.5738 nUMI; P < .001), as well as the NLRP3 inflammasome complex (no CHIP-driver sequence variations, 0.4797 nUMI; DNMT3A, 0.9961 nUMI; P < .001; TET2, 1.2189 nUMI; P < .001), plus upregulation of CD163 (no CHIP-driver sequence variations, 0.5239 nUMI; DNMT3A, 1.4722 nUMI; P < .001; TET2, 1.0684 nUMI; P < .001), a cellular receptor capable of mediating infection, macrophage activation syndrome, and other genes involved in cytokine response syndrome. Gene ontology term analyses of regulated genes revealed that the most significantly upregulated genes encode for leukocyte-activation and interleukin-signaling pathways in monocytes of individuals with DNMT3A (myeloid leukocyte activation: log[Q value], -50.1986; log P value, -54.5177; regulation of cytokine production: log[Q value], -21.0264; log P value, -24.1993; signaling by interleukins: log[Q value], -18.0710; log P value, -21.1597) or TET2 CHIP-driver sequence variations (immune response: log[Q value], -36.3673; log P value, -40.6864; regulation of cytokine production: log[Q value], -13.1733; log P value, -16.3463; signaling by interleukins: log[Q value], -12.6547; log P value, -15.7977). Monocytes of individuals who carry CHIP-driver sequence variations and have cardiovascular disease appear to be primed for excessive inflammatory responses. Further studies are warranted to address potential adverse outcomes of coronavirus disease 2019 in patients with CHIP-driver sequence variations.

Defective Sec61 α 1 underlies a novel cause of autosomal dominant severe congenital neutropenia

Journal of Allergy and Clinical Immunology, The (IF: 12.04), 2020-11, Isabelle Meyts et al.

DOI: 10.1016/j.jaci.2020.03.034 PubMed: 32325141

Single Cell Gene Expression, Human, Immunology.

Abstract

The molecular cause of severe congenital neutropenia (SCN) is unknown in 30% to 50% of patients. SEC61A1 encodes the α -subunit of the Sec61 complex, which governs endoplasmic reticulum protein transport and passive calcium leakage. Recently, mutations in SEC61A1 were reported to be pathogenic in common variable immunodeficiency and glomerulocystic kidney disease. Our aim was to expand the spectrum of SEC61A1-mediated disease to include autosomal dominant SCN. Whole exome sequencing findings were validated, and reported mutations were compared by Western blotting, Ca²⁺flux assays, differentiation of transduced HL-60 cells, in vitro differentiation of primary CD34 cells, quantitative PCR for unfolded protein response (UPR) genes, and single-cell RNA sequencing on whole bone marrow. We identified a novel de novo missense mutation in SEC61A1 (c.A275G;p.Q92R) in a patient with SCN who was born to nonconsanguineous Belgian parents. The mutation results in diminished protein expression, disturbed protein translocation, and an increase in calcium leakage from the endoplasmic reticulum. In vitro differentiation of CD34 cells recapitulated the patient's clinical arrest in granulopoiesis. The impact of Q92R-Sec61 α 1 on neutrophil maturation was validated by using HL-60 cells, in which transduction reduced differentiation into CD11b⁺CD16⁺ cells. A potential mechanism for this defect is the uncontrolled initiation of the unfolded protein stress response, with single-cell analysis of primary bone marrow revealing perturbed UPR in myeloid precursors and in vitro differentiation of primary CD34 cells revealing upregulation of CCAAT/enhancer-binding protein homologous protein and immunoglobulin heavy chain binding protein UPR-response genes. Specific mutations in SEC61A1 cause SCN through dysregulation of the UPR.

dropClust: efficient clustering of ultra-large scRNA-seq data

Nucleic Acids Research (IF: 11.8), 2018-4, Sanghamitra Bandyopadhyay Debarka Sengupta et al.

DOI: 10.1093/nar/gky007 PubMed: 29361178

Single Cell Gene Expression, Human, Computational Method.

Abstract

Droplet based single cell transcriptomics has recently enabled parallel screening of tens of thousands of single cells. Clustering methods that scale for such high dimensional data without compromising accuracy are scarce. We exploit Locality Sensitive Hashing, an approximate nearest neighbour search technique to develop a de novo clustering algorithm for large-scale single cell data. On a number of real datasets, dropClust outperformed the existing best practice methods in terms of execution time, clustering accuracy and detectability of minor cell subtypes.

Transcriptional fates of human-specific segmental duplications in brain

Genome research (IF: 12.01), 2018-10, Alex A Pollen, Evan E Eichler et al. DOI: 10.1101/gr.237610.118

PubMed: 30228200

Single Cell Gene Expression, Human, Genome Analysis.

Abstract

Despite the importance of duplicate genes for evolutionary adaptation, accurate gene annotation is often incomplete, incorrect, or lacking in regions of segmental duplication. We developed an approach combining long-read sequencing and hybridization capture to yield full-length transcript information and confidently distinguish between nearly identical genes/paralogs. We used biotinylated probes to enrich for full-length cDNA from duplicated regions, which were then amplified, size-fractionated, and sequenced using single-molecule, long-read sequencing technology, permitting us to distinguish between highly identical genes by virtue of multiple paralogous sequence variants. We examined 19 gene families as expressed in developing and adult human brain, selected for their high sequence identity (average >99%) and overlap with human-specific segmental duplications (SDs). We characterized the transcriptional differences between related paralogs to better understand the birth-death process of duplicate genes and particularly how the process leads to gene innovation. In 48% of the cases, we find that the expressed duplicates have changed substantially from their ancestral models due to novel sites of transcription initiation, splicing, and polyadenylation, as well as fusion transcripts that connect duplication-derived exons with neighboring genes. We detect unannotated open reading frames in genes currently annotated as pseudogenes, while relegating other duplicates to nonfunctional status. Our method significantly improves gene annotation, specifically defining full-length transcripts, isoforms, and open reading frames for new genes in highly identical SDs. The approach will be more broadly applicable to genes in structurally complex regions of other genomes where the duplication process creates novel genes important for adaptive traits.

Generalizable and Scalable Visualization of Single-Cell Data Using Neural Networks

Cell systems (IF: 11.45), 2018-8, Bonnie Berger et al. DOI: 10.1016/j.cels.2018.05.017 PubMed: 29936184

Single Cell Gene Expression, Human & mouse, Computational Method.

Abstract

Visualization algorithms are fundamental tools for interpreting single-cell data. However, standard methods, such as t-stochastic neighbor embedding (t-SNE), are not scalable to datasets with millions of cells and the resulting visualizations cannot be generalized to analyze new datasets. Here we introduce net-SNE, a generalizable visualization approach that trains a neural network to learn a mapping function from high-dimensional single-cell gene-expression profiles to a low-dimensional visualization. We benchmark net-SNE on 13 different datasets, and show that it achieves visualization quality and clustering accuracy comparable with t-SNE. Additionally we show that the mapping function learned by net-SNE can accurately position entire new subtypes of cells from previously unseen datasets and can also be used to reduce the runtime of visualizing 1.3 million cells by 36-fold (from 1.5 days to an hour). Our work provides a framework for bootstrapping single-cell analysis from existing datasets.

Recovery and analysis of transcriptome subsets from pooled single-cell RNA-seq libraries

Nucleic Acids Research (IF: 11.8), 2019-2, Carol A Sartorius et al. DOI: 10.1093/nar/gky1204 PubMed: 30496484
Single Cell Gene Expression & Single Cell Immune Profiling, Human, Mouse, Assay Method.

Abstract

Single-cell RNA sequencing (scRNA-seq) methods generate sparse gene expression profiles for thousands of single cells in a single experiment. The information in these profiles is sufficient to classify cell types by distinct expression patterns but the high complexity of scRNA-seq libraries often prevents full characterization of transcriptomes from individual cells. To extract more focused gene expression information from scRNA-seq libraries, we developed a strategy to physically recover the DNA molecules comprising transcriptome subsets, enabling deeper interrogation of the isolated molecules by another round of DNA sequencing. We applied the method in cell-centric and gene-centric modes to isolate cDNA fragments from scRNA-seq libraries. First, we resampled the transcriptomes of rare, single megakaryocytes from a complex mixture of lymphocytes and analyzed them in a second round of DNA sequencing, yielding up to 20-fold greater sequencing depth per cell and increasing the number of genes detected per cell from a median of 1313 to 2002. We similarly isolated mRNAs from targeted T cells to improve the reconstruction of their VDJ-rearranged immune receptor mRNAs. Second, we isolated CD3D mRNA fragments expressed across cells in a scRNA-seq library prepared from a clonal T cell line, increasing the number of cells with detected CD3D expression from 59.7% to 100%. Transcriptome resampling is a general approach to recover targeted gene expression information from single-cell RNA sequencing libraries that enhances the utility of these costly experiments, and may be applicable to the targeted recovery of molecules from other single-cell assays.

Bayesian Inference of Allelic Inclusion Rates in the Human T Cell Receptor Repertoire

Cell systems (IF: 11.45), 2019-11, Jonathan B Preall et al. DOI: 10.1016/j.cels.2019.09.006 PubMed: 31677971
Single Cell Immune Profiling, Human, Immunology.

Abstract

A small population of $\alpha\beta$ T cells is characterized by the expression of more than one unique T cell receptor (TCR); this outcome is the result of allelic inclusion; that is, inclusion of both α - or β -chain alleles during V(D)J recombination. Limitations in single-cell sequencing technology, however, have precluded comprehensive enumeration of these dual receptor T cells. Here, we develop and experimentally validate a fully Bayesian inference model capable of reliably estimating the true rate of α and β TCR allelic inclusion across two different emulsion-barcoding single-cell sequencing platforms. We provide a database composed of over 51,000 previously unpublished allelic inclusion TCR sequence sets drawn from eight healthy individuals and show that allelic inclusion contributes a distinct and functionally important set of sequences to the human TCR repertoire. This database and a Python implementation of our statistical inference model are freely available at our Github repository (https://github.com/JasonACarter/Allelic_inclusion).

A pathogenic and clonally expanded B cell transcriptome in active multiple sclerosis

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-9, Stephen L Hauser et al. DOI: 10.1073/pnas.2008523117 PubMed: 32859762

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immunology, Neuroscience.

Abstract

Central nervous system B cells have several potential roles in multiple sclerosis (MS): secretors of proinflammatory cytokines and chemokines, presenters of autoantigens to T cells, producers of pathogenic antibodies, and reservoirs for viruses that trigger demyelination. To interrogate these roles, single-cell RNA sequencing (scRNA-Seq) was performed on paired cerebrospinal fluid (CSF) and blood from subjects with relapsing-remitting MS (RRMS;= 12), other neurologic diseases (ONDs;= 1), and healthy controls (HCs;= 3). Single-cell immunoglobulin sequencing (scIg-Seq) was performed on a subset of these subjects and additional RRMS (= 4), clinically isolated syndrome (= 2), and OND (= 2) subjects. Further, paired CSF and blood B cell subsets (RRMS;= 7) were isolated using fluorescence activated cell sorting for bulk RNA sequencing (RNA-Seq). Independent analyses across technologies demonstrated that nuclear factor kappa B (NF- κ B) and cholesterol biosynthesis pathways were activated, and specific cytokine and chemokine receptors were up-regulated in CSF memory B cells. Further, SMAD/TGF- β 1 signaling was down-regulated in CSF plasmablasts/plasma cells. Clonally expanded, somatically hypermutated IgM+ and IgG1+ CSF B cells were associated with inflammation, blood-brain barrier breakdown, and intrathecal Ig synthesis. While we identified memory B cells and plasmablast/plasma cells with highly similar Ig heavy-chain sequences across MS subjects, similarities were also identified with ONDs and HCs. No viral transcripts, including from Epstein-Barr virus, were detected. Our findings support the hypothesis that in MS, CSF B cells are driven to an inflammatory and clonally expanded memory and plasmablast/plasma cell phenotype.

Human lymphoid organ cDC2 and macrophages play complementary roles in T follicular helper responses

Journal of experimental medicine, The (IF: 11.32), 2019-7, Sebastian AmigorenaElodie Segura

DOI: 10.1084/jem.20181994 PubMed: 31072818

Single Cell Gene Expression, Human, Immunology.

Abstract

CD4T follicular helper (Tfh) cells are essential for inducing efficient humoral responses. T helper polarization is classically orientated by dendritic cells (DCs), which are composed of several subpopulations with distinct functions. Whether human DC subsets display functional specialization for Tfh polarization remains unclear. Here we find that tonsil cDC2 and CD14macrophages are the best inducers of Tfh polarization. This ability is intrinsic to the cDC2 lineage but tissue dependent for macrophages. We further show that human Tfh cells comprise two effector states producing either IL-21 or CXCL13. Distinct mechanisms drive the production of Tfh effector molecules, involving IL-12p70 for IL-21 and activin A and TGF β for CXCL13. Finally, using imaging mass cytometry, we find that tonsil CD14macrophages localize in situ in the B cell follicles, where they can interact with Tfh cells. Our results indicate that human lymphoid organ cDC2 and macrophages play complementary roles in the induction of Tfh responses.

Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-11, Nobuyoshi Hirose et al. DOI: 10.1073/pnas.1907883116 PubMed: 31719197

Single Cell Immune Profiling, Human, Immunology.

Abstract

Supercentenarians, people who have reached 110 y of age, are a great model of healthy aging. Their characteristics of delayed onset of age-related diseases and compression of morbidity imply that their immune system remains functional. Here we performed single-cell transcriptome analysis of 61,202 peripheral blood mononuclear cells (PBMCs), derived from 7 supercentenarians and 5 younger controls. We identified a marked increase of cytotoxic CD4 T cells (CD4 cytotoxic T lymphocytes [CTLs]) as a signature of supercentenarians. Furthermore, single-cell T cell receptor sequencing of 2 supercentenarians revealed that CD4 CTLs had accumulated through massive clonal expansion, with the most frequent clonotypes accounting for 15 to 35% of the entire CD4 T cell population. The CD4 CTLs exhibited substantial heterogeneity in their degree of cytotoxicity as well as a nearly identical transcriptome to that of CD8 CTLs. This indicates that CD4 CTLs utilize the transcriptional program of the CD8 lineage while retaining CD4 expression. Indeed, CD4 CTLs extracted from supercentenarians produced IFN- γ and TNF- α upon ex vivo stimulation. Our study reveals that supercentenarians have unique characteristics in their circulating lymphocytes, which may represent an essential adaptation to achieve exceptional longevity by sustaining immune responses to infections and diseases.

Interferon lambda promotes immune dysregulation and tissue inflammation in TLR7-induced lupus

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-3, Carmelo Carmona-Rivera et al. DOI: 10.1073/pnas.1916897117 PubMed: 32094169

Single Cell Gene Expression, Human & Mouse, Immunology.

Abstract

Type III IFN lambdas (IFN- λ) have recently been described as important mediators of immune responses at barrier surfaces. However, their role in autoimmune diseases such as systemic lupus erythematosus (SLE), a condition characterized by aberrant type I IFN signaling, has not been determined. Here, we identify a nonredundant role for IFN- λ in immune dysregulation and tissue inflammation in a model of TLR7-induced lupus. IFN- λ protein is increased in murine lupus and IFN- λ receptor (Ifnlr1) deficiency significantly reduces immune cell activation and associated organ damage in the skin and kidneys without effects on autoantibody production. Single-cell RNA sequencing in mouse spleen and human peripheral blood revealed that only mouse neutrophils and human B cells are directly responsive to this cytokine. Rather, IFN- λ activates keratinocytes and mesangial cells to produce chemokines that induce immune cell recruitment and promote tissue inflammation. These data provide insights into the immunobiology of SLE and identify type III IFNs as important factors for tissue-specific pathology in this disease.

Putative regulators for the continuum of erythroid differentiation revealed by single-cell transcriptome of human BM and UCB cells

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, Xinghua Pan et al. DOI: 10.1073/pnas.1915085117 PubMed: 32457162

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Fine-resolution differentiation trajectories of adult human hematopoietic stem cells (HSCs) involved in the generation of red cells is critical for understanding dynamic developmental changes that accompany human erythropoiesis. Using single-cell RNA sequencing (scRNA-seq) of primary human terminal erythroid cells (CD34CD235a) isolated directly from adult bone marrow (BM) and umbilical cord blood (UCB), we documented the transcriptome of terminally differentiated human erythroblasts at unprecedented resolution. The insights enabled us to distinguish polychromatic erythroblasts (PolyEs) at the early and late stages of development as well as the different development stages of orthochromatic erythroblasts (OrthoEs). We further identified a set of putative regulators of terminal erythroid differentiation and functionally validated three of the identified genes, and, by monitoring cell differentiation and apoptosis. We documented that knockdown of suppressed the commitment of HSCs to erythroid lineage and cell proliferation and delayed differentiation of colony-forming unit-erythroid (CFU-E) to the proerythroblast stage (ProE). In contrast, the knockdown of and delayed differentiation of PolyE to OrthoE stage. Taken together, the convergence and divergence of the transcriptional continuums at single-cell resolution underscore the transcriptional regulatory networks that underlie human fetal and adult terminal erythroid differentiation.

Single-cell transcription analysis of Plasmodium vivax blood-stage parasites identifies stage- and species-specific profiles of expression

PLOS Biology (IF: 8.88), 2020-5, Thomas E WellemsDavid Serre DOI: 10.1371/journal.pbio.3000711 PubMed: 32365102

Single Cell Gene Expression, Plasmodium, Primate, Cell Atlas, Infectious Disease.

Abstract

Plasmodium vivax and *P. falciparum*, the parasites responsible for most human malaria worldwide, exhibit striking biological differences, which have important clinical consequences. Unfortunately, *P. vivax*, unlike *P. falciparum*, cannot be cultivated continuously in vitro, which limits our understanding of its biology and, consequently, our ability to effectively control vivax malaria. Here, we describe single-cell gene expression profiles of 9,215 *P. vivax* parasites from bloodstream infections of Aotus and Saimiri monkeys. Our results show that transcription of most *P. vivax* genes occurs during short periods of the intraerythrocytic cycle and that this pattern of gene expression is conserved in other *Plasmodium* species. However, we also identify a strikingly high proportion of species-specific transcripts in late schizonts, possibly associated with the specificity of erythrocyte invasion. Our findings provide new and robust markers of blood-stage parasites, including some that are specific to the elusive *P. vivax* male gametocytes, and will be useful for analyzing gene expression data from laboratory and field samples.

Mass Cytometry and Single-Cell RNA-seq Profiling of the Heterogeneity in Human Peripheral Blood Mononuclear Cells Interacting with Silver Nanoparticles

Small (IF: 10.61), 2020-5, Jin-Wu Nam et al. DOI: 10.1002/smll.201907674 PubMed: 32163679

Single Cell Gene Expression, Human, Assay Method, Immunology.

Abstract

Understanding the interactions between nanoparticles (NPs) and human immune cells is necessary for justifying their utilization in consumer products and biomedical applications. However, conventional assays may be insufficient in describing the complexity and heterogeneity of cell-NP interactions. Herein, mass cytometry and single-cell RNA-sequencing (scRNA-seq) are complementarily used to investigate the heterogeneous interactions between silver nanoparticles (AgNPs) and primary immune cells. Mass cytometry reveals the heterogeneous biodistribution of the positively charged polyethylenimine-coated AgNPs in various cell types and finds that monocytes and B cells have higher association with the AgNPs than other populations. scRNA-seq data of these two cell types demonstrate that each type has distinct responses to AgNP treatment: NRF2-mediated oxidative stress is confined to B cells, whereas monocytes show Fcγ-mediated phagocytosis. Besides the between-population heterogeneity, analysis of single-cell dose-response relationships further reveals within-population diversity for the B cells and naïve CD4T cells. Distinct subsets having different levels of cellular responses with respect to their cellular AgNP doses are found. This study demonstrates that the complementary use of mass cytometry and scRNA-seq is helpful for gaining in-depth knowledge on the heterogeneous interactions between immune cells and NPs and can be incorporated into future toxicity assessments of nanomaterials.

Inhibition of ubiquitin-specific protease 7 sensitizes acute myeloid leukemia to chemotherapy

Leukemia (IF: 9.36), 2020-5, Stéphane Manenti et al. DOI: 10.1038/s41375-020-0878 PubMed: 32447346

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Resistance of acute myeloid leukemia (AML) to therapeutic agents is frequent. Consequently, the mechanisms leading to this resistance must be understood and addressed. In this paper, we demonstrate that inhibition of deubiquitylase USP7 significantly reduces cell proliferation in vitro and in vivo, blocks DNA replication progression and increases cell death in AML. Transcriptomic dataset analyses reveal that a USP7 gene signature is highly enriched in cells from AML patients at relapse, as well as in residual blasts from patient-derived xenograft (PDX) models treated with clinically relevant doses of cytarabine, which indicates a relationship between USP7 expression and resistance to therapy. Accordingly, single-cell analysis of AML patient samples at relapse versus at diagnosis showed that a gene signature of the pre-existing subpopulation responsible for relapse is enriched in transcriptomes of patients with a high USP7 level. Furthermore, we found that USP7 interacts and modulates CHK1 protein levels and functions in AML. Finally, we demonstrated that USP7 inhibition acts in synergy with cytarabine to kill AML cell lines and primary cells of patients with high USP7 levels. Altogether, these data demonstrate that USP7 is both a marker of resistance to chemotherapy and a potential therapeutic target in overcoming resistance to treatment.

Myeloperoxidase Modulates Inflammation in Generalized Pustular Psoriasis and Additional Rare Pustular Skin Diseases

American Journal of Human Genetics (IF: 10.5), 2020-9, Silke Frey et al. DOI: 10.1016/j.ajhg.2020.07.001

PubMed: 32758447

Single Cell Gene Expression, Human, Immunology.

Abstract

Generalized pustular psoriasis (GPP) is a severe multi-systemic inflammatory disease characterized by neutrophilic pustulosis and triggered by pro-inflammatory IL-36 cytokines in skin. While 19%-41% of affected individuals harbor bi-allelic mutations in IL36RN, the genetic cause is not known in most cases. To identify and characterize new pathways involved in the pathogenesis of GPP, we performed whole-exome sequencing in 31 individuals with GPP and demonstrated effects of mutations in MPO encoding the neutrophilic enzyme myeloperoxidase (MPO). We discovered eight MPO mutations resulting in MPO deficiency in neutrophils and monocytes. MPO mutations, primarily those resulting in complete MPO deficiency, cumulatively associated with GPP ($p = 1.85 \times 10^{-8}$; OR = 6.47). The number of mutant MPO alleles significantly differed between 82 affected individuals and 4,900 control subjects ($p = 1.04 \times 10^{-9}$); this effect was stronger when including IL36RN mutations (1.48×10^{-13}) and correlated with a younger age of onset ($p = 0.0018$). The activity of four proteases, previously implicated as activating enzymes of IL-36 precursors, correlated with MPO deficiency. Phorbol-myristate-acetate-induced formation of neutrophil extracellular traps (NETs) was reduced in affected cells ($p = 0.015$), and phagocytosis assays in MPO-deficient mice and human cells revealed altered neutrophil function and impaired clearance of neutrophils by monocytes (efferocytosis) allowing prolonged neutrophil persistence in inflammatory skin. MPO mutations contribute significantly to GPP's pathogenesis. We implicate MPO as an inflammatory modulator in humans that regulates protease activity and NET formation and modifies efferocytosis. Our findings indicate possible implications for the application of MPO inhibitors in cardiovascular diseases. MPO and affected pathways represent attractive targets for inducing resolution of inflammation in neutrophil-mediated skin diseases.

Tissue-Specific Macrophage Responses to Remote Injury Impact the Outcome of Subsequent Local Immune Challenge

Immunity (IF: 25.73), 2019-11, Filip K Swirski et al. DOI: 10.1016/j.immuni.2019.10.010 PubMed: 31732166

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Myocardial infarction, stroke, and sepsis trigger systemic inflammation and organism-wide complications that are difficult to manage. Here, we examined the contribution of macrophages residing in vital organs to the systemic response after these injuries. We generated a comprehensive catalog of changes in macrophage number, origin, and gene expression in the heart, brain, liver, kidney, and lung of mice with myocardial infarction, stroke, or sepsis. Predominantly fueled by heightened local proliferation, tissue macrophage numbers increased systemically. Macrophages in the same organ responded similarly to different injuries by altering expression of tissue-specific gene sets. Preceding myocardial infarction improved survival of subsequent pneumonia due to enhanced bacterial clearance, which was caused by IFN γ priming of alveolar macrophages. Conversely, EGF receptor signaling in macrophages exacerbated inflammatory lung injury. Our data suggest that local injury activates macrophages in remote organs and that targeting macrophages could improve resilience against systemic complications following myocardial infarction, stroke, and sepsis.

Changes in Peripheral and Local Tumor Immunity after Neoadjuvant Chemotherapy Reshape Clinical Outcomes in Patients with Breast Cancer

Clinical cancer research (IF: 10.12), 2020-11, Roberto Salgado et al. DOI: 10.1158/1078-0432.CCR-19-3685
PubMed: 32826327

Single Cell Immune Profiling, Human, Cancer Research.

Abstract

The recent approval of anti-programmed death-ligand 1 immunotherapy in combination with nab-paclitaxel for metastatic triple-negative breast cancer (TNBC) highlights the need to understand the role of chemotherapy in modulating the tumor immune microenvironment (TIME). We examined immune-related gene expression patterns before and after neoadjuvant chemotherapy (NAC) in a series of 83 breast tumors, including 44 TNBCs, from patients with residual disease (RD). Changes in gene expression patterns in the TIME were tested for association with recurrence-free (RFS) and overall survival (OS). In addition, we sought to characterize the systemic effects of NAC through single-cell analysis (RNAseq and cytokine secretion) of programmed death-1-high (PD-1) CD8peripheral T cells and examination of a cytolytic gene signature in whole blood. In non-TNBC, no change in expression of any single gene was associated with RFS or OS, while in TNBC upregulation of multiple immune-related genes and gene sets were associated with improved long-term outcome. High cytotoxic T-cell signatures present in the peripheral blood of patients with breast cancer at surgery were associated with persistent disease and recurrence, suggesting active antitumor immunity that may indicate ongoing disease burden. We have characterized the effects of NAC on the TIME, finding that TNBC is uniquely sensitive to the immunologic effects of NAC, and local increases in immune genes/sets are associated with improved outcomes. However, expression of cytotoxic genes in the peripheral blood, as opposed to the TIME, may be a minimally invasive biomarker of persistent micrometastatic disease ultimately leading to recurrence.

Early emergence of T central memory precursors programs clonal dominance during chronic viral infection

Nature Immunology (IF: 22.3), 2020-12, Michael Flossdorf et al. DOI: 10.1038/s41590-020-00807
PubMed: 33106669

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Chronic cytomegalovirus (CMV) infection leads to long-term maintenance of extraordinarily large CMV-specific T cell populations. The magnitude of this so-called 'memory inflation' is thought to mainly depend on antigenic stimulation during the chronic phase of infection. However, by mapping the long-term development of CD8T cell families derived from single naive precursors, we find that fate decisions made during the acute phase of murine CMV infection can alter the level of memory inflation by more than 1,000-fold. Counterintuitively, a T cell family's capacity for memory inflation is not determined by its initial expansion. Instead, those rare T cell families that dominate the chronic phase of infection show an early transcriptomic signature akin to that of established T central memory cells. Accordingly, a T cell family's long-term dominance is best predicted by its early content of T central memory precursors, which later serve as a stem-cell-like source for memory inflation.

The Intestinal Microbiome Restricts Alphavirus Infection and Dissemination through a Bile Acid-Type I IFN Signaling Axis

Cell (IF: 38.62), 2020-8, Thaddeus S Stappenbeck et al. DOI: 10.1016/j.cell.2020.06.029 PubMed: 32668198

Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

Chikungunya virus (CHIKV), an emerging alphavirus, has infected millions of people. However, the factors modulating disease outcome remain poorly understood. Here, we show in germ-free mice or in oral antibiotic-treated conventionally housed mice with depleted intestinal microbiomes that greater CHIKV infection and spread occurs within 1 day of virus inoculation. Alteration of the microbiome alters TLR7-MyD88 signaling in plasmacytoid dendritic cells (pDCs) and blunts systemic production of type I interferon (IFN). Consequently, circulating monocytes express fewer IFN-stimulated genes and become permissive for CHIKV infection. Reconstitution with a single bacterial species, *Clostridium scindens*, or its derived metabolite, the secondary bile acid deoxycholic acid, can restore pDC- and MyD88-dependent type I IFN responses to restrict systemic CHIKV infection and transmission back to vector mosquitoes. Thus, symbiotic intestinal bacteria modulate antiviral immunity and levels of circulating alphaviruses within hours of infection through a bile acid-pDC-IFN signaling axis, which affects viremia, dissemination, and potentially transmission.

TooManyCells identifies and visualizes relationships of single-cell clades

Nature Methods (IF: 36.15), 2020-4, Golnaz Vahedi et al. DOI: 10.1038/s41592-020-0748-5 PubMed: 32123397

Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Identifying and visualizing transcriptionally similar cells is instrumental for accurate exploration of the cellular diversity revealed by single-cell transcriptomics. However, widely used clustering and visualization algorithms produce a fixed number of cell clusters. A fixed clustering 'resolution' hampers our ability to identify and visualize echelons of cell states. We developed TooManyCells, a suite of graph-based algorithms for efficient and unbiased identification and visualization of cell clades. TooManyCells introduces a visualization model built on a concept intentionally orthogonal to dimensionality-reduction methods. TooManyCells is also equipped with an efficient matrix-free divisive hierarchical spectral clustering different from prevalent single-resolution clustering methods. TooManyCells enables multiresolution and multifaceted exploration of single-cell clades. An advantage of this paradigm is the immediate detection of rare and common populations that outperforms popular clustering and visualization algorithms, as demonstrated using existing single-cell transcriptomic data sets and new data modeling drug-resistance acquisition in leukemic T cells.

Single-cell RNA sequencing unveils an IL-10-producing helper subset that sustains humoral immunity during persistent infection

Nature Communications (IF: 13.61), 2018-11, Joseph Craft et al. DOI: 10.1038/s41467-018-07492-4

PubMed: 30487586

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

During chronic viral infection, the inflammatory function of CD4 T-cells becomes gradually attenuated. Concurrently, Th1 cells progressively acquire the capacity to secrete the cytokine IL-10, a potent suppressor of antiviral T cell responses. To determine the transcriptional changes that underlie this adaption process, we applied a single-cell RNA-sequencing approach and assessed the heterogeneity of IL-10-expressing CD4 T-cells during chronic infection. Here we show an IL-10-producing population with a robust Tfh-signature. Using IL-10 and IL-21 double-reporter mice, we further demonstrate that IL-10IL-21co-producing Tfh cells arise predominantly during chronic but not acute LCMV infection. Importantly, depletion of IL-10IL-21co-producing CD4 T-cells or deletion of IL10 specifically in Tfh cells results in impaired humoral immunity and viral control. Mechanistically, B cell-intrinsic IL-10 signaling is required for sustaining germinal center reactions. Thus, our findings elucidate a critical role for Tfh-derived IL-10 in promoting humoral immunity during persistent viral infection.

A committed tissue-resident memory T cell precursor within the circulating CD8+ effector T cell pool

Journal of experimental medicine (IF: 11.32), 2020-10, Joost B Beltman et al. DOI: 10.1084/jem.20191711

PubMed: 32728699

Single Cell Gene Expression, Mouse, Immunology.

Abstract

An increasing body of evidence emphasizes the role of tissue-resident memory T cells (TRM) in the defense against recurring pathogens and malignant neoplasms. However, little is known with regard to the origin of these cells and their kinship to other CD8+ T cell compartments. To address this issue, we followed the antigen-specific progeny of individual naive CD8+ T cells to the T effector (TEFF), T circulating memory (TCIRCM), and TRM pools by lineage-tracing and single-cell transcriptome analysis. We demonstrate that a subset of T cell clones possesses a heightened capacity to form TRM, and that enriched expression of TRM-fate-associated genes is already apparent in the circulating TEFF offspring of such clones. In addition, we demonstrate that the capacity to generate TRM is permanently imprinted at the clonal level, before skin entry. Collectively, these data provide compelling evidence for early stage TRM fate decisions and the existence of committed TRM precursor cells in the circulatory TEFF compartment.

Anti-idiotypic antibodies elicit anti-HIV-1–specific B cell responses

Journal of experimental medicine, The (IF: 11.32), 2019-1, Michel C Nussenzweig et al.

DOI: 10.1084/jem.20190446 PubMed: 31345931

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Human anti-HIV-1 broadly neutralizing antibodies (bNAbs) protect against infection in animal models. However, bNAbs have not been elicited by vaccination in diverse wild-type animals or humans, in part because B cells expressing the precursors of these antibodies do not recognize most HIV-1 envelopes (Envs). Immunogens have been designed that activate these B cell precursors in vivo, but they also activate competing off-target responses. Here we report on a complementary approach to expand specific B cells using an anti-idiotypic antibody, iv8, that selects for naive human B cells expressing immunoglobulin light chains with 5-amino acid complementarity determining region 3s, a key feature of anti-CD4 binding site (CD4bs)-specific VRC01-class antibodies. In mice, iv8 induced target cells to expand and mature in the context of a polyclonal immune system and produced serologic responses targeting the CD4bs on Env. In summary, the results demonstrate that an anti-idiotypic antibody can specifically recognize and expand rare B cells that express VRC01-class antibodies against HIV-1.

Massively parallel single-cell B-cell receptor sequencing enables rapid discovery of diverse antigen-reactive antibodies

Communications Biology (IF: 11.2), 2019-8, Isidro Hötzel et al. DOI: 10.1038/s42003-019-0551

PubMed: 31428692

Single Cell Immune Profiling, Mouse & Rat, Immunology.

Abstract

Obtaining full-length antibody heavy- and light-chain variable regions from individual B cells at scale remains a challenging problem. Here we use high-throughput single-cell B-cell receptor sequencing (scBCR-seq) to obtain accurately paired full-length variable regions in a massively parallel fashion. We sequenced more than 250,000 B cells from rat, mouse and human repertoires to characterize their lineages and expansion. In addition, we immunized rats with chicken ovalbumin and profiled antigen-reactive B cells from lymph nodes of immunized animals. The scBCR-seq data recovered 81% (= 56/69) of B-cell lineages identified from hybridomas generated from the same set of B cells subjected to scBCR-seq. Importantly, scBCR-seq identified an additional 710 candidate lineages not recovered as hybridomas. We synthesized, expressed and tested 93 clones from the identified lineages and found that 99% (= 92/93) of the clones were antigen-reactive. Our results establish scBCR-seq as a powerful tool for antibody discovery.

Resolving medulloblastoma cellular architecture by single-cell genomics

Nature (IF: 46.49), 2019-7, Mario L Suvà et al. DOI: 10.1038/s41586-019-1434-6 PubMed: 31341285

Single Cell Gene Expression, Human & Mouse, Cancer Research.

Abstract

Medulloblastoma is a malignant childhood cerebellar tumour type that comprises distinct molecular subgroups. Whereas genomic characteristics of these subgroups are well defined, the extent to which cellular diversity underlies their divergent biology and clinical behaviour remains largely unexplored. Here we used single-cell transcriptomics to investigate intra- and intertumoral heterogeneity in 25 medulloblastomas spanning all molecular subgroups. WNT, SHH and Group 3 tumours comprised subgroup-specific undifferentiated and differentiated neuronal-like malignant populations, whereas Group 4 tumours consisted exclusively of differentiated neuronal-like neoplastic cells. SHH tumours closely resembled granule neurons of varying differentiation states that correlated with patient age. Group 3 and Group 4 tumours exhibited a developmental trajectory from primitive progenitor-like to more mature neuronal-like cells, the relative proportions of which distinguished these subgroups. Cross-species transcriptomics defined distinct glutamatergic populations as putative cells-of-origin for SHH and Group 4 subtypes. Collectively, these data provide insights into the cellular and developmental states underlying subtype-specific medulloblastoma biology.

MAFG-driven astrocytes promote CNS inflammation

Nature (IF: 46.49), 2020-2, Alexandre Prat et al. DOI: 10.1038/s41586-020-1999-0 PubMed: 32051591

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Multiple sclerosis is a chronic inflammatory disease of the CNS. Astrocytes contribute to the pathogenesis of multiple sclerosis, but little is known about the heterogeneity of astrocytes and its regulation. Here we report the analysis of astrocytes in multiple sclerosis and its preclinical model experimental autoimmune encephalomyelitis (EAE) by single-cell RNA sequencing in combination with cell-specific Ribotag RNA profiling, assay for transposase-accessible chromatin with sequencing (ATAC-seq), chromatin immunoprecipitation with sequencing (ChIP-seq), genome-wide analysis of DNA methylation and in vivo CRISPR-Cas9-based genetic perturbations. We identified astrocytes in EAE and multiple sclerosis that were characterized by decreased expression of NRF2 and increased expression of MAFG, which cooperates with MAT2 α to promote DNA methylation and represses antioxidant and anti-inflammatory transcriptional programs. Granulocyte-macrophage colony-stimulating factor (GM-CSF) signalling in astrocytes drives the expression of MAFG and MAT2 α and pro-inflammatory transcriptional modules, contributing to CNS pathology in EAE and, potentially, multiple sclerosis. Our results identify candidate therapeutic targets in multiple sclerosis.

Decoding the development of the human hippocampus

Nature (IF: 46.49), 2020-1, Qian Wu et al. DOI: 10.1038/s41586-019-1917-5 PubMed: 31942070

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

The hippocampus is an important part of the limbic system in the human brain that has essential roles in spatial navigation and the consolidation of information from short-term memory to long-term memory. Here we use single-cell RNA sequencing and assay for transposase-accessible chromatin using sequencing (ATAC-seq) analysis to illustrate the cell types, cell lineage, molecular features and transcriptional regulation of the developing human hippocampus. Using the transcriptomes of 30,416 cells from the human hippocampus at gestational weeks 16-27, we identify 47 cell subtypes and their developmental trajectories. We also identify the migrating paths and cell lineages of PAX6 and HOPX hippocampal progenitors, and regional markers of CA1, CA3 and dentate gyrus neurons. Multiomic data have uncovered transcriptional regulatory networks of the dentate gyrus marker PROX1. We also illustrate spatially specific gene expression in the developing human prefrontal cortex and hippocampus. The molecular features of the human hippocampus at gestational weeks 16-20 are similar to those of the mouse at postnatal days 0-5 and reveal gene expression differences between the two species. Transient expression of the primate-specific gene NBPF1 leads to a marked increase in PROX1 cells in the mouse hippocampus. These data provides a blueprint for understanding human hippocampal development and a tool for investigating related diseases.

Neuronal vulnerability and multilineage diversity in multiple sclerosis

Nature (IF: 46.49), 2019-7, Arnold R Kriegstein et al. DOI: 10.1038/s41586-019-1404 PubMed: 31316211

Single Cell Gene Expression, Human, Cell Biology, Neuroscience.

Abstract

Multiple sclerosis (MS) is a neuroinflammatory disease with a relapsing-remitting disease course at early stages, distinct lesion characteristics in cortical grey versus subcortical white matter and neurodegeneration at chronic stages. Here we used single-nucleus RNA sequencing to assess changes in expression in multiple cell lineages in MS lesions and validated the results using multiplex in situ hybridization. We found selective vulnerability and loss of excitatory CUX2-expressing projection neurons in upper-cortical layers underlying meningeal inflammation; such MS neuron populations exhibited upregulation of stress pathway genes and long non-coding RNAs. Signatures of stressed oligodendrocytes, reactive astrocytes and activated microglia mapped most strongly to the rim of MS plaques. Notably, single-nucleus RNA sequencing identified phagocytosing microglia and/or macrophages by their ingestion and perinuclear import of myelin transcripts, confirmed by functional mouse and human culture assays. Our findings indicate lineage- and region-specific transcriptomic changes associated with selective cortical neuron damage and glial activation contributing to progression of MS lesions.

Organoid single-cell genomic atlas uncovers human-specific features of brain development

Nature (IF: 46.49), 2019-10, Barbara Treutlein et al. DOI: 10.1038/s41586-019-1654-9 PubMed: 31619793

Single Cell Gene Expression, Human, Primate, Developmental Biology, Neuroscience.

Abstract

The human brain has undergone substantial change since humans diverged from chimpanzees and the other great apes. However, the genetic and developmental programs that underlie this divergence are not fully understood. Here we have analysed stem cell-derived cerebral organoids using single-cell transcriptomics and accessible chromatin profiling to investigate gene-regulatory changes that are specific to humans. We first analysed cell composition and reconstructed differentiation trajectories over the entire course of human cerebral organoid development from pluripotency, through neuroectoderm and neuroepithelial stages, followed by divergence into neuronal fates within the dorsal and ventral forebrain, midbrain and hindbrain regions. Brain-region composition varied in organoids from different iPSC lines, but regional gene-expression patterns remained largely reproducible across individuals. We analysed chimpanzee and macaque cerebral organoids and found that human neuronal development occurs at a slower pace relative to the other two primates. Using pseudotemporal alignment of differentiation paths, we found that human-specific gene expression resolved to distinct cell states along progenitor-to-neuron lineages in the cortex. Chromatin accessibility was dynamic during cortex development, and we identified divergence in accessibility between human and chimpanzee that correlated with human-specific gene expression and genetic change. Finally, we mapped human-specific expression in adult prefrontal cortex using single-nucleus RNA sequencing analysis and identified developmental differences that persist into adulthood, as well as cell-state-specific changes that occur exclusively in the adult brain. Our data provide a temporal cell atlas of great ape forebrain development, and illuminate dynamic gene-regulatory features that are unique to humans.

Human CNS barrier-forming organoids with cerebrospinal fluid production

Science (IF: 44.37), 2020-7, Mark Skehel et al. DOI: 10.1126/science.aaz5626 PubMed: 32527923

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Cerebrospinal fluid (CSF) is a vital liquid, providing nutrients and signaling molecules and clearing out toxic by-products from the brain. The CSF is produced by the choroid plexus (ChP), a protective epithelial barrier that also prevents free entry of toxic molecules or drugs from the blood. Here, we establish human ChP organoids with a selective barrier and CSF-like fluid secretion in self-contained compartments. We show that this in vitro barrier exhibits the same selectivity to small molecules as the ChP in vivo and that ChP-CSF organoids can predict central nervous system (CNS) permeability of new compounds. The transcriptomic and proteomic signatures of ChP-CSF organoids reveal a high degree of similarity to the ChP in vivo. Finally, the intersection of single-cell transcriptomics and proteomic analysis uncovers key human CSF components produced by previously unidentified specialized epithelial subtypes.

Single-cell transcriptomic analysis of Alzheimer's disease

Nature (IF: 46.49), 2019-5, Manolis KellisLi-Huei Tsai et al. DOI: 10.1038/s41586-019-1195-2 PubMed: 31042697
Single Cell Gene Expression, Human, Neuroscience.

Abstract

Alzheimer's disease is a pervasive neurodegenerative disorder, the molecular complexity of which remains poorly understood. Here, we analysed 80,660 single-nucleus transcriptomes from the prefrontal cortex of 48 individuals with varying degrees of Alzheimer's disease pathology. Across six major brain cell types, we identified transcriptionally distinct subpopulations, including those associated with pathology and characterized by regulators of myelination, inflammation, and neuron survival. The strongest disease-associated changes appeared early in pathological progression and were highly cell-type specific, whereas genes upregulated at late stages were common across cell types and primarily involved in the global stress response. Notably, we found that female cells were overrepresented in disease-associated subpopulations, and that transcriptional responses were substantially different between sexes in several cell types, including oligodendrocytes. Overall, myelination-related processes were recurrently perturbed in multiple cell types, suggesting that myelination has a key role in Alzheimer's disease pathophysiology. Our single-cell transcriptomic resource provides a blueprint for interrogating the molecular and cellular basis of Alzheimer's disease.

Histone H3.3G34-Mutant Interneuron Progenitors Co-opt PDGFRA for Gliomagenesis

Cell (IF: 38.62), 2020-12, Claudia L Kleinman et al. DOI: 10.1016/j.cell.2020.11.012 PubMed: 33259802
Single Cell Gene Expression, Human, Cancer Research.

Abstract

Histone H3.3 glycine 34 to arginine/valine (G34R/V) mutations drive deadly gliomas and show exquisite regional and temporal specificity, suggesting a developmental context permissive to their effects. Here we show that 50% of G34R/V tumors (n = 95) bear activating PDGFRA mutations that display strong selection pressure at recurrence. Although considered gliomas, G34R/V tumors actually arise in GSX2/DLX-expressing interneuron progenitors, where G34R/V mutations impair neuronal differentiation. The lineage of origin may facilitate PDGFRA co-option through a chromatin loop connecting PDGFRA to GSX2 regulatory elements, promoting PDGFRA overexpression and mutation. At the single-cell level, G34R/V tumors harbor dual neuronal/astroglial identity and lack oligodendroglial programs, actively repressed by GSX2/DLX-mediated cell fate specification. G34R/V may become dispensable for tumor maintenance, whereas mutant-PDGFRA is potentially oncogenic. Collectively, our results open novel research avenues in deadly tumors. G34R/V gliomas are neuronal malignancies where interneuron progenitors are stalled in differentiation by G34R/V mutations and malignant gliogenesis is promoted by co-option of a potentially targetable pathway, PDGFRA signaling.

Altered human oligodendrocyte heterogeneity in multiple sclerosis

Nature (IF: 46.49), 2019-1, Anna WilliamsGonçalo Castelo-Branco et al. DOI: 10.1038/s41586-019-0903-2

PubMed: 30747918

Single Cell Gene Expression, Human, Genetic Health, Neuroscience.

Abstract

Oligodendrocyte pathology is increasingly implicated in neurodegenerative diseases as oligodendrocytes both myelinate and provide metabolic support to axons. In multiple sclerosis (MS), demyelination in the central nervous system thus leads to neurodegeneration, but the severity of MS between patients is very variable. Disability does not correlate well with the extent of demyelination, which suggests that other factors contribute to this variability. One such factor may be oligodendrocyte heterogeneity. Not all oligodendrocytes are the same—those from the mouse spinal cord inherently produce longer myelin sheaths than those from the cortex, and single-cell analysis of the mouse central nervous system identified further differences. However, the extent of human oligodendrocyte heterogeneity and its possible contribution to MS pathology remain unknown. Here we performed single-nucleus RNA sequencing from white matter areas of post-mortem human brain from patients with MS and from unaffected controls. We identified subclusters of oligodendroglia in control human white matter, some with similarities to mouse, and defined new markers for these cell states. Notably, some subclusters were underrepresented in MS tissue, whereas others were more prevalent. These differences in mature oligodendrocyte subclusters may indicate different functional states of oligodendrocytes in MS lesions. We found similar changes in normal-appearing white matter, showing that MS is a more diffuse disease than its focal demyelination suggests. Our findings of an altered oligodendroglial heterogeneity in MS may be important for understanding disease progression and developing therapeutic approaches.

Single-cell genomics identifies cell type-specific molecular changes in autism

Science (IF: 44.37), 2019-5, David H RowitchArnold R Kriegstein et al. DOI: 10.1126/science.aav8130

PubMed: 31097668

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Despite the clinical and genetic heterogeneity of autism, bulk gene expression studies show that changes in the neocortex of autism patients converge on common genes and pathways. However, direct assessment of specific cell types in the brain affected by autism has not been feasible until recently. We used single-nucleus RNA sequencing of cortical tissue from patients with autism to identify autism-associated transcriptomic changes in specific cell types. We found that synaptic signaling of upper-layer excitatory neurons and the molecular state of microglia are preferentially affected in autism. Moreover, our results show that dysregulation of specific groups of genes in cortico-cortical projection neurons correlates with clinical severity of autism. These findings suggest that molecular changes in upper-layer cortical circuits are linked to behavioral manifestations of autism.

Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease

Nature Medicine (IF: 36.23), 2020-1, Maxim N Artyomov et al. DOI: 10.1038/s41591-019-0695-9

PubMed: 31932797

Single Cell Gene Expression & Single Cell Immune Profiling, Human & Mouse, Neuroscience.

Abstract

Glia have been implicated in Alzheimer's disease (AD) pathogenesis. Variants of the microglia receptor triggering receptor expressed on myeloid cells 2 (TREM2) increase AD risk, and activation of disease-associated microglia (DAM) is dependent on TREM2 in mouse models of AD. We surveyed gene-expression changes associated with AD pathology and TREM2 in 5XFAD mice and in human AD by single-nucleus RNA sequencing. We confirmed the presence of Trem2-dependent DAM and identified a previously undiscovered Serpina3nC4b reactive oligodendrocyte population in mice. Interestingly, remarkably different glial phenotypes were evident in human AD. Microglia signature was reminiscent of IRF8-driven reactive microglia in peripheral-nerve injury. Oligodendrocyte signatures suggested impaired axonal myelination and metabolic adaptation to neuronal degeneration. Astrocyte profiles indicated weakened metabolic coordination with neurons. Notably, the reactive phenotype of microglia was less evident in TREM2-R47H and TREM2-R62H carriers than in non-carriers, demonstrating a TREM2 requirement in both mouse and human AD, despite the marked species-specific differences.

Origins and Proliferative States of Human Oligodendrocyte Precursor Cells

Cell (IF: 38.62), 2020-8, David H Rowitch et al. DOI: 10.1016/j.cell.2020.06.027 PubMed: 32679030

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Human cerebral cortex size and complexity has increased greatly during evolution. While increased progenitor diversity and enhanced proliferative potential play important roles in human neurogenesis and gray matter expansion, the mechanisms of human oligodendrogenesis and white matter expansion remain largely unknown. Here, we identify EGFR-expressing ;Pre-OPCs that originate from outer radial glial cells (oRGs) and undergo mitotic somal translocation (MST) during division. oRG-derived Pre-OPCs provide an additional source of human cortical oligodendrocyte precursor cells (OPCs) and define a lineage trajectory. We further show that human OPCs undergo consecutive symmetric divisions to exponentially increase the progenitor pool size. Additionally, we find that the OPC-enriched gene, PCDH15, mediates daughter cell repulsion and facilitates proliferation. These findings indicate properties of OPC derivation, proliferation, and dispersion important for human white matter expansion and myelination.

Immune profiling of human tumors identifies CD73 as a combinatorial target in glioblastoma

Nature Medicine (IF: 36.23), 2019-12, Dana Pe'er et al. DOI: 10.1038/s41591-019-0694 PubMed: 31873309

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Immune checkpoint therapy with anti-CTLA-4 and anti-PD-1/PD-L1 has revolutionized the treatment of many solid tumors. However, the clinical efficacy of immune checkpoint therapy is limited to a subset of patients with specific tumor types. Multiple clinical trials with combinatorial immune checkpoint strategies are ongoing; however, the mechanistic rationale for tumor-specific targeting of immune checkpoints is elusive. To garner an insight into tumor-specific immunomodulatory targets, we analyzed 94 patients representing five different cancer types, including those that respond relatively well to immune checkpoint therapy and those that do not, such as glioblastoma multiforme, prostate cancer and colorectal cancer. Through mass cytometry and single-cell RNA sequencing, we identified a unique population of CD73macrophages in glioblastoma multiforme that persists after anti-PD-1 treatment. To test if targeting CD73 would be important for a successful combination strategy in glioblastoma multiforme, we performed reverse translational studies using CD73mice. We found that the absence of CD73 improved survival in a murine model of glioblastoma multiforme treated with anti-CTLA-4 and anti-PD-1. Our data identified CD73 as a specific immunotherapeutic target to improve antitumor immune responses to immune checkpoint therapy in glioblastoma multiforme and demonstrate that comprehensive human and reverse translational studies can be used for rational design of combinatorial immune checkpoint strategies.

Deep generative modeling for single-cell transcriptomics

Nature Methods (IF: 36.15), 2018-11, Michael I Jordan et al. DOI: 10.1038/s41592-018-0229-2 PubMed: 30504886

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

Single-cell transcriptome measurements can reveal unexplored biological diversity, but they suffer from technical noise and bias that must be modeled to account for the resulting uncertainty in downstream analyses. Here we introduce single-cell variational inference (scVI), a ready-to-use scalable framework for the probabilistic representation and analysis of gene expression in single cells (<https://github.com/YosefLab/scVI>). scVI uses stochastic optimization and deep neural networks to aggregate information across similar cells and genes and to approximate the distributions that underlie observed expression values, while accounting for batch effects and limited sensitivity. We used scVI for a range of fundamental analysis tasks including batch correction, visualization, clustering, and differential expression, and achieved high accuracy for each task.

Engineering of human brain organoids with a functional vascular-like system

Nature Methods (IF: 36.15), 2019-10, Young-Sup Yoon et al. DOI: 10.1038/s41592-019-0586-5 PubMed: 31591580
Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Human cortical organoids (hCOs), derived from human embryonic stem cells (hESCs), provide a platform to study human brain development and diseases in complex three-dimensional tissue. However, current hCOs lack microvasculature, resulting in limited oxygen and nutrient delivery to the inner-most parts of hCOs. We engineered hESCs to ectopically express human ETS variant 2 (ETV2). ETV2-expressing cells in hCOs contributed to forming a complex vascular-like network in hCOs. Importantly, the presence of vasculature-like structures resulted in enhanced functional maturation of organoids. We found that vascularized hCOs (vhCOs) acquired several blood-brain barrier characteristics, including an increase in the expression of tight junctions, nutrient transporters and trans-endothelial electrical resistance. Finally, ETV2-induced endothelium supported the formation of perfused blood vessels in vivo. These vhCOs form vasculature-like structures that resemble the vasculature in early prenatal brain, and they present a robust model to study brain disease in vitro.

Stalled developmental programs at the root of pediatric brain tumors

Nature Genetics (IF: 30.33), 2019-11, Nada Jabado et al. DOI: 10.1038/s41588-019-0531-7 PubMed: 31768071
Single Cell Gene Expression, Human & Mouse, Cell Atlas, Developmental Biology.

Abstract

Childhood brain tumors have suspected prenatal origins. To identify vulnerable developmental states, we generated a single-cell transcriptome atlas of >65,000 cells from embryonal pons and forebrain, two major tumor locations. We derived signatures for 191 distinct cell populations and defined the regional cellular diversity and differentiation dynamics. Projection of bulk tumor transcriptomes onto this dataset shows that WNT medulloblastomas match the rhombic lip-derived mossy fiber neuronal lineage and embryonal tumors with multilayered rosettes fully recapitulate a neuronal lineage, while group 2a/b atypical teratoid/rhabdoid tumors may originate outside the neuroectoderm. Importantly, single-cell tumor profiles reveal highly defined cell hierarchies that mirror transcriptional programs of the corresponding normal lineages. Our findings identify impaired differentiation of specific neural progenitors as a common mechanism underlying these pediatric cancers and provide a rational framework for future modeling and therapeutic interventions.

Neuronal impact of patient-specific aberrant NRXN1 α splicing

Nature Genetics (IF: 30.33), 2019-11, Gang Fang et al. DOI: 10.1038/s41588-019-0539 PubMed: 31784728

Single Cell Gene Expression, Human, Genetic Health, Neuroscience.

Abstract

NRXN1 undergoes extensive alternative splicing, and non-recurrent heterozygous deletions in NRXN1 are strongly associated with neuropsychiatric disorders. We establish that human induced pluripotent stem cell (hiPSC)-derived neurons well represent the diversity of NRXN1 α alternative splicing observed in the human brain, cataloguing 123 high-confidence in-frame human NRXN1 α isoforms. Patient-derived NRXN1hiPSC-neurons show a greater than twofold reduction in half of the wild-type NRXN1 α isoforms and express dozens of novel isoforms from the mutant allele. Reduced neuronal activity in patient-derived NRXN1hiPSC-neurons is ameliorated by overexpression of individual control isoforms in a genotype-dependent manner, whereas individual mutant isoforms decrease neuronal activity levels in control hiPSC-neurons. In a genotype-dependent manner, the phenotypic impact of patient-specific NRXN1 mutations can occur through a reduction in wild-type NRXN1 α isoform levels as well as the presence of mutant NRXN1 α isoforms.

Single-cell epigenomic analyses implicate candidate causal variants at inherited risk loci for Alzheimer's and Parkinson's diseases

Nature Genetics (IF: 30.33), 2020-11, Howard Y Chang et al. DOI: 10.1038/s41588-020-00721 PubMed: 33106633

Single Cell ATAC, Human, Neuroscience.

Abstract

Genome-wide association studies of neurological diseases have identified thousands of variants associated with disease phenotypes. However, most of these variants do not alter coding sequences, making it difficult to assign their function. Here, we present a multi-omic epigenetic atlas of the adult human brain through profiling of single-cell chromatin accessibility landscapes and three-dimensional chromatin interactions of diverse adult brain regions across a cohort of cognitively healthy individuals. We developed a machine-learning classifier to integrate this multi-omic framework and predict dozens of functional SNPs for Alzheimer's and Parkinson's diseases, nominating target genes and cell types for previously orphaned loci from genome-wide association studies. Moreover, we dissected the complex inverted haplotype of the MAPT (encoding tau) Parkinson's disease risk locus, identifying putative ectopic regulatory interactions in neurons that may mediate this disease association. This work expands understanding of inherited variation and provides a roadmap for the epigenomic dissection of causal regulatory variation in disease.

The Phenotypes of Proliferating Glioblastoma Cells Reside on a Single Axis of Variation

Cancer Discovery (IF: 28.3), 2019-12, Manish K Agghi et al. DOI: 10.1158/2159-8290.CD-19-0329

PubMed: 31554641

Single Cell Gene Expression, Human, Cancer Research, Neuroscience.

Abstract

Although tumor-propagating cells can be derived from glioblastomas (GBM) of the proneural and mesenchymal subtypes, a glioma stem-like cell (GSC) of the classic subtype has not been identified. It is unclear whether mesenchymal GSCs (mGSC) and/or proneural GSCs (pGSC) alone are sufficient to generate the heterogeneity observed in GBM. We performed single-cell/single-nucleus RNA sequencing of 28 gliomas, and single-cell ATAC sequencing for 8 cases. We found that GBM GSCs reside on a single axis of variation, ranging from proneural to mesenchymal. Lineage tracing using both transcriptomics and genetics supports mGSCs as the progenitors of pGSCs. Dual inhibition of pGSC-enriched and mGSC-enriched growth and survival pathways provides a more complete treatment than combinations targeting one GSC phenotype alone. This study sheds light on a long-standing debate regarding lineage relationships among GSCs and presents a paradigm by which personalized combination therapies can be derived from single-cell RNA signatures, to overcome intratumor heterogeneity. SIGNIFICANCE: Tumor-propagating cells can be derived from mesenchymal and proneural glioblastomas. However, a stem cell of the classic subtype has yet to be demonstrated. We show that classic-subtype gliomas are comprised of proneural and mesenchymal cells. This study sheds light on a long-standing debate regarding lineage relationships between glioma cell types...

Fast interpolation-based t-SNE for improved visualization of single-cell RNA-seq data

Nature Methods (IF: 36.15), 2019-2, Stefan Steinerberger et al. DOI: 10.1038/s41592-018-0308-4

PubMed: 30742040

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

t-distributed stochastic neighbor embedding (t-SNE) is widely used for visualizing single-cell RNA-sequencing (scRNA-seq) data, but it scales poorly to large datasets. We dramatically accelerate t-SNE, obviating the need for data downsampling, and hence allowing visualization of rare cell populations. Furthermore, we implement a heatmap-style visualization for scRNA-seq based on one-dimensional t-SNE for simultaneously visualizing the expression patterns of thousands of genes. Software is available at <https://github.com/KlugerLab/Flt-SNE> and <https://github.com/KlugerLab/t-SNE-Heatmaps>.

Single-Cell Transcriptomics Uncovers Glial Progenitor Diversity and Cell Fate Determinants during Development and Gliomagenesis

Cell Stem Cell (IF: 23.45), 2019-5, Guoqiang Yu et al. DOI: 10.1016/j.stem.2019.03.006 PubMed: 30982771

Single Cell Gene Expression, Human & Mouse, Developmental Biology, Neuroscience.

Abstract

The identity and degree of heterogeneity of glial progenitors and their contributions to brain tumor malignancy remain elusive. By applying lineage-targeted single-cell transcriptomics, we uncover an unanticipated diversity of glial progenitor pools with unique molecular identities in developing brain. Our analysis identifies distinct transitional intermediate states and their divergent developmental trajectories in astroglial and oligodendroglial lineages. Moreover, intersectional analysis uncovers analogous intermediate progenitors during brain tumorigenesis, wherein oligodendrocyte-progenitor intermediates are abundant, hyper-proliferative, and progressively reprogrammed toward a stem-like state susceptible to further malignant transformation. Similar actively cycling intermediate progenitors are prominent components in human gliomas with distinct driver mutations. We further unveil lineage-driving networks underlying glial fate specification and identify Zfp36l1 as necessary for oligodendrocyte-astrocyte lineage transition and glioma growth. Together, our results resolve the dynamic repertoire of common and divergent glial progenitors during development and tumorigenesis and highlight Zfp36l1 as a molecular nexus for balancing glial cell-fate decision and controlling gliomagenesis.

Outer Radial Glia-like Cancer Stem Cells Contribute to Heterogeneity of Glioblastoma

Cell Stem Cell (IF: 23.45), 2020-1, David Ronan Raleigh et al. DOI: 10.1016/j.stem.2019.11.015 PubMed: 31901251

Single Cell Gene Expression, Human, Developmental Biology, Genetic Health.

Abstract

Glioblastoma is a devastating form of brain cancer. To identify aspects of tumor heterogeneity that may illuminate drivers of tumor invasion, we created a glioblastoma tumor cell atlas with single-cell transcriptomics of cancer cells mapped onto a reference framework of the developing and adult human brain. We find that multiple GSC subtypes exist within a single tumor. Within these GSCs, we identify an invasive cell population similar to outer radial glia (oRG), a fetal cell type that expands the stem cell niche in normal human cortex. Using live time-lapse imaging of primary resected tumors, we discover that tumor-derived oRG-like cells undergo characteristic mitotic somal translocation behavior previously only observed in human development, suggesting a reactivation of developmental programs. In addition, we show that PTPRZ1 mediates both mitotic somal translocation and glioblastoma tumor invasion. These data suggest that the presence of heterogeneous GSCs may underlie glioblastoma's rapid progression and invasion.

Single-nucleus transcriptomics of the prefrontal cortex in major depressive disorder implicates oligodendrocyte precursor cells and excitatory neurons

Nature Neuroscience (IF: 21.5), 2020-6, Jiannis Ragoussis et al. DOI: 10.1038/s41593-020-0621

PubMed: 32341540

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Major depressive disorder (MDD) has an enormous impact on global disease burden, affecting millions of people worldwide and ranking as a leading cause of disability for almost three decades. Past molecular studies of MDD employed bulk homogenates of postmortem brain tissue, which obscures gene expression changes within individual cell types. Here we used single-nucleus transcriptomics to examine ~80,000 nuclei from the dorsolateral prefrontal cortex of male individuals with MDD (n = 17) and of healthy controls (n = 17). We identified 26 cellular clusters, and over 60% of these showed differential gene expression between groups. We found that the greatest dysregulation occurred in deep layer excitatory neurons and immature oligodendrocyte precursor cells (OPCs), and these contributed almost half (47%) of all changes in gene expression. These results highlight the importance of dissecting cell-type-specific contributions to the disease and offer opportunities to identify new avenues of research and novel targets for treatment.

Complex Oscillatory Waves Emerging from Cortical Organoids Model Early Human Brain Network Development

Cell Stem Cell (IF: 23.45), 2019-10, Bradley Voytek et al. DOI: 10.1016/j.stem.2019.08.002 PubMed: 31474560

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Structural and transcriptional changes during early brain maturation follow fixed developmental programs defined by genetics. However, whether this is true for functional network activity remains unknown, primarily due to experimental inaccessibility of the initial stages of the living human brain. Here, we developed human cortical organoids that dynamically change cellular populations during maturation and exhibited consistent increases in electrical activity over the span of several months. The spontaneous network formation displayed periodic and regular oscillatory events that were dependent on glutamatergic and GABAergic signaling. The oscillatory activity transitioned to more spatiotemporally irregular patterns, and synchronous network events resembled features similar to those observed in preterm human electroencephalography. These results show that the development of structured network activity in a human neocortex model may follow stable genetic programming. Our approach provides opportunities for investigating and manipulating the role of network activity in the developing human cortex.

Profiling gene expression in the human dentate gyrus granule cell layer reveals insights into schizophrenia and its genetic risk

Nature Neuroscience (IF: 21.5), 2020-4, Mitsuyuki Matsumoto et al. DOI: 10.1038/s41593-020-0604

PubMed: 32203495

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Specific cell populations may have unique contributions to schizophrenia but may be missed in studies of homogenate tissue. Here laser capture microdissection followed by RNA sequencing (LCM-seq) was used to transcriptomically profile the granule cell layer of the dentate gyrus (DG-GCL) in human hippocampus and contrast these data to those obtained from bulk hippocampal homogenate. We identified widespread cell-type-enriched aging and genetic effects in the DG-GCL that were either absent or directionally discordant in bulk hippocampus data. Of the ~9 million expression quantitative trait loci identified in the DG-GCL, 15% were not detected in bulk hippocampus, including 15 schizophrenia risk variants. We created transcriptome-wide association study genetic weights from the DG-GCL, which identified many schizophrenia-associated genetic signals not found in transcriptome-wide association studies from bulk hippocampus, including GRM3 and CACNA1C. These results highlight the improved biological resolution provided by targeted sampling strategies like LCM and complement homogenate and single-nucleus approaches in human brain.

Stem-cell-derived human microglia transplanted in mouse brain to study human disease

Nature Neuroscience (IF: 21.5), 2019-10, Mark Fiers et al. DOI: 10.1038/s41593-019-0525 PubMed: 31659342

Single Cell Gene Expression, Human & Mouse, Neuroscience.

Abstract

Although genetics highlights the role of microglia in Alzheimer's disease, one-third of putative Alzheimer's disease risk genes lack adequate mouse orthologs. Here we successfully engraft human microglia derived from embryonic stem cells in the mouse brain. The cells recapitulate transcriptionally human primary microglia ex vivo and show expression of human-specific Alzheimer's disease risk genes. Oligomeric amyloid- β induces a divergent response in human versus mouse microglia. This model can be used to study the role of microglia in neurological diseases.

A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation

Nature Neuroscience (IF: 21.5), 2019-11, Enrico Petretto et al. DOI: 10.1038/s41593-019-0539-4

PubMed: 31768052

Single Cell Gene Expression, Human, Neuroscience.

Abstract

There is currently little information available about how individual cell types contribute to Alzheimer's disease. Here we applied single-nucleus RNA sequencing to entorhinal cortex samples from control and Alzheimer's disease brains (n = 6 per group), yielding a total of 13,214 high-quality nuclei. We detail cell-type-specific gene expression patterns, unveiling how transcriptional changes in specific cell subpopulations are associated with Alzheimer's disease. We report that the Alzheimer's disease risk gene **APOE** is specifically repressed in Alzheimer's disease oligodendrocyte progenitor cells and astrocyte subpopulations and upregulated in an Alzheimer's disease-specific microglial subpopulation. Integrating transcription factor regulatory modules with Alzheimer's disease risk loci revealed drivers of cell-type-specific state transitions towards Alzheimer's disease. For example, transcription factor EB, a master regulator of lysosomal function, regulates multiple disease genes in a specific Alzheimer's disease astrocyte subpopulation. These results provide insights into the coordinated control of Alzheimer's disease risk genes and their cell-type-specific contribution to disease susceptibility. These results are available at <http://adsn.ddnetbio.com>.

Single-cell analysis of olfactory neurogenesis and differentiation in adult humans

Nature Neuroscience (IF: 21.5), 2020-3, Hiroaki Matsunami et al. DOI: 10.1038/s41593-020-0587-9

PubMed: 32066986

Single Cell Gene Expression, Human, Neuroscience.

Abstract

The presence of active neurogenic niches in adult humans is controversial. We focused attention to the human olfactory neuroepithelium, an extracranial site supplying input to the olfactory bulbs of the brain. Using single-cell RNA sequencing analyzing 28,726 cells, we identified neural stem cell and neural progenitor cell pools and neurons. Additionally, we detailed the expression of 140 olfactory receptors. These data from the olfactory neuroepithelium niche provide evidence that neuron production may continue for decades in humans.

Dysregulation of BRD4 Function Underlies the Functional Abnormalities of MeCP2 Mutant Neurons

Molecular Cell (IF: 16.13), 2020-7, Sherman M Weissman et al. DOI: 10.1016/j.molcel.2020.05.016

PubMed: 32526163

Single Cell Gene Expression, Human, Genetic Health, Neuroscience.

Abstract

Rett syndrome (RTT), mainly caused by mutations in methyl-CpG binding protein 2 (MeCP2), is one of the most prevalent intellectual disorders without effective therapies. Here, we used 2D and 3D human brain cultures to investigate MeCP2 function. We found that MeCP2 mutations cause severe abnormalities in human interneurons (INs). Surprisingly, treatment with a BET inhibitor, JQ1, rescued the molecular and functional phenotypes of MeCP2 mutant INs. We uncovered that abnormal increases in chromatin binding of BRD4 and enhancer-promoter interactions underlie the abnormal transcription in MeCP2 mutant INs, which were recovered to normal levels by JQ1. We revealed cell-type-specific transcriptome impairment in MeCP2 mutant region-specific human brain organoids that were rescued by JQ1. Finally, JQ1 ameliorated RTT-like phenotypes in mice. These data demonstrate that BRD4 dysregulation is a critical driver for RTT etiology and suggest that targeting BRD4 could be a potential therapeutic opportunity for RTT.

Direct pericyte-to-neuron reprogramming via unfolding of a neural stem cell-like program

Nature Neuroscience (IF: 21.5), 2018-6, Barbara TreutleinBenedikt Berninger et al. DOI: 10.1038/s41593-018-0168-3 PubMed: 29915193

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Ectopic expression of defined transcription factors can force direct cell-fate conversion from one lineage to another in the absence of cell division. Several transcription factor cocktails have enabled successful reprogramming of various somatic cell types into induced neurons (iNs) of distinct neurotransmitter phenotype. However, the nature of the intermediate states that drive the reprogramming trajectory toward distinct iN types is largely unknown. Here we show that successful direct reprogramming of adult human brain pericytes into functional iNs by Ascl1 and Sox2 encompasses transient activation of a neural stem cell-like gene expression program that precedes bifurcation into distinct neuronal lineages. During this transient state, key signaling components relevant for neural induction and neural stem cell maintenance are regulated by and functionally contribute to iN reprogramming and maturation. Thus, Ascl1- and Sox2-mediated reprogramming into a broad spectrum of iN types involves the unfolding of a developmental program via neural stem cell-like intermediates.

Cell Type-Specific Transcriptomics Reveals that Mutant Huntingtin Leads to Mitochondrial RNA Release and Neuronal Innate Immune Activation

Neuron (IF: 16), 2020-9, Matthew J LaVoie et al. DOI: 10.1016/j.neuron.2020.06.021 PubMed: 32681824
Single Cell Gene Expression, Human & Mouse, Genetic Health, Neuroscience.

Abstract

The mechanisms by which mutant huntingtin (mHTT) leads to neuronal cell death in Huntington's disease (HD) are not fully understood. To gain new molecular insights, we used single nuclear RNA sequencing (snRNA-seq) and translating ribosome affinity purification (TRAP) to conduct transcriptomic analyses of caudate/putamen (striatal) cell type-specific gene expression changes in human HD and mouse models of HD. In striatal spiny projection neurons, the most vulnerable cell type in HD, we observe a release of mitochondrial RNA (mtRNA) (a potent mitochondrial-derived innate immunogen) and a concomitant upregulation of innate immune signaling in spiny projection neurons. Further, we observe that the released mtRNAs can directly bind to the innate immune sensor protein kinase R (PKR). We highlight the importance of studying cell type-specific gene expression dysregulation in HD pathogenesis and reveal that the activation of innate immune signaling in the most vulnerable HD neurons provides a novel framework to understand the basis of mHTT toxicity and raises new therapeutic opportunities.

CD49f Is a Novel Marker of Functional and Reactive Human iPSC-Derived Astrocytes

Neuron (IF: 16), 2020-8, Shane A Liddelow et al. DOI: 10.1016/j.neuron.2020.05.014 PubMed: 32485136
Single Cell Gene Expression, Human, Neuroscience.

Abstract

New methods for investigating human astrocytes are urgently needed, given their critical role in the central nervous system. Here we show that CD49f is a novel marker for human astrocytes, expressed in fetal and adult brains from healthy and diseased individuals. CD49f can be used to purify fetal astrocytes and human induced pluripotent stem cell (hiPSC)-derived astrocytes. We provide single-cell and bulk transcriptome analyses of CD49fhiPSC-astrocytes and demonstrate that they perform key astrocytic functions in vitro, including trophic support of neurons, glutamate uptake, and phagocytosis. Notably, CD49fhiPSC-astrocytes respond to inflammatory stimuli, acquiring an A1-like reactive state, in which they display impaired phagocytosis and glutamate uptake and fail to support neuronal maturation. Most importantly, we show that conditioned medium from human reactive A1-like astrocytes is toxic to human and rodent neurons. CD49fhiPSC-astrocytes are thus a valuable resource for investigating human astrocyte function and dysfunction in health and disease.

Glioma-derived IL-33 orchestrates an inflammatory brain tumor microenvironment that accelerates glioma progression

Nature Communications (IF: 13.61), 2020-10, Stephen M Robbins et al. DOI: 10.1038/s41467-020-18569-4
PubMed: 33020472

Single Cell Gene Expression, Human & Mouse, Cancer Research.

Abstract

Despite a deeper molecular understanding, human glioblastoma remains one of the most treatment refractory and fatal cancers. It is known that the presence of macrophages and microglia impact glioblastoma tumorigenesis and prevent durable response. Herein we identify the dual function cytokine IL-33 as an orchestrator of the glioblastoma microenvironment that contributes to tumorigenesis. We find that IL-33 expression in a large subset of human glioma specimens and murine models correlates with increased tumor-associated macrophages/monocytes/microglia. In addition, nuclear and secreted functions of IL-33 regulate chemokines that collectively recruit and activate circulating and resident innate immune cells creating a pro-tumorigenic environment. Conversely, loss of nuclear IL-33 cripples recruitment, dramatically suppresses glioma growth, and increases survival. Our data supports the paradigm that recruitment and activation of immune cells, when instructed appropriately, offer a therapeutic strategy that switches the focus from the cancer cell alone to one that includes the normal host environment.

Nuclei multiplexing with barcoded antibodies for single-nucleus genomics

Nature Communications (IF: 13.61), 2019-7, Orit Rozenblatt-Rosen et al. DOI: 10.1038/s41467-019-10756-2
PubMed: 31266958

Single Cell Gene Expression, Human & Mouse, Assay Method, Neuroscience.

Abstract

Single-nucleus RNA-seq (snRNA-seq) enables the interrogation of cellular states in complex tissues that are challenging to dissociate or are frozen, and opens the way to human genetics studies, clinical trials, and precise cell atlases of large organs. However, such applications are currently limited by batch effects, processing, and costs. Here, we present an approach for multiplexing snRNA-seq, using sample-barcoded antibodies to uniquely label nuclei from distinct samples. Comparing human brain cortex samples profiled with or without hashing antibodies, we demonstrate that nucleus hashing does not significantly alter recovered profiles. We develop DemuxEM, a computational tool that detects inter-sample multiplets and assigns singlets to their sample of origin, and validate its accuracy using sex-specific gene expression, species-mixing and natural genetic variation. Our approach will facilitate tissue atlases of isogenic model organisms or from multiple biopsies or longitudinal samples of one donor, and large-scale perturbation screens.

Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis

Nature Communications (IF: 13.61), 2020-10, Peter V Kharchenko et al. DOI: 10.1038/s41467-020-18752-7
PubMed: 33028830

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Epilepsy is one of the most common neurological disorders, yet its pathophysiology is poorly understood due to the high complexity of affected neuronal circuits. To identify dysfunctional neuronal subtypes underlying seizure activity in the human brain, we have performed single-nucleus transcriptomics analysis of >110,000 neuronal transcriptomes derived from temporal cortex samples of multiple temporal lobe epilepsy and non-epileptic subjects. We found that the largest transcriptomic changes occur in distinct neuronal subtypes from several families of principal neurons (L5-6_Fezf2 and L2-3_Cux2) and GABAergic interneurons (Sst and Pvalb), whereas other subtypes in the same families were less affected. Furthermore, the subtypes with the largest epilepsy-related transcriptomic changes may belong to the same circuit, since we observed coordinated transcriptomic shifts across these subtypes. Glutamate signaling exhibited one of the strongest dysregulations in epilepsy, highlighted by layer-wise transcriptional changes in multiple glutamate receptor genes and strong upregulation of genes coding for AMPA receptor auxiliary subunits. Overall, our data reveal a neuronal subtype-specific molecular phenotype of epilepsy.

Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease

Nature Communications (IF: 13.61), 2020-11, Elizabeth M Bradshaw et al. DOI: 10.1038/s41467-020-19737-2
PubMed: 33257666

Single Cell Gene Expression, Human, Neuroscience.

Abstract

The extent of microglial heterogeneity in humans remains a central yet poorly explored question in light of the development of therapies targeting this cell type. Here, we investigate the population structure of live microglia purified from human cerebral cortex samples obtained at autopsy and during neurosurgical procedures. Using single cell RNA sequencing, we find that some subsets are enriched for disease-related genes and RNA signatures. We confirm the presence of four of these microglial subpopulations histologically and illustrate the utility of our data by characterizing further microglial cluster 7, enriched for genes depleted in the cortex of individuals with Alzheimer's disease (AD). Histologically, these cluster 7 microglia are reduced in frequency in AD tissue, and we validate this observation in an independent set of single nucleus data. Thus, our live human microglia identify a range of subtypes, and we prioritize one of these as being altered in AD.

A single-cell atlas of the human substantia nigra reveals cell-specific pathways associated with neurological disorders

Nature Communications (IF: 13.61), 2020-8, Richard Wade-Martins et al. DOI: 10.1038/s41467-020-17876-0
PubMed: 32826893

Single Cell Gene Expression, Human, Cell Atlas, Neuroscience.

Abstract

We describe a human single-nuclei transcriptomic atlas for the substantia nigra (SN), generated by sequencing approximately 17,000 nuclei from matched cortical and SN samples. We show that the common genetic risk for Parkinson's disease (PD) is associated with dopaminergic neuron (DaN)-specific gene expression, including mitochondrial functioning, protein folding and ubiquitination pathways. We identify a distinct cell type association between PD risk and oligodendrocyte-specific gene expression. Unlike Alzheimer's disease (AD), we find no association between PD risk and microglia or astrocytes, suggesting that neuroinflammation plays a less causal role in PD than AD. Beyond PD, we find associations between SN DaNs and GABAergic neuron gene expression and multiple neuropsychiatric disorders. Conditional analysis reveals that distinct neuropsychiatric disorders associate with distinct sets of neuron-specific genes but converge onto shared loci within oligodendrocytes and oligodendrocyte precursors. This atlas guides our aetiological understanding by associating SN cell type expression profiles with specific disease risk.

Realistic in silico generation and augmentation of single-cell RNA-seq data using generative adversarial networks

Nature Communications (IF: 13.61), 2020-1, Christian F Krebs et al. DOI: 10.1038/s41467-019-14018
PubMed: 31919373

Single Cell Gene Expression, Human, Computational Method.

Abstract

A fundamental problem in biomedical research is the low number of observations available, mostly due to a lack of available biosamples, prohibitive costs, or ethical reasons. Augmenting few real observations with generated in silico samples could lead to more robust analysis results and a higher reproducibility rate. Here, we propose the use of conditional single-cell generative adversarial neural networks (cscGAN) for the realistic generation of single-cell RNA-seq data. cscGAN learns non-linear gene-gene dependencies from complex, multiple cell type samples and uses this information to generate realistic cells of defined types. Augmenting sparse cell populations with cscGAN generated cells improves downstream analyses such as the detection of marker genes, the robustness and reliability of classifiers, the assessment of novel analysis algorithms, and might reduce the number of animal experiments and costs in consequence. cscGAN outperforms existing methods for single-cell RNA-seq data generation in quality and hold great promise for the realistic generation and augmentation of other biomedical data types.

Accurate estimation of cell composition in bulk expression through robust integration of single-cell information

Nature Communications (IF: 13.61), 2020-4, Päivi Pajukanta et al. DOI: 10.1038/s41467-020-15816-6

PubMed: 32332754

Single Cell Gene Expression, Human, Computational Method.

Abstract

We present Bisque, a tool for estimating cell type proportions in bulk expression. Bisque implements a regression-based approach that utilizes single-cell RNA-seq (scRNA-seq) or single-nucleus RNA-seq (snRNA-seq) data to generate a reference expression profile and learn gene-specific bulk expression transformations to robustly decompose RNA-seq data. These transformations significantly improve decomposition performance compared to existing methods when there is significant technical variation in the generation of the reference profile and observed bulk expression. Importantly, compared to existing methods, our approach is extremely efficient, making it suitable for the analysis of large genomic datasets that are becoming ubiquitous. When applied to subcutaneous adipose and dorsolateral prefrontal cortex expression datasets with both bulk RNA-seq and snRNA-seq data, Bisque replicates previously reported associations between cell type proportions and measured phenotypes across abundant and rare cell types. We further propose an additional mode of operation that merely requires a set of known marker genes.

Transcriptomic and clonal characterization of T cells in the human central nervous system

Science Immunology (IF: 13.41), 2020-9, David van Dijk et al. DOI: 10.1126/sciimmunol.abb8786

PubMed: 32948672

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immunology, Neuroscience.

Abstract

T cells provide critical immune surveillance to the central nervous system (CNS), and the cerebrospinal fluid (CSF) is thought to be a main route for their entry. Further characterization of the state of T cells in the CSF in healthy individuals is important for understanding how T cells provide protective immune surveillance without damaging the delicate environment of the CNS and providing tissue-specific context for understanding immune dysfunction in neuroinflammatory disease. Here, we have profiled T cells in the CSF of healthy human donors and have identified signatures related to cytotoxic capacity and tissue adaptation that are further exemplified in clonally expanded CSF T cells. By comparing profiles of clonally expanded T cells obtained from the CSF of patients with multiple sclerosis (MS) and healthy donors, we report that clonally expanded T cells from the CSF of patients with MS have heightened expression of genes related to T cell activation and cytotoxicity.

Immature excitatory neurons develop during adolescence in the human amygdala

Nature Communications (IF: 13.61), 2019-6, Eric J Huang et al. DOI: 10.1038/s41467-019-10765-1

PubMed: 31227709

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

The human amygdala grows during childhood, and its abnormal development is linked to mood disorders. The primate amygdala contains a large population of immature neurons in the paralamina nuclei (PL), suggesting protracted development and possibly neurogenesis. Here we studied human PL development from embryonic stages to adulthood. The PL develops next to the caudal ganglionic eminence, which generates inhibitory interneurons, yet most PL neurons express excitatory markers. In children, most PL cells are immature (DCX+PSA-NCAM+), and during adolescence many transition into mature (TBR1+VGLUT2+) neurons. Immature PL neurons persist into old age, yet local progenitor proliferation sharply decreases in infants. Using single nuclei RNA sequencing, we identify the transcriptional profile of immature excitatory neurons in the human amygdala between 4-15 years. We conclude that the human PL contains excitatory neurons that remain immature for decades, a possible substrate for persistent plasticity at the interface of the hippocampus and amygdala.

Single-cell-resolution transcriptome map of human, chimpanzee, bonobo, and macaque brains

Genome research (IF: 12.01), 2020-5, Barbara Treutlein et al. DOI: 10.1101/gr.256958.119

PubMed: 32424074

Single Cell Gene Expression, Human, Primate, Cell Atlas, Neuroscience.

Abstract

Identification of gene expression traits unique to the human brain sheds light on the molecular mechanisms underlying human evolution. Here, we searched for uniquely human gene expression traits by analyzing 422 brain samples from humans, chimpanzees, bonobos, and macaques representing 33 anatomical regions, as well as 88,047 cell nuclei composing three of these regions. Among 33 regions, cerebral cortex areas, hypothalamus, and cerebellar gray and white matter evolved rapidly in humans. At the cellular level, astrocytes and oligodendrocyte progenitors displayed more differences in the human evolutionary lineage than the neurons. Comparison of the bulk tissue and single-nuclei sequencing revealed that conventional RNA sequencing did not detect up to two-thirds of cell-type-specific evolutionary differences.

Silencing Trisomy 21 with XIST in Neural Stem Cells Promotes Neuronal Differentiation

Developmental Cell (IF: 10.13), 2020-2, Jan Tomasz Czermiński et al. DOI: 10.1016/j.devcel.2019.12.015

PubMed: 31978324

Single Cell Gene Expression, Human, Developmental Biology, Functional Genomics.

Abstract

The ability of XIST to dosage compensate a trisomic autosome presents unique experimental opportunities and potentially transformative therapeutic prospects. However, it is currently thought that XIST requires the natural context surrounding pluripotency to initiate chromosome silencing. Here, we demonstrate that XIST RNA induced in differentiated neural cells can trigger chromosome-wide silencing of chromosome 21 in Down syndrome patient-derived cells. Use of this tightly controlled system revealed a deficiency in differentiation of trisomic neural stem cells to neurons, correctable by inducing XIST at different stages of neurogenesis. Single-cell transcriptomics and other analyses strongly implicate elevated Notch signaling due to trisomy 21, thereby promoting neural stem cell cycling that delays terminal differentiation. These findings have significance for illuminating the epigenetic plasticity of cells during development, the understanding of how human trisomy 21 effects Down syndrome neurobiology, and the translational potential of XIST, a unique non-coding RNA.

Comprehensive Identification and Spatial Mapping of Habenular Neuronal Types Using Single-Cell RNA-Seq

Current Biology (IF: 10.17), 2018-4, Aviv Regev Alexander F Schier DOI: 10.1016/j.cub.2018.02.040

PubMed: 29576475

Single Cell Gene Expression, Zebrafish, Developmental Biology, Neuroscience.

Abstract

The identification of cell types and marker genes is critical for dissecting neural development and function, but the size and complexity of the brain has hindered the comprehensive discovery of cell types. We combined single-cell RNA-seq (scRNA-seq) with anatomical brain registration to create a comprehensive map of the zebrafish habenula, a conserved forebrain hub involved in pain processing and learning. Single-cell transcriptomes of ~13,000 habenular cells with 4× cellular coverage identified 18 neuronal types and dozens of marker genes. Registration of marker genes onto a reference atlas created a resource for anatomical and functional studies and enabled the mapping of active neurons onto neuronal types following aversive stimuli. Strikingly, despite brain growth and functional maturation, cell types were retained between the larval and adult habenula. This study provides a gene expression atlas to dissect habenular development and function and offers a general framework for the comprehensive characterization of other brain regions.

Generalizable and Scalable Visualization of Single-Cell Data Using Neural Networks

Cell systems (IF: 11.45), 2018-8, Bonnie Berger et al. DOI: 10.1016/j.cels.2018.05.017 PubMed: 29936184

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

Visualization algorithms are fundamental tools for interpreting single-cell data. However, standard methods, such as t-stochastic neighbor embedding (t-SNE), are not scalable to datasets with millions of cells and the resulting visualizations cannot be generalized to analyze new datasets. Here we introduce net-SNE, a generalizable visualization approach that trains a neural network to learn a mapping function from high-dimensional single-cell gene-expression profiles to a low-dimensional visualization. We benchmark net-SNE on 13 different datasets, and show that it achieves visualization quality and clustering accuracy comparable with t-SNE. Additionally we show that the mapping function learned by net-SNE can accurately position entire new subtypes of cells from previously unseen datasets and can also be used to reduce the runtime of visualizing 1.3 million cells by 36-fold (from 1.5 days to an hour). Our work provides a framework for bootstrapping single-cell analysis from existing datasets.

Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-10, Amy K Y Fu et al. DOI: 10.1073/pnas.2008762117 PubMed: 32989152

Single Cell Gene Expression, Human, Neuroscience.

Abstract

Alzheimer's disease (AD) is the most common form of dementia but has no effective treatment. A comprehensive investigation of cell type-specific responses and cellular heterogeneity in AD is required to provide precise molecular and cellular targets for therapeutic development. Accordingly, we perform single-nucleus transcriptome analysis of 169,496 nuclei from the prefrontal cortical samples of AD patients and normal control (NC) subjects. Differential analysis shows that the cell type-specific transcriptomic changes in AD are associated with the disruption of biological processes including angiogenesis, immune activation, synaptic signaling, and myelination. Subcluster analysis reveals that compared to NC brains, AD brains contain fewer neuroprotective astrocytes and oligodendrocytes. Importantly, our findings show that a subpopulation of angiogenic endothelial cells is induced in the brain in patients with AD. These angiogenic endothelial cells exhibit increased expression of angiogenic growth factors and their receptors (i.e., *VEGF* and *Ang-1*) and antigen-presentation machinery (i.e., *HLA*). This suggests that these endothelial cells contribute to angiogenesis and immune response in AD pathogenesis. Thus, our comprehensive molecular profiling of brain samples from patients with AD reveals previously unknown molecular changes as well as cellular targets that potentially underlie the functional dysregulation of endothelial cells, astrocytes, and oligodendrocytes in AD, providing important insights for therapeutic development.

Bidirectional perisomatic inhibitory plasticity of a Fos neuronal network

Nature (IF: 46.49), 2020-12, Christopher D Harvey et al. DOI: 10.1038/s41586-020-3031-0 PubMed: 33299180
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Behavioural experiences activate the FOS transcription factor in sparse populations of neurons that are critical for encoding and recalling specific events. However, there is limited understanding of the mechanisms by which experience drives circuit reorganization to establish a network of Fos-activated cells. It is also not known whether FOS is required in this process beyond serving as a marker of recent neural activity and, if so, which of its many gene targets underlie circuit reorganization. Here we demonstrate that when mice engage in spatial exploration of novel environments, perisomatic inhibition of Fos-activated hippocampal CA1 pyramidal neurons by parvalbumin-expressing interneurons is enhanced, whereas perisomatic inhibition by cholecystokinin-expressing interneurons is weakened. This bidirectional modulation of inhibition is abolished when the function of the FOS transcription factor complex is disrupted. Single-cell RNA-sequencing, ribosome-associated mRNA profiling and chromatin analyses, combined with electrophysiology, reveal that FOS activates the transcription of *Scg2*, a gene that encodes multiple distinct neuropeptides, to coordinate these changes in inhibition. As parvalbumin- and cholecystokinin-expressing interneurons mediate distinct features of pyramidal cell activity, the SCG2-dependent reorganization of inhibitory synaptic input might be predicted to affect network function in vivo. Consistent with this prediction, hippocampal gamma rhythms and pyramidal cell coupling to theta phase are significantly altered in the absence of *Scg2*. These findings reveal an instructive role for FOS and SCG2 in establishing a network of Fos-activated neurons via the rewiring of local inhibition to form a selectively modulated state. The opposing plasticity mechanisms acting on distinct inhibitory pathways may support the consolidation of memories over time.

Single-cell analysis reveals T cell infiltration in old neurogenic niches

Nature (IF: 46.49), 2019-7, Mark M Davis et al. DOI: 10.1038/s41586-019-1362-5 PubMed: 31270459
Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

The mammalian brain contains neurogenic niches that comprise neural stem cells and other cell types. Neurogenic niches become less functional with age, but how they change during ageing remains unclear. Here we perform single-cell RNA sequencing of young and old neurogenic niches in mice. The analysis of 14,685 single-cell transcriptomes reveals a decrease in activated neural stem cells, changes in endothelial cells and microglia, and an infiltration of T cells in old neurogenic niches. T cells in old brains are clonally expanded and are generally distinct from those in old blood, which suggests that they may experience specific antigens. T cells in old brains also express interferon- γ , and the subset of neural stem cells that has a high interferon response shows decreased proliferation in vivo. We find that T cells can inhibit the proliferation of neural stem cells in co-cultures and in vivo, in part by secreting interferon- γ . Our study reveals an interaction between T cells and neural stem cells in old brains, opening potential avenues through which to counteract age-related decline in brain function.

Phenotypic variation of transcriptomic cell types in mouse motor cortex

Nature (IF: 46.49), 2020-11, Philipp Berens et al. DOI: 10.1038/s41586-020-2907-3 PubMed: 33184512

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Cortical neurons exhibit extreme diversity in gene expression as well as in morphological and electrophysiological properties. Most existing neural taxonomies are based on either transcriptomic or morpho-electric criteria, as it has been technically challenging to study both aspects of neuronal diversity in the same set of cells. Here we used Patch-seq to combine patch-clamp recording, biocytin staining, and single-cell RNA sequencing of more than 1,300 neurons in adult mouse primary motor cortex, providing a morpho-electric annotation of almost all transcriptomically defined neural cell types. We found that, although broad families of transcriptomic types (those expressing Vip, Pvalb, Sst and so on) had distinct and essentially non-overlapping morpho-electric phenotypes, individual transcriptomic types within the same family were not well separated in the morpho-electric space. Instead, there was a continuum of variability in morphology and electrophysiology, with neighbouring transcriptomic cell types showing similar morpho-electric features, often without clear boundaries between them. Our results suggest that neuronal types in the neocortex do not always form discrete entities. Instead, neurons form a hierarchy that consists of distinct non-overlapping branches at the level of families, but can form continuous and correlated transcriptomic and morpho-electrical landscapes within families.

The cellular basis of distinct thirst modalities

Nature (IF: 46.49), 2020-12, John Ngai et al. DOI: 10.1038/s41586-020-2821-8 PubMed: 33057193

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Fluid intake is an essential innate behaviour that is mainly caused by two distinct types of thirst. Increased blood osmolality induces osmotic thirst that drives animals to consume pure water. Conversely, the loss of body fluid induces hypovolaemic thirst, in which animals seek both water and minerals (salts) to recover blood volume. Circumventricular organs in the lamina terminalis are critical sites for sensing both types of thirst-inducing stimulus. However, how different thirst modalities are encoded in the brain remains unknown. Here we employed stimulus-to-cell-type mapping using single-cell RNA sequencing to identify the cellular substrates that underlie distinct types of thirst. These studies revealed diverse types of excitatory and inhibitory neuron in each circumventricular organ structure. We show that unique combinations of these neuron types are activated under osmotic and hypovolaemic stresses. These results elucidate the cellular logic that underlies distinct thirst modalities. Furthermore, optogenetic gain of function in thirst-modality-specific cell types recapitulated water-specific and non-specific fluid appetite caused by the two distinct dipsogenic stimuli. Together, these results show that thirst is a multimodal physiological state, and that different thirst states are mediated by specific neuron types in the mammalian brain.

Negative feedback control of neuronal activity by microglia

Nature (IF: 46.49), 2020-10, Viviana Gradinaru et al. DOI: 10.1038/s41586-020-2777-8 PubMed: 32999463

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Microglia, the brain's resident macrophages, help to regulate brain function by removing dying neurons, pruning non-functional synapses, and producing ligands that support neuronal survival. Here we show that microglia are also critical modulators of neuronal activity and associated behavioural responses in mice. Microglia respond to neuronal activation by suppressing neuronal activity, and ablation of microglia amplifies and synchronizes the activity of neurons, leading to seizures. Suppression of neuronal activation by microglia occurs in a highly region-specific fashion and depends on the ability of microglia to sense and catabolize extracellular ATP, which is released upon neuronal activation by neurons and astrocytes. ATP triggers the recruitment of microglial protrusions and is converted by the microglial ATP/ADP hydrolysing ectoenzyme CD39 into AMP; AMP is then converted into adenosine by CD73, which is expressed on microglia as well as other brain cells. Microglial sensing of ATP, the ensuing microglia-dependent production of adenosine, and the adenosine-mediated suppression of neuronal responses via the adenosine receptor AR are essential for the regulation of neuronal activity and animal behaviour. Our findings suggest that this microglia-driven negative feedback mechanism operates similarly to inhibitory neurons and is essential for protecting the brain from excessive activation in health and disease.

Physiological blood-brain transport is impaired with age by a shift in transcytosis

Nature (IF: 46.49), 2020-7, Michelle L James et al. DOI: 10.1038/s41586-020-2453 PubMed: 32612231

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The vascular interface of the brain, known as the blood-brain barrier (BBB), is understood to maintain brain function in part via its low transcellular permeability. Yet, recent studies have demonstrated that brain ageing is sensitive to circulatory proteins. Thus, it is unclear whether permeability to individually injected exogenous tracers—as is standard in BBB studies—fully represents blood-to-brain transport. Here we label hundreds of proteins constituting the mouse blood plasma proteome, and upon their systemic administration, study the BBB with its physiological ligand. We find that plasma proteins readily permeate the healthy brain parenchyma, with transport maintained by BBB-specific transcriptional programmes. Unlike IgG antibody, plasma protein uptake diminishes in the aged brain, driven by an age-related shift in transport from ligand-specific receptor-mediated to non-specific caveolar transcytosis. This age-related shift occurs alongside a specific loss of pericyte coverage. Pharmacological inhibition of the age-upregulated phosphatase ALPL, a predicted negative regulator of transport, enhances brain uptake of therapeutically relevant transferrin, transferrin receptor antibody and plasma. These findings reveal the extent of physiological protein transcytosis to the healthy brain, a mechanism of widespread BBB dysfunction with age and a strategy for enhanced drug delivery.

Neurons that regulate mouse torpor

Nature (IF: 46.49), 2020-7, Eric C Griffith et al. DOI: 10.1038/s41586-020-2387-5 PubMed: 32528180

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The advent of endothermy, which is achieved through the continuous homeostatic regulation of body temperature and metabolism, is a defining feature of mammalian and avian evolution. However, when challenged by food deprivation or harsh environmental conditions, many mammalian species initiate adaptive energy-conserving survival strategies-including torpor and hibernation-during which their body temperature decreases far below its homeostatic set-point. How homeothermic mammals initiate and regulate these hypothermic states remains largely unknown. Here we show that entry into mouse torpor, a fasting-induced state with a greatly decreased metabolic rate and a body temperature as low as 20 °C, is regulated by neurons in the medial and lateral preoptic area of the hypothalamus. We show that restimulation of neurons that were activated during a previous bout of torpor is sufficient to initiate the key features of torpor, even in mice that are not calorically restricted. Among these neurons we identify a population of glutamatergic *Adcyap1*-positive cells, the activity of which accurately determines when mice naturally initiate and exit torpor, and the inhibition of which disrupts the natural process of torpor entry, maintenance and arousal. Taken together, our results reveal a specific neuronal population in the mouse hypothalamus that serves as a core regulator of torpor. This work forms a basis for the future exploration of mechanisms and circuitry that regulate extreme hypothermic and hypometabolic states, and enables genetic access to monitor, initiate, manipulate and study these ancient adaptations of homeotherm biology.

Molecular design of hypothalamus development

Nature (IF: 46.49), 2020-6, Erik Keimpema et al. DOI: 10.1038/s41586-020-2266-0 PubMed: 32499648

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

A wealth of specialized neuroendocrine command systems intercalated within the hypothalamus control the most fundamental physiological needs in vertebrates. Nevertheless, we lack a developmental blueprint that integrates the molecular determinants of neuronal and glial diversity along temporal and spatial scales of hypothalamus development. Here we combine single-cell RNA sequencing of 51,199 mouse cells of ectodermal origin, gene regulatory network (GRN) screens in conjunction with genome-wide association study-based disease phenotyping, and genetic lineage reconstruction to show that nine glial and thirty-three neuronal subtypes are generated by mid-gestation under the control of distinct GRNs. Combinatorial molecular codes that arise from neurotransmitters, neuropeptides and transcription factors are minimally required to decode the taxonomical hierarchy of hypothalamic neurons. The differentiation of γ -aminobutyric acid (GABA) and dopamine neurons, but not glutamate neurons, relies on quasi-stable intermediate states, with a pool of GABA progenitors giving rise to dopamine cells. We found an unexpected abundance of chemotropic proliferation and guidance cues that are commonly implicated in dorsal (cortical) patterning in the hypothalamus. In particular, loss of SLIT-ROBO signalling impaired both the production and positioning of periventricular dopamine neurons. Overall, we identify molecular principles that shape the developmental architecture of the hypothalamus and show how neuronal heterogeneity is transformed into a multimodal neural unit to provide virtually infinite adaptive potential throughout life.

The microbiota regulate neuronal function and fear extinction learning

Nature (IF: 46.49), 2019-10, Conor Liston et al. DOI: 10.1038/s41586-019-1644 PubMed: 31645720

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Multicellular organisms have co-evolved with complex consortia of viruses, bacteria, fungi and parasites, collectively referred to as the microbiota. In mammals, changes in the composition of the microbiota can influence many physiologic processes (including development, metabolism and immune cell function) and are associated with susceptibility to multiple diseases. Alterations in the microbiota can also modulate host behaviours-such as social activity, stress, and anxiety-related responses-that are linked to diverse neuropsychiatric disorders. However, the mechanisms by which the microbiota influence neuronal activity and host behaviour remain poorly defined. Here we show that manipulation of the microbiota in antibiotic-treated or germ-free adult mice results in significant deficits in fear extinction learning. Single-nucleus RNA sequencing of the medial prefrontal cortex of the brain revealed significant alterations in gene expression in excitatory neurons, glia and other cell types. Transcranial two-photon imaging showed that deficits in extinction learning after manipulation of the microbiota in adult mice were associated with defective learning-related remodelling of postsynaptic dendritic spines and reduced activity in cue-encoding neurons in the medial prefrontal cortex. In addition, selective re-establishment of the microbiota revealed a limited neonatal developmental window in which microbiota-derived signals can restore normal extinction learning in adulthood. Finally, unbiased metabolomic analysis identified four metabolites that were significantly downregulated in germ-free mice and have been reported to be related to neuropsychiatric disorders in humans and mouse models, suggesting that microbiota-derived compounds may directly affect brain function and behaviour. Together, these data indicate that fear extinction learning requires microbiota-derived signals both during early postnatal neurodevelopment and in adult mice, with implications for our understanding of how diet, infection, and lifestyle influence brain health and subsequent susceptibility to neuropsychiatric disorders.

Scalable analysis of cell-type composition from single-cell transcriptomics using deep recurrent learning

Nature Methods (IF: 36.15), 2019-3, Lani F Wu et al. DOI: 10.1038/s41592-019-0353-7 PubMed: 30886411

Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Recent advances in large-scale single-cell RNA-seq enable fine-grained characterization of phenotypically distinct cellular states in heterogeneous tissues. We present scScope, a scalable deep-learning-based approach that can accurately and rapidly identify cell-type composition from millions of noisy single-cell gene-expression profiles.

Childhood cerebellar tumours mirror conserved fetal transcriptional programs

Nature (IF: 46.49), 2019-5, Lincoln Stein et al. DOI: 10.1038/s41586-019-1158-7 PubMed: 31043743

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Study of the origin and development of cerebellar tumours has been hampered by the complexity and heterogeneity of cerebellar cells that change over the course of development. Here we use single-cell transcriptomics to study more than 60,000 cells from the developing mouse cerebellum and show that different molecular subgroups of childhood cerebellar tumours mirror the transcription of cells from distinct, temporally restricted cerebellar lineages. The Sonic Hedgehog medulloblastoma subgroup transcriptionally mirrors the granule cell hierarchy as expected, while group 3 medulloblastoma resembles Nestin stem cells, group 4 medulloblastoma resembles unipolar brush cells, and PFA/PFB ependymoma and cerebellar pilocytic astrocytoma resemble the prenatal gliogenic progenitor cells. Furthermore, single-cell transcriptomics of human childhood cerebellar tumours demonstrates that many bulk tumours contain a mixed population of cells with divergent differentiation. Our data highlight cerebellar tumours as a disorder of early brain development and provide a proximate explanation for the peak incidence of cerebellar tumours in early childhood.

Developmental diversification of cortical inhibitory interneurons

Nature (IF: 46.49), 2018-3, Gord Fishell et al. DOI: 10.1038/nature25999 PubMed: 29513653

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

Diverse subsets of cortical interneurons have vital roles in higher-order brain functions. To investigate how this diversity is generated, here we used single-cell RNA sequencing to profile the transcriptomes of mouse cells collected along a developmental time course. Heterogeneity within mitotic progenitors in the ganglionic eminences is driven by a highly conserved maturation trajectory, alongside eminence-specific transcription factor expression that seeds the emergence of later diversity. Upon becoming postmitotic, progenitors diverge and differentiate into transcriptionally distinct states, including an interneuron precursor state. By integrating datasets across developmental time points, we identified shared sources of transcriptomic heterogeneity between adult interneurons and their precursors, and uncovered the embryonic emergence of cardinal interneuron subtypes. Our analysis revealed that the transcription factor Mef2c, which is linked to various neuropsychiatric and neurodevelopmental disorders, delineates early precursors of parvalbumin-expressing neurons, and is essential for their development. These findings shed new light on the molecular diversification of early inhibitory precursors, and identify gene modules that may influence the specification of human interneuron subtypes.

CNS-Native Myeloid Cells Drive Immune Suppression in the Brain Metastatic Niche through Cxcl10

Cell (IF: 38.62), 2020-11, Gary E Landreth et al. DOI: 10.1016/j.cell.2020.09.064 PubMed: 33113353

Single Cell Gene Expression, Mouse, Cancer Research, Neuroscience.

Abstract

Brain metastasis (br-met) develops in an immunologically unique br-met niche. Central nervous system-native myeloid cells (CNS-myeloids) and bone-marrow-derived myeloid cells (BMDMs) cooperatively regulate brain immunity. The phenotypic heterogeneity and specific roles of these myeloid subsets in shaping the br-met niche to regulate br-met outgrowth have not been fully revealed. Applying multimodal single-cell analyses, we elucidated a heterogeneous but spatially defined CNS-myeloid response during br-met outgrowth. We found Ccr2BMDMs minimally influenced br-met while CNS-myeloid promoted br-met outgrowth. Additionally, br-met-associated CNS-myeloid exhibited downregulation of Cx3cr1. Cx3cr1 knockout in CNS-myeloid increased br-met incidence, leading to an enriched interferon response signature and Cxcl10 upregulation. Significantly, neutralization of Cxcl10 reduced br-met, while rCxcl10 increased br-met and recruited VISTA⁺PD-L1⁺CNS-myeloid to br-met lesions. Inhibiting VISTA- and PD-L1-signaling relieved immune suppression and reduced br-met burden. Our results demonstrate that loss of Cx3cr1 in CNS-myeloid triggers a Cxcl10-mediated vicious cycle, cultivating a br-met-promoting, immune-suppressive niche.

Visualization and analysis of gene expression in tissue sections by spatial transcriptomics

Science (IF: 44.37), 2016-7, Joakim Lundeberg et al. DOI: 10.1126/science.aaf2403 PubMed: 27365449

Spatial Gene Expression, Mouse, Assay Method.

Abstract

Analysis of the pattern of proteins or messengerRNAs (mRNAs) in histological tissue sections is a cornerstone in biomedical research and diagnostics. This typically involves the visualization of a few proteins or expressed genes at a time. We have devised a strategy, which we call spatial transcriptomics; that allows visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections. By positioning histological sections on arrayed reverse transcription primers with unique positional barcodes, we demonstrate high-quality RNA-sequencing data with maintained two-dimensional positional information from the mouse brain and human breast cancer. Spatial transcriptomics provides quantitative gene expression data and visualization of the distribution of mRNAs within tissue sections and enables novel types of bioinformatics analyses, valuable in research and diagnostics.

Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and Chromatin

Cell (IF: 38.62), 2020-11, Aviv Regev et al. DOI: 10.1016/j.cell.2020.09.056 PubMed: 33098772

Single Cell Gene Expression & Single Cell ATAC, Mouse, Method Comparison, Single Cell Multiomics Method.

Abstract

Cell differentiation and function are regulated across multiple layers of gene regulation, including modulation of gene expression by changes in chromatin accessibility. However, differentiation is an asynchronous process precluding a temporal understanding of regulatory events leading to cell fate commitment. Here we developed simultaneous high-throughput ATAC and RNA expression with sequencing (SHARE-seq), a highly scalable approach for measurement of chromatin accessibility and gene expression in the same single cell, applicable to different tissues. Using 34,774 joint profiles from mouse skin, we develop a computational strategy to identify cis-regulatory interactions and define domains of regulatory chromatin (DORCs) that significantly overlap with super-enhancers. During lineage commitment, chromatin accessibility at DORCs precedes gene expression, suggesting that changes in chromatin accessibility may prime cells for lineage commitment. We computationally infer chromatin potential as a quantitative measure of chromatin lineage-priming and use it to predict cell fate outcomes. SHARE-seq is an extensible platform to study regulatory circuitry across diverse cells in tissues.

Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T

Immunotherapies

Cell (IF: 38.62), 2020-10, Avery D Posey et al. DOI: 10.1016/j.cell.2020.08.022 PubMed: 32961131

Single Cell Immune Profiling, Mouse, Immuno-oncology.

Abstract

CD19-directed immunotherapies are clinically effective for treating B cell malignancies but also cause a high incidence of neurotoxicity. A subset of patients treated with chimeric antigen receptor (CAR) T cells or bispecific T cell engager (BiTE) antibodies display severe neurotoxicity, including fatal cerebral edema associated with T cell infiltration into the brain. Here, we report that mural cells, which surround the endothelium and are critical for blood-brain-barrier integrity, express CD19. We identify CD19 expression in brain mural cells using single-cell RNA sequencing data and confirm perivascular staining at the protein level. CD19 expression in the brain begins early in development alongside the emergence of mural cell lineages and persists throughout adulthood across brain regions. Mouse mural cells demonstrate lower levels of Cd19 expression, suggesting limitations in preclinical animal models of neurotoxicity. These data suggest an on-target mechanism for neurotoxicity in CD19-directed therapies and highlight the utility of human single-cell atlases for designing immunotherapies.

Ubiquitin Ligase COP1 Suppresses Neuroinflammation by Degrading c/EBP β in Microglia

Cell (IF: 38.62), 2020-9, Kim Newton et al. DOI: 10.1016/j.cell.2020.07.011 PubMed: 32795415

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Dysregulated microglia are intimately involved in neurodegeneration, including Alzheimer's disease (AD) pathogenesis, but the mechanisms controlling pathogenic microglial gene expression remain poorly understood. The transcription factor CCAAT/enhancer binding protein beta (c/EBP β) regulates pro-inflammatory genes in microglia and is upregulated in AD. We show expression of c/EBP β in microglia is regulated post-translationally by the ubiquitin ligase COP1 (also called RFWD2). In the absence of COP1, c/EBP β accumulates rapidly and drives a potent pro-inflammatory and neurodegeneration-related gene program, evidenced by increased neurotoxicity in microglia-neuronal co-cultures. Antibody blocking studies reveal that neurotoxicity is almost entirely attributable to complement. Remarkably, loss of a single allele of Cebpb prevented the pro-inflammatory phenotype. COP1-deficient microglia markedly accelerated tau-mediated neurodegeneration in a mouse model where activated microglia play a deleterious role. Thus, COP1 is an important suppressor of pathogenic c/EBP β -dependent gene expression programs in microglia.

Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease

Cell (IF: 38.62), 2020-8, Mark Fiers et al. DOI: 10.1016/j.cell.2020.06.038 PubMed: 32702314

Spatial Gene Expression, Mouse, Neuroscience.

Abstract

Although complex inflammatory-like alterations are observed around the amyloid plaques of Alzheimer's disease (AD), little is known about the molecular changes and cellular interactions that characterize this response. We investigate here, in an AD mouse model, the transcriptional changes occurring in tissue domains in a 100- μ m diameter around amyloid plaques using spatial transcriptomics. We demonstrate early alterations in a gene co-expression network enriched for myelin and oligodendrocyte genes (OLIGs), whereas a multicellular gene co-expression network of plaque-induced genes (PIGs) involving the complement system, oxidative stress, lysosomes, and inflammation is prominent in the later phase of the disease. We confirm the majority of the observed alterations at the cellular level using in situ sequencing on mouse and human brain sections. Genome-wide spatial transcriptomics analysis provides an unprecedented approach to untangle the dysregulated cellular network in the vicinity of pathogenic hallmarks of AD and other brain diseases.

Microglia Require CD4 T Cells to Complete the Fetal-to-Adult Transition

Cell (IF: 38.62), 2020-8, James Dooley et al. DOI: 10.1016/j.cell.2020.06.026 PubMed: 32702313

Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

The brain is a site of relative immune privilege. Although CD4 T cells have been reported in the central nervous system, their presence in the healthy brain remains controversial, and their function remains largely unknown. We used a combination of imaging, single cell, and surgical approaches to identify a CD69CD4 T cell population in both the mouse and human brain, distinct from circulating CD4 T cells. The brain-resident population was derived through in situ differentiation from activated circulatory cells and was shaped by self-antigen and the peripheral microbiome. Single-cell sequencing revealed that in the absence of murine CD4 T cells, resident microglia remained suspended between the fetal and adult states. This maturation defect resulted in excess immature neuronal synapses and behavioral abnormalities. These results illuminate a role for CD4 T cells in brain development and a potential interconnected dynamic between the evolution of the immunological and neurological systems. VIDEO ABSTRACT.

Microglial Remodeling of the Extracellular Matrix Promotes Synapse Plasticity

Cell (IF: 38.62), 2020-7, Mazen A Kheirbek et al. DOI: 10.1016/j.cell.2020.05.050 PubMed: 32615087

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Synapse remodeling is essential to encode experiences into neuronal circuits. Here, we define a molecular interaction between neurons and microglia that drives experience-dependent synapse remodeling in the hippocampus. We find that the cytokine interleukin-33 (IL-33) is expressed by adult hippocampal neurons in an experience-dependent manner and defines a neuronal subset primed for synaptic plasticity. Loss of neuronal IL-33 or the microglial IL-33 receptor leads to impaired spine plasticity, reduced newborn neuron integration, and diminished precision of remote fear memories. Memory precision and neuronal IL-33 are decreased in aged mice, and IL-33 gain of function mitigates age-related decreases in spine plasticity. We find that neuronal IL-33 instructs microglial engulfment of the extracellular matrix (ECM) and that its loss leads to impaired ECM engulfment and a concomitant accumulation of ECM proteins in contact with synapses. These data define a cellular mechanism through which microglia regulate experience-dependent synapse remodeling and promote memory consolidation.

High-Throughput Mapping of Long-Range Neuronal Projection Using In Situ Sequencing

Cell (IF: 38.62), 2019-10, Jesse Gillis et al. DOI: 10.1016/j.cell.2019.09.023 PubMed: 31626774

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Understanding neural circuits requires deciphering interactions among myriad cell types defined by spatial organization, connectivity, gene expression, and other properties. Resolving these cell types requires both single-neuron resolution and high throughput, a challenging combination with conventional methods. Here, we introduce barcoded anatomy resolved by sequencing (BARseq), a multiplexed method based on RNA barcoding for mapping projections of thousands of spatially resolved neurons in a single brain and relating those projections to other properties such as gene or Cre expression. Mapping the projections to 11 areas of 3,579 neurons in mouse auditory cortex using BARseq confirmed the laminar organization of the three top classes (intratelencephalic [IT], pyramidal tract-like [PT-like], and corticothalamic [CT]) of projection neurons. In depth analysis uncovered a projection type restricted almost exclusively to transcriptionally defined subtypes of IT neurons. By bridging anatomical and transcriptomic approaches at cellular resolution with high throughput, BARseq can potentially uncover the organizing principles underlying the structure and formation of neural circuits.

Identification of ALK in Thinness

Cell (IF: 38.62), 2020-6, Nele Gheldof et al. DOI: 10.1016/j.cell.2020.04.034 PubMed: 32442405

Single Cell Gene Expression, Mouse, Genetic Health.

Abstract

There is considerable inter-individual variability in susceptibility to weight gain despite an equally obesogenic environment in large parts of the world. Whereas many studies have focused on identifying the genetic susceptibility to obesity, we performed a GWAS on metabolically healthy thin individuals (lowest 6percentile of the population-wide BMI spectrum) in a uniquely phenotyped Estonian cohort. We discovered anaplastic lymphoma kinase (ALK) as a candidate thinness gene. In *Drosophila*, RNAi mediated knockdown of Alk led to decreased triglyceride levels. In mice, genetic deletion of Alk resulted in thin animals with marked resistance to diet- and leptin-mutation-induced obesity. Mechanistically, we found that ALK expression in hypothalamic neurons controls energy expenditure via sympathetic control of adipose tissue lipolysis. Our genetic and mechanistic experiments identify ALK as a thinness gene, which is involved in the resistance to weight gain.

Repopulating Microglia Promote Brain Repair in an IL-6-Dependent Manner

Cell (IF: 38.62), 2020-3, Marc J Ruitenberg et al. DOI: 10.1016/j.cell.2020.02.013 PubMed: 32142677

Spatial Gene Expression, Mouse, Neuroscience.

Abstract

Cognitive dysfunction and reactive microglia are hallmarks of traumatic brain injury (TBI), yet whether these cells contribute to cognitive deficits and secondary inflammatory pathology remains poorly understood. Here, we show that removal of microglia from the mouse brain has little effect on the outcome of TBI, but inducing the turnover of these cells through either pharmacologic or genetic approaches can yield a neuroprotective microglial phenotype that profoundly aids recovery. The beneficial effects of these repopulating microglia are critically dependent on interleukin-6 (IL-6) trans-signaling via the soluble IL-6 receptor (IL-6R) and robustly support adult neurogenesis, specifically by augmenting the survival of newborn neurons that directly support cognitive function. We conclude that microglia in the mammalian brain can be manipulated to adopt a neuroprotective and pro-regenerative phenotype that can aid repair and alleviate the cognitive deficits arising from brain injury.

Stress-Induced Metabolic Disorder in Peripheral CD4⁺ T Cells Leads to Anxiety-like Behavior

Cell (IF: 38.62), 2019-10, Ren-Jie Chai et al. DOI: 10.1016/j.cell.2019.10.001 PubMed: 31675497

Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Physical or mental stress leads to neuroplasticity in the brain and increases the risk of depression and anxiety. Stress exposure causes the dysfunction of peripheral T lymphocytes. However, the pathological role and underlying regulatory mechanism of peripheral T lymphocytes in mood disorders have not been well established. Here, we show that the lack of CD4T cells protects mice from stress-induced anxiety-like behavior. Physical stress-induced leukotriene B4 triggers severe mitochondrial fission in CD4T cells, which further leads to a variety of behavioral abnormalities including anxiety, depression, and social disorders. Metabolomic profiles and single-cell transcriptome reveal that CD4T cell-derived xanthine acts on oligodendrocytes in the left amygdala via adenosine receptor A1. Mitochondrial fission promotes the de novo synthesis of purine via interferon regulatory factor 1 accumulation in CD4T cells. Our study implicates a critical link between a purine metabolic disorder in CD4T cells and stress-driven anxiety-like behavior.

Multimodal Analysis of Cell Types in a Hypothalamic Node Controlling Social Behavior

Cell (IF: 38.62), 2019-10, Hongkui Zeng et al. DOI: 10.1016/j.cell.2019.09.020 PubMed: 31626771

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The ventrolateral subdivision of the ventromedial hypothalamus (VMHvl) contains ~4,000 neurons that project to multiple targets and control innate social behaviors including aggression and mounting. However, the number of cell types in VMHvl and their relationship to connectivity and behavioral function are unknown. We performed single-cell RNA sequencing using two independent platforms-SMART-seq (~4,500 neurons) and 10x (~78,000 neurons)-and investigated correspondence between transcriptomic identity and axonal projections or behavioral activation, respectively. Canonical correlation analysis (CCA) identified 17 transcriptomic types (T-types), including several sexually dimorphic clusters, the majority of which were validated by seqFISH. Immediate early gene analysis identified T-types exhibiting preferential responses to intruder males versus females but only rare examples of behavior-specific activation. Unexpectedly, many VMHvl T-types comprise a mixed population of neurons with different projection target preferences. Overall our analysis revealed that, surprisingly, few VMHvl T-types exhibit a clear correspondence with behavior-specific activation and connectivity.

Rapid Generation of Somatic Mouse Mosaics with Locus-Specific, Stably Integrated Transgenic Elements

Cell (IF: 38.62), 2019-9, Moise Danielpour et al. DOI: 10.1016/j.cell.2019.08.013 PubMed: 31539496

Single Cell Gene Expression & Single Cell ATAC, Mouse, Cancer Research, Functional Genomics.

Abstract

In situ transgenesis methods such as viruses and electroporation can rapidly create somatic transgenic mice but lack control over copy number, zygosity, and locus specificity. Here we establish mosaic analysis by dual recombinase-mediated cassette exchange (MADR), which permits stable labeling of mutant cells expressing transgenic elements from precisely defined chromosomal loci. We provide a toolkit of MADR elements for combination labeling, inducible and reversible transgene manipulation, VCre recombinase expression, and transgenesis of human cells. Further, we demonstrate the versatility of MADR by creating glioma models with mixed reporter-identified zygosity or with personalized; driver mutations from pediatric glioma. MADR is extensible to thousands of existing mouse lines, providing a flexible platform to democratize the generation of somatic mosaic mice.

Single-Cell Transcriptomics in Medulloblastoma Reveals Tumor-Initiating Progenitors and Oncogenic Cascades during Tumorigenesis and Relapse

Cancer Cell (IF: 30.24), 2019-9, William A Weiss et al. DOI: 10.1016/j.ccell.2019.07.009 PubMed: 31474569

Single Cell Gene Expression, Mouse, Cancer Research, Neuroscience.

Abstract

Progenitor heterogeneity and identities underlying tumor initiation and relapse in medulloblastomas remain elusive. Utilizing single-cell transcriptomic analysis, we demonstrated a developmental hierarchy of progenitor pools in Sonic Hedgehog (SHH) medulloblastomas, and identified OLIG2-expressing glial progenitors as transit-amplifying cells at the tumorigenic onset. Although OLIG2progenitors become quiescent stem-like cells in full-blown tumors, they are highly enriched in therapy-resistant and recurrent medulloblastomas. Depletion of mitotic Olig2progenitors or Olig2 ablation impeded tumor initiation. Genomic profiling revealed that OLIG2 modulates chromatin landscapes and activates oncogenic networks including HIPPO-YAP/TAZ and AURORA-A/MYCN pathways. Co-targeting these oncogenic pathways induced tumor growth arrest. Together, our results indicate that glial lineage-associated OLIG2progenitors are tumor-initiating cells during medulloblastoma tumorigenesis and relapse, suggesting OLIG2-driven oncogenic networks as potential therapeutic targets.

Molecular Architecture of the Mouse Nervous System

Cell (IF: 38.62), 2018-8, Ulrika Marklund et al. DOI: 10.1016/j.cell.2018.06.021 PubMed: 30096314

Single Cell Gene Expression, Mouse, Cell Atlas, Neuroscience.

Abstract

The mammalian nervous system executes complex behaviors controlled by specialized, precisely positioned, and interacting cell types. Here, we used RNA sequencing of half a million single cells to create a detailed census of cell types in the mouse nervous system. We mapped cell types spatially and derived a hierarchical, data-driven taxonomy. Neurons were the most diverse and were grouped by developmental anatomical units and by the expression of neurotransmitters and neuropeptides. Neuronal diversity was driven by genes encoding cell identity, synaptic connectivity, neurotransmission, and membrane conductance. We discovered seven distinct, regionally restricted astrocyte types that obeyed developmental boundaries and correlated with the spatial distribution of key glutamate and glycine neurotransmitters. In contrast, oligodendrocytes showed a loss of regional identity followed by a secondary diversification. The resource presented here lays a solid foundation for understanding the molecular architecture of the mammalian nervous system and enables genetic manipulation of specific cell types.

Tumor Microenvironment Is Critical for the Maintenance of Cellular States Found in Primary Glioblastomas

Cancer Discovery (IF: 28.3), 2020-7, Olivier Elemento et al. DOI: 10.1158/2159-8290.CD-20-0057

PubMed: 32253265

Single Cell Gene Expression, Mouse, Cancer Research, Neuroscience.

Abstract

Glioblastoma (GBM), an incurable tumor, remains difficult to model and more importantly to treat due to its genetic/epigenetic heterogeneity and plasticity across cellular states. The ability of current tumor models to recapitulate the cellular states found in primary tumors remains unexplored. To address this issue, we compared single-cell RNA sequencing of tumor cells from 5 patients across four patient-specific glioblastoma stem cell (GSC)-derived model types, including glioma spheres, tumor organoids, glioblastoma cerebral organoids (GLICO), and patient-derived xenografts. We find that GSCs within the GLICO model are enriched for a neural progenitor-like cell subpopulation and recapitulate the cellular states and their plasticity found in the corresponding primary parental tumors. These data demonstrate how the contribution of a neuroanatomically accurate human microenvironment is critical and sufficient for recapitulating the cellular states found in human primary GBMs, a principle that may likely apply to other tumor models. SIGNIFICANCE: It has been unclear how well different patient-derived GBM models are able to recreate the full heterogeneity of primary tumors. Here, we provide a complete transcriptomic characterization of the major model types. We show that the microenvironment is crucial for recapitulating GSC cellular states, highlighting the importance of tumor-host cell interactions...

Interleukin-10 Prevents Pathological Microglia Hyperactivation following Peripheral Endotoxin Challenge

Immunity (IF: 25.73), 2020-11, Kiavash Movahedi et al. DOI: 10.1016/j.immuni.2020.09.018 PubMed: 33049219

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Microglia, the resident macrophages of the brain parenchyma, are key players in central nervous system (CNS) development, homeostasis, and disorders. Distinct brain pathologies seem associated with discrete microglia activation modules. How microglia regain quiescence following challenges remains less understood. Here, we explored the role of the interleukin-10 (IL-10) axis in restoring murine microglia homeostasis following a peripheral endotoxin challenge. Specifically, we show that lipopolysaccharide (LPS)-challenged mice harboring IL-10 receptor-deficient microglia displayed neuronal impairment and succumbed to fatal sickness. Addition of a microglial tumor necrosis factor (TNF) deficiency rescued these animals, suggesting a microglia-based circuit driving pathology. Single cell transcriptome analysis revealed various IL-10 producing immune cells in the CNS, including most prominently Ly49DNK cells and neutrophils, but not microglia. Collectively, we define kinetics of the microglia response to peripheral endotoxin challenge, including their activation and robust silencing, and highlight the critical role of non-microglial IL-10 in preventing deleterious microglia hyperactivation.

Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes

Immunity (IF: 25.73), 2019-1, Steven A McCarroll et al. DOI: 10.1016/j.immuni.2018.11.004 PubMed: 30471926
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Microglia, the resident immune cells of the brain, rapidly change states in response to their environment, but we lack molecular and functional signatures of different microglial populations. Here, we analyzed the RNA expression patterns of more than 76,000 individual microglia in mice during development, in old age, and after brain injury. Our analysis uncovered at least nine transcriptionally distinct microglial states, which expressed unique sets of genes and were localized in the brain using specific markers. The greatest microglial heterogeneity was found at young ages; however, several states-including chemokine-enriched inflammatory microglia-persisted throughout the lifespan or increased in the aged brain. Multiple reactive microglial subtypes were also found following demyelinating injury in mice, at least one of which was also found in human multiple sclerosis lesions. These distinct microglia signatures can be used to better understand microglia function and to identify and manipulate specific subpopulations in health and disease.

Glucose-Dependent Insulinotropic Polypeptide Receptor-Expressing Cells in the Hypothalamus Regulate Food Intake

Cell Metabolism (IF: 24.29), 2019-11, Fiona M Gribble et al. DOI: 10.1016/j.cmet.2019.07.013 PubMed: 31447324
Single Cell Gene Expression, Mouse, Functional Genomics.

Abstract

Ambiguity regarding the role of glucose-dependent insulinotropic polypeptide (GIP) in obesity arises from conflicting reports asserting that both GIP receptor (GIPR) agonism and antagonism are effective strategies for inhibiting weight gain. To enable identification and manipulation of *Gipr*-expressing (*Gipr*) cells, we created *Gipr*-Cre knockin mice. As GIPR-agonists have recently been reported to suppress food intake, we aimed to identify central mediators of this effect. *Gipr* cells were identified in the arcuate, dorsomedial, and paraventricular nuclei of the hypothalamus, as confirmed by RNAscope in mouse and human. Single-cell RNA-seq identified clusters of hypothalamic *Gipr* cells exhibiting transcriptomic signatures for vascular, glial, and neuronal cells, the latter expressing somatostatin but little pro-opiomelanocortin or agouti-related peptide. Activation of G-DREADDs in hypothalamic *Gipr* cells suppressed food intake in vivo, which was not obviously additive with concomitant GLP1R activation. These data identify hypothalamic GIPR as a target for the regulation of energy balance.

A Widespread Neurogenic Potential of Neocortical Astrocytes Is Induced by Injury

Cell Stem Cell (IF: 23.45), 2020-10, Jens Peter Magnusson et al. DOI: 10.1016/j.stem.2020.07.006

PubMed: 32758425

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Parenchymal astrocytes have emerged as a potential reservoir for new neurons in non-neurogenic brain regions. It is currently unclear how astrocyte neurogenesis is controlled molecularly. Here we show that Notch signaling-deficient astrocytes can generate new neurons after injury. Using single-cell RNA sequencing, we found that, when Notch signaling is blocked, astrocytes transition to a neural stem cell-like state. However, only after injury do a few of these primed astrocytes unfold a neurogenic program, including a self-amplifying progenitor-like state. Further, reconstruction of the trajectories of individual cells allowed us to uncouple astrocyte neurogenesis from reactive gliosis, which occur along independent branches. Finally, we show that cortical neurogenesis molecularly recapitulates canonical subventricular zone neurogenesis with remarkable fidelity. Our study supports a widespread potential of parenchymal astrocytes to function as dormant neural stem cells.

Meningeal $\gamma\delta$ T cells regulate anxiety-like behavior via IL-17a signaling in neurons

Nature Immunology (IF: 22.3), 2020-11, Jasmin Herz et al. DOI: 10.1038/s41590-020-0776-4 PubMed: 32929273

Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Interleukin (IL)-17a has been highly conserved during evolution of the vertebrate immune system and widely studied in contexts of infection and autoimmunity. Studies suggest that IL-17a promotes behavioral changes in experimental models of autism and aggregation behavior in worms. Here, through a cellular and molecular characterization of meningeal $\gamma\delta$ 17 T cells, we defined the nearest central nervous system-associated source of IL-17a under homeostasis. Meningeal $\gamma\delta$ T cells express high levels of the chemokine receptor CXCR6 and seed meninges shortly after birth. Physiological release of IL-17a by these cells was correlated with anxiety-like behavior in mice and was partially dependent on T cell receptor engagement and commensal-derived signals. IL-17a receptor was expressed in cortical glutamatergic neurons under steady state and its genetic deletion decreased anxiety-like behavior in mice. Our findings suggest that IL-17a production by meningeal $\gamma\delta$ 17 T cells represents an evolutionary bridge between this conserved anti-pathogen molecule and survival behavioral traits in vertebrates.

Neuroblast senescence in the aged brain augments natural kill cell cytotoxicity leading to impaired neurogenesis and cognition

Nature Neuroscience (IF: 21.5), 2020-11, Fu-Dong Shi et al. DOI: 10.1038/s41593-020-00745 PubMed: 33257875
Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Normal aging is accompanied by escalating systemic inflammation. Yet the potential impact of immune homeostasis on neurogenesis and cognitive decline during brain aging have not been previously addressed. Here we report that natural killer (NK) cells of the innate immune system reside in the dentate gyrus neurogenic niche of aged brains in humans and mice. In situ expansion of these cells contributes to their abundance, which dramatically exceeds that of other immune subsets. Neuroblasts within the aged dentate gyrus display a senescence-associated secretory phenotype and reinforce NK cell activities and surveillance functions, which result in NK cell elimination of aged neuroblasts. Genetic or antibody-mediated depletion of NK cells leads to sustained improvements in neurogenesis and cognitive function during normal aging. These results demonstrate that NK cell accumulation in the aging brain impairs neurogenesis, which may serve as a therapeutic target to improve cognition in the aged population.

Viral manipulation of functionally distinct interneurons in mice, non-human primates and humans

Nature Neuroscience (IF: 21.5), 2020-12, Gord Fishell et al. DOI: 10.1038/s41593-020-0692-9 PubMed: 32807948
Single Cell ATAC, Mouse, Neuroscience.

Abstract

Recent success in identifying gene-regulatory elements in the context of recombinant adeno-associated virus vectors has enabled cell-type-restricted gene expression. However, within the cerebral cortex these tools are largely limited to broad classes of neurons. To overcome this limitation, we developed a strategy that led to the identification of multiple new enhancers to target functionally distinct neuronal subtypes. By investigating the regulatory landscape of the disease gene *Scn1a*, we discovered enhancers selective for parvalbumin (PV) and vasoactive intestinal peptide-expressing interneurons. Demonstrating the functional utility of these elements, we show that the PV-specific enhancer allowed for the selective targeting and manipulation of these neurons across vertebrate species, including humans. Finally, we demonstrate that our selection method is generalizable and characterizes additional PV-specific enhancers with exquisite specificity within distinct brain regions. Altogether, these viral tools can be used for cell-type-specific circuit manipulation and hold considerable promise for use in therapeutic interventions.

A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment

Nature Neuroscience (IF: 21.5), 2019-5, Yvan Saeys et al. DOI: 10.1038/s41593-019-0393-4 PubMed: 31061494
Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

While the roles of parenchymal microglia in brain homeostasis and disease are fairly clear, other brain-resident myeloid cells remain less well understood. By dissecting border regions and combining single-cell RNA-sequencing with high-dimensional cytometry, bulk RNA-sequencing, fate-mapping and microscopy, we reveal the diversity of non-parenchymal brain macrophages. Border-associated macrophages (BAMs) residing in the dura mater, subdural meninges and choroid plexus consisted of distinct subsets with tissue-specific transcriptional signatures, and their cellular composition changed during postnatal development. BAMs exhibited a mixed ontogeny, and subsets displayed distinct self-renewal capacity following depletion and repopulation. Single-cell and fate-mapping analysis both suggested that there is a unique microglial subset residing on the apical surface of the choroid plexus epithelium. Finally, gene network analysis and conditional deletion revealed IRF8 as a master regulator that drives the maturation and diversity of brain macrophages. Our results provide a framework for understanding host-macrophage interactions in both the healthy and diseased brain.

Disease-associated astrocytes in Alzheimer's disease and aging

Nature Neuroscience (IF: 21.5), 2020-6, Aviv Regev et al. DOI: 10.1038/s41593-020-0624-8 PubMed: 32341542
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The role of non-neuronal cells in Alzheimer's disease progression has not been fully elucidated. Using single-nucleus RNA sequencing, we identified a population of disease-associated astrocytes in an Alzheimer's disease mouse model. These disease-associated astrocytes appeared at early disease stages and increased in abundance with disease progression. We discovered that similar astrocytes appeared in aged wild-type mice and in aging human brains, suggesting their linkage to genetic and age-related factors.

Spatiotemporal single-cell analysis of gene expression in the mouse suprachiasmatic nucleus

Nature Neuroscience (IF: 21.5), 2020-3, Haifang Wang et al. DOI: 10.1038/s41593-020-0586 PubMed: 32066983
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Mammalian circadian behaviors are orchestrated by the suprachiasmatic nucleus (SCN) in the ventral hypothalamus, but the number of SCN cell types and their functional roles remain unclear. We have used single-cell RNA-sequencing to identify the basic cell types in the mouse SCN and to characterize their circadian and light-induced gene expression patterns. We identified eight major cell types, with each type displaying a specific pattern of circadian gene expression. Five SCN neuronal subtypes, each with specific combinations of markers, differ in their spatial distribution, circadian rhythmicity and light responsiveness. Through a complete three-dimensional reconstruction of the mouse SCN at single-cell resolution, we obtained a standardized SCN atlas containing the spatial distribution of these subtypes and gene expression. Furthermore, we observed heterogeneous circadian gene expression between SCN neuron subtypes. Such a spatiotemporal pattern of gene regulation within the SCN may have an important function in the circadian pacemaker.

Single-cell transcriptomic profiling of the aging mouse brain

Nature Neuroscience (IF: 21.5), 2019-9, Joshua Z Levin et al. DOI: 10.1038/s41593-019-0491-3
PubMed: 31551601
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The mammalian brain is complex, with multiple cell types performing a variety of diverse functions, but exactly how each cell type is affected in aging remains largely unknown. Here we performed a single-cell transcriptomic analysis of young and old mouse brains. We provide comprehensive datasets of aging-related genes, pathways and ligand-receptor interactions in nearly all brain cell types. Our analysis identified gene signatures that vary in a coordinated manner across cell types and gene sets that are regulated in a cell-type specific manner, even at times in opposite directions. These data reveal that aging, rather than inducing a universal program, drives a distinct transcriptional course in each cell population, and they highlight key molecular processes, including ribosome biogenesis, underlying brain aging. Overall, these large-scale datasets (accessible online at https://portals.broadinstitute.org/single_cell/study/aging-mouse-brain) provide a resource for the neuroscience community that will facilitate additional discoveries directed towards understanding and modifying the aging process.

Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons

Nature Neuroscience (IF: 21.5), 2019-3, Paul Robson et al. DOI: 10.1038/s41593-019-0349-8 PubMed: 30858605
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The lateral hypothalamic area (LHA) coordinates an array of fundamental behaviors, including sleeping, waking, feeding, stress and motivated behavior. The wide spectrum of functions ascribed to the LHA may be explained by a heterogeneous population of neurons, the full diversity of which is poorly understood. We employed a droplet-based single-cell RNA-sequencing approach to develop a comprehensive census of molecularly distinct cell types in the mouse LHA. Neuronal populations were classified based on fast neurotransmitter phenotype and expression of neuropeptides, transcription factors and synaptic proteins, among other gene categories. We define 15 distinct populations of glutamatergic neurons and 15 of GABAergic neurons, including known and novel cell types. We further characterize a novel population of somatostatin-expressing neurons through anatomical and behavioral approaches, identifying a role for these neurons in specific forms of innate locomotor behavior. This study lays the groundwork for better understanding the circuit-level underpinnings of LHA function.

Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing

Nature Neuroscience (IF: 21.5), 2018-2, Peter Lönnerberg et al. DOI: 10.1038/s41593-017-0056-2 PubMed: 29335606

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The dentate gyrus of the hippocampus is a brain region in which neurogenesis persists into adulthood; however, the relationship between developmental and adult dentate gyrus neurogenesis has not been examined in detail. Here we used single-cell RNA sequencing to reveal the molecular dynamics and diversity of dentate gyrus cell types in perinatal, juvenile, and adult mice. We found distinct quiescent and proliferating progenitor cell types, linked by transient intermediate states to neuroblast stages and fully mature granule cells. We observed shifts in the molecular identity of quiescent and proliferating radial glia and granule cells during the postnatal period that were then maintained through adult stages. In contrast, intermediate progenitor cells, neuroblasts, and immature granule cells were nearly indistinguishable at all ages. These findings demonstrate the fundamental similarity of postnatal and adult neurogenesis in the hippocampus and pinpoint the early postnatal transformation of radial glia from embryonic progenitors to adult quiescent stem cells.

An Immunocompetent Mouse Model of Zika Virus Infection

Cell Host & Microbe (IF: 17.77), 2018-5, Adolfo Garcia-Sastre et al. DOI: 10.1016/j.chom.2018.04.003

PubMed: 29746837

Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

Progress toward understanding Zika virus (ZIKV) pathogenesis is hindered by lack of immunocompetent small animal models, in part because ZIKV fails to effectively antagonize Stat2-dependent interferon (IFN) responses in mice. To address this limitation, we first passaged an African ZIKV strain (ZIKV-Dak-41525) through Rag1 mice to obtain a mouse-adapted virus (ZIKV-Dak-MA) that was more virulent than ZIKV-Dak-41525 in mice treated with an anti-Ifnar1 antibody. A G18R substitution in NS4B was the genetic basis for the increased replication, and resulted in decreased IFN- β production, diminished IFN-stimulated gene expression, and the greater brain infection observed with ZIKV-Dak-MA. To generate a fully immunocompetent mouse model of ZIKV infection, human STAT2 was introduced into the mouse Stat2 locus (hSTAT2 KI). Subcutaneous inoculation of pregnant hSTAT2 KI mice with ZIKV-Dak-MA resulted in spread to the placenta and fetal brain. An immunocompetent mouse model of ZIKV infection may prove valuable for evaluating countermeasures to limit disease.

Macrophage-tumor cell interaction promotes ATRT progression and chemoresistance

Acta Neuropathologica (IF: 16.29), 2020-5, Thomas K Albert et al. DOI: 10.1007/s00401-019-02116-7

PubMed: 31848709

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Atypical teratoid/rhabdoid tumors (ATRT) are known for their heterogeneity concerning pathophysiology and outcome. However, predictive factors within distinct subgroups still need to be uncovered. Using multiplex immunofluorescent staining and single-cell RNA sequencing we unraveled distinct compositions of the immunological tumor microenvironment (TME) across ATRT subgroups. CD68 cells predominantly infiltrate ATRT-SHH and ATRT-MYC and are a negative prognostic factor for patients' survival. Within the murine ATRT-MYC and ATRT-SHH TME, Cd68 macrophages are core to intercellular communication with tumor cells. In ATRT-MYC distinct tumor cell phenotypes express macrophage marker genes. These cells are involved in the acquisition of chemotherapy resistance in our relapse xenograft mouse model. In conclusion, the tumor cell-macrophage interaction contributes to ATRT-MYC heterogeneity and potentially to tumor recurrence.

Dissecting Cell-Type Composition and Activity-Dependent Transcriptional State in Mammalian Brains by Massively Parallel Single-Nucleus RNA-Seq

Molecular Cell (IF: 16.13), 2017-12, Zhaolan Zhou et al. DOI: 10.1016/j.molcel.2017.11.017 PubMed: 29220646
Single Cell Gene Expression, Mouse, Assay Method, Neuroscience.

Abstract

Massively parallel single-cell RNA sequencing can precisely resolve cellular diversity in a high-throughput manner at low cost, but unbiased isolation of intact single cells from complex tissues such as adult mammalian brains is challenging. Here, we integrate sucrose-gradient-assisted purification of nuclei with droplet microfluidics to develop a highly scalable single-nucleus RNA-seq approach (sNucDrop-seq), which is free of enzymatic dissociation and nucleus sorting. By profiling ~18,000 nuclei isolated from cortical tissues of adult mice, we demonstrate that sNucDrop-seq not only accurately reveals neuronal and non-neuronal subtype composition with high sensitivity but also enables in-depth analysis of transient transcriptional states driven by neuronal activity, at single-cell resolution, in vivo.

Context-Specific Striatal Astrocyte Molecular Responses Are Phenotypically Exploitable

Neuron (IF: 16), 2020-10, M Madan Babu et al. DOI: 10.1016/j.neuron.2020.09.021 PubMed: 33086039
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Astrocytes tile the central nervous system and are widely implicated in brain diseases, but the molecular mechanisms by which astrocytes contribute to brain disorders remain incompletely explored. By performing astrocyte gene expression analyses following 14 experimental perturbations of relevance to the striatum, we discovered that striatal astrocytes mount context-specific molecular responses at the level of genes, pathways, and upstream regulators. Through data mining, we also identified astrocyte pathways in Huntington's disease (HD) that were reciprocally altered with respect to the activation of striatal astrocyte G protein-coupled receptor (GPCR) signaling. Furthermore, selective striatal astrocyte stimulation of the G-GPCR pathway in vivo corrected several HD-associated astrocytic, synaptic, and behavioral phenotypes, with accompanying improvement of HD-associated astrocyte signaling pathways, including those related to synaptogenesis and neuroimmune functions. Overall, our data show that astrocytes are malleable, using context-specific responses that can be dissected molecularly and used for phenotypic benefit in brain disorders.

MCH Neurons Regulate Permeability of the Median Eminence Barrier

Neuron (IF: 16), 2020-7, Vincent Prevot et al. DOI: 10.1016/j.neuron.2020.04.020 PubMed: 32407670

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Melanin-concentrating hormone (MCH)-expressing neurons are key regulators of energy and glucose homeostasis. Here, we demonstrate that they provide dense projections to the median eminence (ME) in close proximity to tanycytes and fenestrated vessels. Chemogenetic activation of MCH neurons as well as optogenetic stimulation of their projections in the ME enhance permeability of the ME by increasing fenestrated vascular loops and enhance leptin action in the arcuate nucleus of the hypothalamus (ARC). Unbiased phosphoRiboTrap-based assessment of cell activation upon chemogenetic MCH neuron activation reveals MCH-neuron-dependent regulation of endothelial cells. MCH neurons express the vascular endothelial growth factor A (VEGFA), and blocking VEGF-R signaling attenuates the leptin-sensitizing effect of MCH neuron activation. Our experiments reveal that MCH neurons directly regulate permeability of the ME barrier, linking the activity of energy state and sleep regulatory neurons to the regulation of hormone accessibility to the ARC.

An Activity-Mediated Transition in Transcription in Early Postnatal Neurons

Neuron (IF: 16), 2020-9, Emi Ling et al. DOI: 10.1016/j.neuron.2020.06.008 PubMed: 32589877

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The maturation of the mammalian brain occurs after birth, and this stage of neuronal development is frequently impaired in neurological disorders, such as autism and schizophrenia. However, the mechanisms that regulate postnatal brain maturation are poorly defined. By purifying neuronal subpopulations across brain development in mice, we identify a postnatal switch in the transcriptional regulatory circuits that operates in the maturing mammalian brain. We show that this developmental transition includes the formation of hundreds of cell-type-specific neuronal enhancers that appear to be modulated by neuronal activity. Once selected, these enhancers are active throughout adulthood, suggesting that their formation in early life shapes neuronal identity and regulates mature brain function.

Transcriptional and Spatial Resolution of Cell Types in the Mammalian Habenula

Neuron (IF: 16), 2020-6, Omar R Ahmad et al. DOI: 10.1016/j.neuron.2020.03.011 PubMed: 32272058

Single Cell Gene Expression, Mouse & Zebrafish, Neuroscience.

Abstract

The habenula complex is appreciated as a critical regulator of motivated and pathological behavioral states via its output to midbrain nuclei. Despite this, transcriptional definition of cell populations that comprise both the medial habenular (MHb) and lateral habenular (LHb) subregions in mammals remain undefined. To resolve this, we performed single-cell transcriptional profiling and highly multiplexed in situ hybridization experiments of the mouse habenula complex in naive mice and those exposed to an acute aversive stimulus. Transcriptionally distinct neuronal cell types identified within the MHb and LHb, were spatially defined, differentially engaged by aversive stimuli, and had distinct electrophysiological properties. Cell types identified in mice also displayed a high degree of transcriptional similarity to those previously described in zebrafish, highlighting the well-conserved nature of habenular cell types across the phylum. These data identify key molecular targets within habenular cell types and provide a critical resource for future studies.

Genome-wide In Vivo CNS Screening Identifies Genes that Modify CNS Neuronal Survival and mHTT Toxicity

Neuron (IF: 16), 2020-4, John G Doench et al. DOI: 10.1016/j.neuron.2020.01.004 PubMed: 32004439

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Unbiased in vivo genome-wide genetic screening is a powerful approach to elucidate new molecular mechanisms, but such screening has not been possible to perform in the mammalian central nervous system (CNS). Here, we report the results of the first genome-wide genetic screens in the CNS using both short hairpin RNA (shRNA) and CRISPR libraries. Our screens identify many classes of CNS neuronal essential genes and demonstrate that CNS neurons are particularly sensitive not only to perturbations to synaptic processes but also autophagy, proteostasis, mRNA processing, and mitochondrial function. These results reveal a molecular logic for the common implication of these pathways across multiple neurodegenerative diseases. To further identify disease-relevant genetic modifiers, we applied our screening approach to two mouse models of Huntington's disease (HD). Top mutant huntingtin toxicity modifier genes included several Nme genes and several genes involved in methylation-dependent chromatin silencing and dopamine signaling, results that reveal new HD therapeutic target pathways.

TREM2 Regulates Microglial Cholesterol Metabolism upon Chronic Phagocytic Challenge

Neuron (IF: 16), 2020-3, Kathryn M Monroe et al. DOI: 10.1016/j.neuron.2019.12.007 PubMed: 31902528

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Loss-of-function (LOF) variants of TREM2, an immune receptor expressed in microglia, increase Alzheimer's disease risk. TREM2 senses lipids and mediates myelin phagocytosis, but its role in microglial lipid metabolism is unknown. Combining chronic demyelination paradigms and cell sorting with RNA sequencing and lipidomics, we find that wild-type microglia acquire a disease-associated transcriptional state, while TREM2-deficient microglia remain largely homeostatic, leading to neuronal damage. TREM2-deficient microglia phagocytose myelin debris but fail to clear myelin cholesterol, resulting in cholesteryl ester (CE) accumulation. CE increase is also observed in APOE-deficient glial cells, reflecting impaired brain cholesterol transport. This finding replicates in myelin-treated TREM2-deficient murine macrophages and human iPSC-derived microglia, where it is rescued by an ACAT1 inhibitor and LXR agonist. Our studies identify TREM2 as a key transcriptional regulator of cholesterol transport and metabolism under conditions of chronic myelin phagocytic activity, as TREM2 LOF causes pathogenic lipid accumulation in microglia.

Recapitulation and Reversal of Schizophrenia-Related Phenotypes in Setd1a-Deficient Mice

Neuron (IF: 16), 2019-11, Bin Xu et al. DOI: 10.1016/j.neuron.2019.09.014 PubMed: 31606247

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

SETD1A, a lysine-methyltransferase, is a key schizophrenia susceptibility gene. Mice carrying a heterozygous loss-of-function mutation of the orthologous gene exhibit alterations in axonal branching and cortical synaptic dynamics accompanied by working memory deficits. We show that Setd1a binds both promoters and enhancers with a striking overlap between Setd1a and Mef2 on enhancers. Setd1a targets are highly expressed in pyramidal neurons and display a complex pattern of transcriptional up- and downregulations shaped by presumed opposing functions of Setd1a on promoters and Mef2-bound enhancers. Notably, evolutionarily conserved Setd1a targets are associated with neuropsychiatric genetic risk burden. Reinstating Setd1a expression in adulthood rescues cognitive deficits. Finally, we identify LSD1 as a major counteracting demethylase for Setd1a and show that its pharmacological antagonism results in a full rescue of the behavioral and morphological deficits in Setd1a-deficient mice. Our findings advance understanding of how SETD1A mutations predispose to schizophrenia (SCZ) and point to novel therapeutic interventions.

Neocortical Projection Neurons Instruct Inhibitory Interneuron Circuit Development in a Lineage-Dependent Manner

Neuron (IF: 16), 2019-6, Timothy J Petros et al. DOI: 10.1016/j.neuron.2019.03.036 PubMed: 31027966

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Neocortical circuits consist of stereotypical motifs that must self-assemble during development. Recent evidence suggests that the subtype identity of both excitatory projection neurons (PNs) and inhibitory interneurons (INs) is important for this process. We knocked out the transcription factor *Satb2* in PNs to induce those of the intratelencephalic (IT) type to adopt a pyramidal tract (PT)-type identity. Loss of IT-type PNs selectively disrupted the lamination and circuit integration of INs derived from the caudal ganglionic eminence (CGE). Strikingly, reprogrammed PNs demonstrated reduced synaptic targeting of CGE-derived INs relative to controls. In control mice, IT-type PNs targeted neighboring CGE INs, while PT-type PNs did not in deep layers, confirming this lineage-dependent motif. Finally, single-cell RNA sequencing revealed that major CGE IN subtypes were conserved after loss of IT PNs, but with differential transcription of synaptic proteins and signaling molecules. Thus, IT-type PNs influence CGE-derived INs in a non-cell-autonomous manner during cortical development.

A Population of Navigator Neurons Is Essential for Olfactory Map Formation during the Critical Period

Neuron (IF: 16), 2018-12, Anoja Perera et al. DOI: 10.1016/j.neuron.2018.09.051 PubMed: 30482691

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

In the developing brain, heightened plasticity during the critical period enables the proper formation of neural circuits. Here, we identify the navigator; neurons, a group of perinatally born olfactory sensory neurons, as playing an essential role in establishing the olfactory map during the critical period. The navigator axons project circuitously in the olfactory bulb and traverse multiple glomeruli before terminating in perspective glomeruli. These neurons undergo a phase of exuberant axon growth and exhibit a shortened lifespan. Single-cell transcriptome analyses reveal distinct molecular signatures for the navigators. Extending their lifespan prolongs the period of exuberant growth and perturbs axon convergence. Conversely, a genetic ablation experiment indicates that, despite postnatal neurogenesis, only the navigators are endowed with the ability to establish a convergent map. The presence and the proper removal of the navigator neurons are both required to establish tight axon convergence into the glomeruli.

PDGFR β Cells Rapidly Relay Inflammatory Signal from the Circulatory System to Neurons via Chemokine CCL2

Neuron (IF: 16), 2018-10, Wen-Hao Zhou et al. DOI: 10.1016/j.neuron.2018.08.030 PubMed: 30269986

Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Acute infection, if not kept in check, can lead to systemic inflammatory responses in the brain. Here, we show that within 2 hr of systemic inflammation, PDGFR β mural cells of blood vessels rapidly secrete chemokine CCL2, which in turn increases total neuronal excitability by promoting excitatory synaptic transmission in glutamatergic neurons of multiple brain regions. By single-cell RNA sequencing, we identified Col1a1 and Rgs5 subgroups of PDGFR β cells as the main source of CCL2. Lipopolysaccharide (LPS)- or Poly(I:C)-treated pericyte culture medium induced similar effects in a CCL2-dependent manner. Importantly, in *Pdgfrb-Cre;Ccl2* mice, LPS-induced increase in excitatory synaptic transmission was significantly attenuated. These results demonstrate in vivo that PDGFR β cells function as initial sensors of external insults by secreting CCL2, which relays the signal to the central nervous system. Through their gateway position in the brain, PDGFR β cells are ideally positioned to respond rapidly to environmental changes and to coordinate responses.

Graded Arrays of Spinal and Supraspinal V2a Interneuron Subtypes Underlie Forelimb and Hindlimb Motor Control

Neuron (IF: 16), 2018-2, Kamal Sharma et al. DOI: 10.1016/j.neuron.2018.01.023 PubMed: 29398364

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The spinal cord contains neural networks that enable regionally distinct motor outputs along the body axis. Nevertheless, it remains unclear how segment-specific motor computations are processed because the cardinal interneuron classes that control motor neurons appear uniform at each level of the spinal cord. V2a interneurons are essential to both forelimb and hindlimb movements, and here we identify two major types that emerge during development: type I neurons marked by high *Chx10* form recurrent networks with neighboring spinal neurons and type II neurons that downregulate *Chx10* and project to supraspinal structures. Types I and II V2a interneurons are arrayed in counter-gradients, and this network activates different patterns of motor output at cervical and lumbar levels. Single-cell RNA sequencing (RNA-seq) revealed type I and II V2a neurons are each comprised of multiple subtypes. Our findings uncover a molecular and anatomical organization of V2a interneurons reminiscent of the orderly way motor neurons are divided into columns and pools.

Molecular atlas of the adult mouse brain

Science Advances (IF: 14.09), 2020-6, Joakim Lundeberg et al. DOI: 10.1126/sciadv.abb3446 PubMed: 32637622
Spatial Gene Expression, Mouse, Cell Atlas, Neuroscience.

Abstract

Brain maps are essential for integrating information and interpreting the structure-function relationship of circuits and behavior. We aimed to generate a systematic classification of the adult mouse brain based purely on the unbiased identification of spatially defining features by employing whole-brain spatial transcriptomics. We found that the molecular information was sufficient to deduce the complex and detailed neuroanatomical organization of the brain. The unsupervised (non-expert, data-driven) classification revealed new area- and layer-specific subregions, for example in isocortex and hippocampus, and new subdivisions of striatum. The molecular atlas further supports the characterization of the spatial identity of neurons from their single-cell RNA profile, and provides a resource for annotating the brain using a minimal gene set-a brain palette. In summary, we have established a molecular atlas to formally define the spatial organization of brain regions, including the molecular code for mapping and targeting of discrete neuroanatomical domains.

Pharmacologically reversible zonation-dependent endothelial cell transcriptomic changes with neurodegenerative disease associations in the aged brain

Nature Communications (IF: 13.61), 2020-9, Vincent C T Mok et al. DOI: 10.1038/s41467-020-18249-3
PubMed: 32887883
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The molecular signatures of cells in the brain have been revealed in unprecedented detail, yet the ageing-associated genome-wide expression changes that may contribute to neurovascular dysfunction in neurodegenerative diseases remain elusive. Here, we report zonation-dependent transcriptomic changes in aged mouse brain endothelial cells (ECs), which prominently implicate altered immune/cytokine signaling in ECs of all vascular segments, and functional changes impacting the blood-brain barrier (BBB) and glucose/energy metabolism especially in capillary ECs (capECs). An overrepresentation of Alzheimer disease (AD) GWAS genes is evident among the human orthologs of the differentially expressed genes of aged capECs, while comparative analysis revealed a subset of concordantly downregulated, functionally important genes in human AD brains. Treatment with exenatide, a glucagon-like peptide-1 receptor agonist, strongly reverses aged mouse brain EC transcriptomic changes and BBB leakage, with associated attenuation of microglial priming. We thus revealed transcriptomic alterations underlying brain EC ageing that are complex yet pharmacologically reversible.

The cellular and molecular landscape of hypothalamic patterning and differentiation from embryonic to late postnatal development

Nature Communications (IF: 13.61), 2020-8, Lizhi Jiang et al. DOI: 10.1038/s41467-020-18231 PubMed: 32868762
Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The hypothalamus is a central regulator of many innate behaviors essential for survival, but the molecular mechanisms controlling hypothalamic patterning and cell fate specification are poorly understood. To identify genes that control hypothalamic development, we have used single-cell RNA sequencing (scRNA-Seq) to profile mouse hypothalamic gene expression across 12 developmental time points between embryonic day 10 and postnatal day 45. This identified genes that delineated clear developmental trajectories for all major hypothalamic cell types, and readily distinguished major regional subdivisions of the developing hypothalamus. By using our developmental dataset, we were able to rapidly annotate previously unidentified clusters from existing scRNA-Seq datasets collected during development and to identify the developmental origins of major neuronal populations of the ventromedial hypothalamus. We further show that our approach can rapidly and comprehensively characterize mutants that have altered hypothalamic patterning, identifying Nkx2.1 as a negative regulator of prethalamic identity. These data serve as a resource for further studies of hypothalamic development, physiology, and dysfunction.

VEGFR2 signaling drives meningeal vascular regeneration upon head injury

Nature Communications (IF: 13.61), 2020-7, Gou Young Koh et al. DOI: 10.1038/s41467-020-17545-2 PubMed: 32737287
Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Upon severe head injury (HI), blood vessels of the meninges and brain parenchyma are inevitably damaged. While limited vascular regeneration of the injured brain has been studied extensively, our understanding of meningeal vascular regeneration following head injury is quite limited. Here, we identify key pathways governing meningeal vascular regeneration following HI. Rapid and complete vascular regeneration in the meninges is predominantly driven by VEGFR2 signaling. Substantial increase of VEGFR2 is observed in both human patients and mouse models of HI, and endothelial cell-specific deletion of Vegfr2 in the latter inhibits meningeal vascular regeneration. We further identify the facilitating, stabilizing and arresting roles of Tie2, PDGFR β and Dll4 signaling, respectively, in meningeal vascular regeneration. Prolonged inhibition of this angiogenic process following HI compromises immunological and stromal integrity of the injured meninges. These findings establish a molecular framework for meningeal vascular regeneration after HI, and may guide development of wound healing therapeutics.

KAT3-dependent acetylation of cell type-specific genes maintains neuronal identity in the adult mouse brain

Nature Communications (IF: 13.61), 2020-5, Jose P Lopez-Atalaya et al. DOI: 10.1038/s41467-020-16246-0
PubMed: 32444594

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The lysine acetyltransferases type 3 (KAT3) family members CBP and p300 are important transcriptional co-activators, but their specific functions in adult post-mitotic neurons remain unclear. Here, we show that the combined elimination of both proteins in forebrain excitatory neurons of adult mice resulted in a rapidly progressing neurological phenotype associated with severe ataxia, dendritic retraction and reduced electrical activity. At the molecular level, we observed the downregulation of neuronal genes, as well as decreased H3K27 acetylation and pro-neural transcription factor binding at the promoters and enhancers of canonical neuronal genes. The combined deletion of CBP and p300 in hippocampal neurons resulted in the rapid loss of neuronal molecular identity without de- or transdifferentiation. Restoring CBP expression or lysine acetylation rescued neuronal-specific transcription in cultured neurons. Together, these experiments show that KAT3 proteins maintain the excitatory neuron identity through the regulation of histone acetylation at cell type-specific promoter and enhancer regions.

Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain

Nature Communications (IF: 13.61), 2020-3, Ronald P Hart et al. DOI: 10.1038/s41467-020-15411-9
PubMed: 32221280

Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Microglia, the brain-resident macrophages, exhibit highly dynamic functions in neurodevelopment and neurodegeneration. Human microglia possess unique features as compared to mouse microglia, but our understanding of human microglial functions is largely limited by an inability to obtain human microglia under homeostatic states. Here, we develop a human pluripotent stem cell (hPSC)-based microglial chimeric mouse brain model by transplanting hPSC-derived primitive macrophage progenitors into neonatal mouse brains. Single-cell RNA-sequencing of the microglial chimeric mouse brains reveals that xenografted hPSC-derived microglia largely retain human microglial identity, as they exhibit signature gene expression patterns consistent with physiological human microglia and recapitulate heterogeneity of adult human microglia. Importantly, the engrafted hPSC-derived microglia exhibit dynamic response to cuprizone-induced demyelination and species-specific transcriptomic differences in the expression of neurological disease-risk genes in microglia. This model will serve as a tool to study the role of human microglia in brain development and degeneration.

Longitudinal assessment of tumor development using cancer avatars derived from genetically engineered pluripotent stem cells

Nature Communications (IF: 13.61), 2020-1, Gene W Yeo et al. DOI: 10.1038/s41467-020-14312-1

PubMed: 31992716

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

Many cellular models aimed at elucidating cancer biology do not recapitulate pathobiology including tumor heterogeneity, an inherent feature of cancer that underlies treatment resistance. Here we introduce a cancer modeling paradigm using genetically engineered human pluripotent stem cells (hiPSCs) that captures authentic cancer pathobiology. Orthotopic engraftment of the neural progenitor cells derived from hiPSCs that have been genome-edited to contain tumor-associated genetic driver mutations revealed by The Cancer Genome Atlas project for glioblastoma (GBM) results in formation of high-grade gliomas. Similar to patient-derived GBM, these models harbor inter-tumor heterogeneity resembling different GBM molecular subtypes, intra-tumor heterogeneity, and extrachromosomal DNA amplification. Re-enugraftment of these primary tumor neurospheres generates secondary tumors with features characteristic of patient samples and present mutation-dependent patterns of tumor evolution. These cancer avatar models provide a platform for comprehensive longitudinal assessment of human tumor development as governed by molecular subtype mutations and lineage-restricted differentiation.

Cell type-specific transcriptional programs in mouse prefrontal cortex during adolescence and addiction

Nature Communications (IF: 13.61), 2019-9, Luis M Tuesta et al. DOI: 10.1038/s41467-019-12054-3

PubMed: 31519873

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Coordinated activity-induced transcriptional changes across multiple neuron subtypes of the prefrontal cortex (PFC) play a pivotal role in encoding and regulating major cognitive behaviors. Yet, the specific transcriptional programs in each neuron subtype remain unknown. Using single-cell RNA sequencing (scRNA-seq), here we comprehensively classify all unique cell subtypes in the PFC. We analyze transcriptional dynamics of each cell subtype under a naturally adaptive and an induced condition. Adaptive changes during adolescence (between P21 and P60), a highly dynamic phase of postnatal neuroplasticity, profoundly impacted transcription in each neuron subtype, including cell type-specific regulation of genes implicated in major neuropsychiatric disorders. On the other hand, an induced plasticity evoked by chronic cocaine addiction resulted in progressive transcriptional changes in multiple neuron subtypes and became most pronounced upon prolonged drug withdrawal. Our findings lay a foundation for understanding cell type-specific postnatal transcriptional dynamics under normal PFC function and in neuropsychiatric disease states.

Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets

Nature Communications (IF: 13.61), 2019-11, Josef Spidlen et al. DOI: 10.1038/s41467-019-13055

PubMed: 31780669

Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Accurate and comprehensive extraction of information from high-dimensional single cell datasets necessitates faithful visualizations to assess biological populations. A state-of-the-art algorithm for non-linear dimension reduction, t-SNE, requires multiple heuristics and fails to produce clear representations of datasets when millions of cells are projected. We develop opt-SNE, an automated toolkit for t-SNE parameter selection that utilizes Kullback-Leibler divergence evaluation in real time to tailor the early exaggeration and overall number of gradient descent iterations in a dataset-specific manner. The precise calibration of early exaggeration together with opt-SNE adjustment of gradient descent learning rate dramatically improves computation time and enables high-quality visualization of large cytometry and transcriptomics datasets, overcoming limitations of analysis tools with hard-coded parameters that often produce poorly resolved or misleading maps of fluorescent and mass cytometry data. In summary, opt-SNE enables superior data resolution in t-SNE space and thereby more accurate data interpretation.

Single cell transcriptomics identifies stem cell-derived graft composition in a model of Parkinson's disease

Nature Communications (IF: 13.61), 2020-5, Thomas PerlmannMalin Parmar et al. DOI: 10.1038/s41467-020-16225-5 PubMed: 32415072

Single Cell Gene Expression, Rat, Neuroscience.

Abstract

Cell replacement is a long-standing and realistic goal for the treatment of Parkinson's disease (PD). Cells for transplantation can be obtained from fetal brain tissue or from stem cells. However, after transplantation, dopamine (DA) neurons are seen to be a minor component of grafts, and it has remained difficult to determine the identity of other cell types. Here, we report analysis by single-cell RNA sequencing (scRNA-seq) combined with comprehensive histological analyses to characterize intracerebral grafts from human embryonic stem cells (hESCs) and fetal tissue after functional maturation in a pre-clinical rat PD model. We show that neurons and astrocytes are major components in both fetal and stem cell-derived grafts. Additionally, we identify a cell type closely resembling a class of recently identified perivascular-like cells in stem cell-derived grafts. Thus, this study uncovers previously unknown cellular diversity in a clinically relevant cell replacement PD model.

An ultra high-throughput method for single-cell joint analysis of open chromatin and transcriptome

Nature Structural & Molecular Biology (IF: 12.26), 2019-11, Ming Hu et al. DOI: 10.1038/s41594-019-0323
PubMed: 31695190

Single Cell Gene Expression & Single Cell ATAC, Mouse, Method Comparison, Neuroscience.

Abstract

Simultaneous profiling of transcriptome and chromatin accessibility within single cells is a powerful approach to dissect gene regulatory programs in complex tissues. However, current tools are limited by modest throughput. We now describe an ultra high-throughput method, Paired-seq, for parallel analysis of transcriptome and accessible chromatin in millions of single cells. We demonstrate the utility of Paired-seq for analyzing the dynamic and cell-type-specific gene regulatory programs in complex tissues by applying it to mouse adult cerebral cortex and fetal forebrain. The joint profiles of a large number of single cells allowed us to deconvolute the transcriptome and open chromatin landscapes in the major cell types within these brain tissues, infer putative target genes of candidate enhancers, and reconstruct the trajectory of cellular lineages within the developing forebrain.

bigScale: an analytical framework for big-scale single-cell data

Genome research (IF: 12.01), 2018-6, Ivo Gut et al. DOI: 10.1101/gr.230771.117 PubMed: 29724792

Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Single-cell RNA sequencing (scRNA-seq) has significantly deepened our insights into complex tissues, with the latest techniques capable of processing tens of thousands of cells simultaneously. Analyzing increasing numbers of cells, however, generates extremely large data sets, extending processing time and challenging computing resources. Current scRNA-seq analysis tools are not designed to interrogate large data sets and often lack sensitivity to identify marker genes. With bigScale, we provide a scalable analytical framework to analyze millions of cells, which addresses the challenges associated with large data sets. To handle the noise and sparsity of scRNA-seq data, bigScale uses large sample sizes to estimate an accurate numerical model of noise. The framework further includes modules for differential expression analysis, cell clustering, and marker identification. A directed convolution strategy allows processing of extremely large data sets, while preserving transcript information from individual cells. We evaluated the performance of bigScale using both a biological model of aberrant gene expression in patient-derived neuronal progenitor cells and simulated data sets, which underlines the speed and accuracy in differential expression analysis. To test its applicability for large data sets, we applied bigScale to assess 1.3 million cells from the mouse developing forebrain. Its directed down-sampling strategy accumulates information from single cells into index cell transcriptomes, thereby defining cellular clusters with improved resolution. Accordingly, index cell clusters identified rare populations, such as reelin (-)-positive Cajal-Retzius neurons, for which we report previously unrecognized heterogeneity associated with distinct differentiation stages, spatial organization, and cellular function. Together, bigScale presents a solution to address future challenges of large single-cell data sets.

Agonist-induced functional analysis and cell sorting associated with single-cell transcriptomics characterizes cell subtypes in normal and pathological brain

Genome research (IF: 12.01), 2020-11, Barbara Bardoni et al. DOI: 10.1101/gr.262717.120 PubMed: 32973039

Single Cell Gene Expression, Mouse, Assay Method, Neuroscience.

Abstract

To gain better insight into the dynamic interaction between cells and their environment, we developed the agonist-induced functional analysis and cell sorting (aiFACS) technique, which allows the simultaneous recording and sorting of cells in real-time according to their immediate and individual response to a stimulus. By modulating the aiFACS selection parameters, testing different developmental times, using various stimuli, and multiplying the analysis of readouts, it is possible to analyze cell populations of any normal or pathological tissue. The association of aiFACS with single-cell transcriptomics allows the construction of functional tissue cartography based on specific pharmacological responses of cells. As a proof of concept, we used aiFACS on the dissociated mouse brain, a highly heterogeneous tissue, enriching it in interneurons by stimulation with KCl or with AMPA, an agonist of the glutamate receptors, followed by sorting based on calcium levels. After AMPA stimulus, single-cell transcriptomics of these aiFACS-selected interneurons resulted in a nine-cluster classification. Furthermore, we used aiFACS on interneurons derived from the brain of the-KO mouse, a rodent model of fragile X syndrome. We showed that these interneurons manifest a generalized defective response to AMPA compared with wild-type cells, affecting all the analyzed cell clusters at one specific postnatal developmental time.

Single-cell and spatial transcriptomics enables probabilistic inference of cell type topography

Communications Biology (IF: 11.2), 2020-10, José Fernández Navarro et al. DOI: 10.1038/s42003-020-01247

PubMed: 33037292

Single Cell Gene Expression & Spatial Gene Expression, Mouse, Computational Method.

Abstract

The field of spatial transcriptomics is rapidly expanding, and with it the repertoire of available technologies. However, several of the transcriptome-wide spatial assays do not operate on a single cell level, but rather produce data comprised of contributions from a - potentially heterogeneous - mixture of cells. Still, these techniques are attractive to use when examining complex tissue specimens with diverse cell populations, where complete expression profiles are required to properly capture their richness. Motivated by an interest to put gene expression into context and delineate the spatial arrangement of cell types within a tissue, we here present a model-based probabilistic method that uses single cell data to deconvolve the cell mixtures in spatial data. To illustrate the capacity of our method, we use data from different experimental platforms and spatially map cell types from the mouse brain and developmental heart, which arrange as expected.

Latent cellular analysis robustly reveals subtle diversity in large-scale single-cell RNA-seq data

Nucleic Acids Research (IF: 11.8), 2019-12, Wenan Chen et al. DOI: 10.1093/nar/gkz826 PubMed: 31566233

Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Single-cell RNA sequencing (scRNA-seq) is a powerful tool for characterizing the cell-to-cell variation and cellular dynamics in populations which appear homogeneous otherwise in basic and translational biological research. However, significant challenges arise in the analysis of scRNA-seq data, including the low signal-to-noise ratio with high data sparsity, potential batch effects, scalability problems when hundreds of thousands of cells are to be analyzed among others. The inherent complexities of scRNA-seq data and dynamic nature of cellular processes lead to suboptimal performance of many currently available algorithms, even for basic tasks such as identifying biologically meaningful heterogeneous subpopulations. In this study, we developed the Latent Cellular Analysis (LCA), a machine learning-based analytical pipeline that combines cosine-similarity measurement by latent cellular states with a graph-based clustering algorithm. LCA provides heuristic solutions for population number inference, dimension reduction, feature selection, and control of technical variations without explicit gene filtering. We show that LCA is robust, accurate, and powerful by comparison with multiple state-of-the-art computational methods when applied to large-scale real and simulated scRNA-seq data. Importantly, the ability of LCA to learn from representative subsets of the data provides scalability, thereby addressing a significant challenge posed by growing sample sizes in scRNA-seq data analysis.

Anti-human TREM2 induces microglia proliferation and reduces pathology in an Alzheimer's disease model

Journal of experimental medicine (IF: 11.32), 2020-9, Tina Schwabe et al. DOI: 10.1084/jem.20200785

PubMed: 32579671

Single Cell Gene Expression, Mouse, Genetic Health, Neuroscience.

Abstract

TREM2 is a receptor for lipids expressed in microglia. The R47H variant of human TREM2 impairs ligand binding and increases Alzheimer's disease (AD) risk. In mouse models of amyloid β (A β) accumulation, defective TREM2 function affects microglial response to A β plaques, exacerbating tissue damage, whereas TREM2 overexpression attenuates pathology. Thus, AD may benefit from TREM2 activation. Here, we examined the impact of an anti-human TREM2 agonistic mAb, AL002c, in a mouse AD model expressing either the common variant (CV) or the R47H variant of TREM2. Single-cell RNA-seq of microglia after acute systemic administration of AL002c showed induction of proliferation in both CV- and R47H-transgenic mice. Prolonged administration of AL002c reduced filamentous plaques and neurite dystrophy, impacted behavior, and tempered microglial inflammatory response. We further showed that a variant of AL002c is safe and well tolerated in a first-in-human phase I clinical trial and engages TREM2 based on cerebrospinal fluid biomarkers. We conclude that AL002 is a promising candidate for AD therapy.

Single-Cell Transcriptomic Analyses of the Developing Meninges Reveal Meningeal Fibroblast Diversity and Function

Developmental Cell (IF: 10.13), 2020-7, Kenneth L Jones et al. DOI: 10.1016/j.devcel.2020.06.009

PubMed: 32634398

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The meninges are a multilayered structure composed of fibroblasts, blood and lymphatic vessels, and immune cells. Meningeal fibroblasts secrete a variety of factors that control CNS development, yet strikingly little is known about their heterogeneity or development. Using single-cell sequencing, we report distinct transcriptional signatures for fibroblasts in the embryonic dura, arachnoid, and pia. We define new markers for meningeal layers and show conservation in human meninges. We find that embryonic meningeal fibroblasts are transcriptionally distinct between brain regions and identify a regionally localized pial subpopulation marked by the expression of μ -crystallin. Developmental analysis reveals a progressive, ventral-to-dorsal maturation of telencephalic meninges. Our studies have generated an unparalleled view of meningeal fibroblasts, providing molecular profiles of embryonic meningeal fibroblasts by layer and yielding insights into the mechanisms of meninges development and function.

Activation of group 2 innate lymphoid cells alleviates aging-associated cognitive decline

Journal of experimental medicine (IF: 11.32), 2020-4, Kristen L Zuloaga et al. DOI: 10.1084/jem.20190915

PubMed: 32022838

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Increasing evidence has challenged the traditional view about the immune privilege of the brain, but the precise roles of immune cells in regulating brain physiology and function remain poorly understood. Here, we report that tissue-resident group 2 innate lymphoid cells (ILC2) accumulate in the choroid plexus of aged brains. ILC2 in the aged brain are long-lived, are relatively resistant to cellular senescence and exhaustion, and are capable of switching between cell cycle dormancy and proliferation. They are functionally quiescent at homeostasis but can be activated by IL-33 to produce large amounts of type 2 cytokines and other effector molecules in vitro and in vivo. Intracerebroventricular transfer of activated ILC2 revitalized the aged brain and enhanced the cognitive function of aged mice. Administration of IL-5, a major ILC2 product, was sufficient to repress aging-associated neuroinflammation and alleviate aging-associated cognitive decline. Targeting ILC2 in the aged brain may provide new avenues to combat aging-associated neurodegenerative disorders.

Novel Alzheimer risk genes determine the microglia response to amyloid- β but not to TAU pathology

EMBO Molecular Medicine (IF: 10.37), 2020-3, Bart De Strooper et al. DOI: 10.15252/emmm.201910606

PubMed: 31951107

Single Cell Gene Expression, Mouse, Genetic Health, Neuroscience.

Abstract

Polygenic risk scores have identified that genetic variants without genome-wide significance still add to the genetic risk of developing Alzheimer's disease (AD). Whether and how subthreshold risk loci translate into relevant disease pathways is unknown. We investigate here the involvement of AD risk variants in the transcriptional responses of two mouse models: APPswe/PS1 and Thy-TAU22. A unique gene expression module, highly enriched for AD risk genes, is specifically responsive to A β but not TAU pathology. We identify in this module 7 established AD risk genes (APOE, CLU, INPP5D, CD33, PLCG2, SPI1, and FCER1G) and 11 AD GWAS genes below the genome-wide significance threshold (GPC2, TREML2, SYK, GRN, SLC2A5, SAMS1, PYDC1, HEXB, RRB1, LYN, and BLNK), that become significantly upregulated when exposed to A β . Single microglia sequencing confirms that A β , not TAU, pathology induces marked transcriptional changes in microglia, including increased proportions of activated microglia. We conclude that genetic risk of AD functionally translates into different microglia pathway responses to A β pathology, placing AD genetic risk downstream of the amyloid pathway but upstream of TAU pathology.

A Single-Cell Transcriptional Atlas of the Developing Murine Cerebellum

Current Biology (IF: 10.17), 2018-9, Paul A Northcott et al. DOI: 10.1016/j.cub.2018.07.062 PubMed: 30220501

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The cerebellum develops from a restricted number of cell types that precisely organize to form the circuitry that controls sensory-motor coordination and some higher-order cognitive processes. To acquire an enhanced understanding of the molecular processes that mediate cerebellar development, we performed single-cell RNA-sequencing of 39,245 murine cerebellar cells at twelve critical developmental time points. Using recognized lineage markers, we confirmed that the single-cell data accurately recapitulate cerebellar development. We then followed distinct populations from emergence through migration and differentiation, and determined the associated transcriptional cascades. After identifying key lineage commitment decisions, focused analyses uncovered waves of transcription factor expression at those branching points. Finally, we created Cell Seek, a flexible online interface that facilitates exploration of the dataset. Our study provides a transcriptional summarization of cerebellar development at single-cell resolution that will serve as a valuable resource for future investigations of cerebellar development, neurobiology, and disease.

A conserved dendritic-cell regulatory program limits antitumour immunity

Nature (IF: 46.49), 2020-4, Brian D Brown et al. DOI: 10.1038/s41586-020-2134 PubMed: 32269339

Single Cell Gene Expression, Human, Mouse, Immuno-oncology.

Abstract

Checkpoint blockade therapies have improved cancer treatment, but such immunotherapy regimens fail in a large subset of patients. Conventional type 1 dendritic cells (DC1s) control the response to checkpoint blockade in preclinical models and are associated with better overall survival in patients with cancer, reflecting the specialized ability of these cells to prime the responses of CD8T cells. Paradoxically, however, DC1s can be found in tumours that resist checkpoint blockade, suggesting that the functions of these cells may be altered in some lesions. Here, using single-cell RNA sequencing in human and mouse non-small-cell lung cancers, we identify a cluster of dendritic cells (DCs) that we name "mature DCs enriched in immunoregulatory molecules" (mregDCs), owing to their coexpression of immunoregulatory genes (Cd274, Pdcd1lg2 and Cd200) and maturation genes (Cd40, Ccr7 and Il12b). We find that the mregDC program is expressed by canonical DC1s and DC2s upon uptake of tumour antigens. We further find that upregulation of the programmed death ligand 1 protein-a key checkpoint molecule-in mregDCs is induced by the receptor tyrosine kinase AXL, while upregulation of interleukin (IL)-12 depends strictly on interferon- γ and is controlled negatively by IL-4 signalling. Blocking IL-4 enhances IL-12 production by tumour-antigen-bearing mregDC1s, expands the pool of tumour-infiltrating effector T cells and reduces tumour burden. We have therefore uncovered a regulatory module associated with tumour-antigen uptake that reduces DC1 functionality in human and mouse cancers.

Host-Viral Infection Maps Reveal Signatures of Severe COVID-19 Patients

Cell (IF: 38.62), 2020-6, Zheng Zhang et al. DOI: 10.1016/j.cell.2020.05.006 PubMed: 32479746

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Computational Method, Infectious Disease.

Abstract

Viruses are a constant threat to global health as highlighted by the current COVID-19 pandemic. Currently, lack of data underlying how the human host interacts with viruses, including the SARS-CoV-2 virus, limits effective therapeutic intervention. We introduce Viral-Track, a computational method that globally scans unmapped single-cell RNA sequencing (scRNA-seq) data for the presence of viral RNA, enabling transcriptional cell sorting of infected versus bystander cells. We demonstrate the sensitivity and specificity of Viral-Track to systematically detect viruses from multiple models of infection, including hepatitis B virus, in an unsupervised manner. Applying Viral-Track to bronchoalveolar-lavage samples from severe and mild COVID-19 patients reveals a dramatic impact of the virus on the immune system of severe patients compared to mild cases. Viral-Track detects an unexpected co-infection of the human metapneumovirus, present mainly in monocytes perturbed in type-I interferon (IFN)-signaling. Viral-Track provides a robust technology for dissecting the mechanisms of viral-infection and pathology.

Regenerative Metaplastic Clones in COPD Lung Drive Inflammation and Fibrosis

Cell (IF: 38.62), 2020-5, Frank D McKeon et al. DOI: 10.1016/j.cell.2020.03.047 PubMed: 32298651

Single Cell Gene Expression, Human, Immunology.

Abstract

Chronic obstructive pulmonary disease (COPD) is a progressive condition of chronic bronchitis, small airway obstruction, and emphysema that represents a leading cause of death worldwide. While inflammation, fibrosis, mucus hypersecretion, and metaplastic epithelial lesions are hallmarks of this disease, their origins and dependent relationships remain unclear. Here we apply single-cell cloning technologies to lung tissue of patients with and without COPD. Unlike control lungs, which were dominated by normal distal airway progenitor cells, COPD lungs were inundated by three variant progenitors epigenetically committed to distinct metaplastic lesions. When transplanted to immunodeficient mice, these variant clones induced pathology akin to the mucous and squamous metaplasia, neutrophilic inflammation, and fibrosis seen in COPD. Remarkably, similar variants pre-exist as minor constituents of control and fetal lung and conceivably act in normal processes of immune surveillance. However, these same variants likely catalyze the pathologic and progressive features of COPD when expanded to high numbers.

Regenerative lineages and immune-mediated pruning in lung cancer metastasis

Nature Medicine (IF: 36.23), 2020-2, Dana Pe'er et al. DOI: 10.1038/s41591-019-0750-6 PubMed: 32042191

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Developmental processes underlying normal tissue regeneration have been implicated in cancer, but the degree of their enactment during tumor progression and under the selective pressures of immune surveillance, remain unknown. Here we show that human primary lung adenocarcinomas are characterized by the emergence of regenerative cell types, typically seen in response to lung injury, and by striking infidelity among transcription factors specifying most alveolar and bronchial epithelial lineages. In contrast, metastases are enriched for key endoderm and lung-specifying transcription factors, SOX2 and SOX9, and recapitulate more primitive transcriptional programs spanning stem-like to regenerative pulmonary epithelial progenitor states. This developmental continuum mirrors the progressive stages of spontaneous outbreak from metastatic dormancy in a mouse model and exhibits SOX9-dependent resistance to natural killer cells. Loss of developmental stage-specific constraint in macrometastases triggered by natural killer cell depletion suggests a dynamic interplay between developmental plasticity and immune-mediated pruning during metastasis.

A cellular census of human lungs identifies novel cell states in health and in asthma

Nature Medicine (IF: 36.23), 2019-6, Martijn C Nawijn Sarah A Teichmann DOI: 10.1038/s41591-019-0468-5 PubMed: 31209336

Single Cell Gene Expression, Human, Cell Atlas, Immunology.

Abstract

Human lungs enable efficient gas exchange and form an interface with the environment, which depends on mucosal immunity for protection against infectious agents. Tightly controlled interactions between structural and immune cells are required to maintain lung homeostasis. Here, we use single-cell transcriptomics to chart the cellular landscape of upper and lower airways and lung parenchyma in healthy lungs, and lower airways in asthmatic lungs. We report location-dependent airway epithelial cell states and a novel subset of tissue-resident memory T cells. In the lower airways of patients with asthma, mucous cell hyperplasia is shown to stem from a novel mucous ciliated cell state, as well as goblet cell hyperplasia. We report the presence of pathogenic effector type 2 helper T cells (T2) in asthmatic lungs and find evidence for type 2 cytokines in maintaining the altered epithelial cell states. Unbiased analysis of cell-cell interactions identifies a shift from airway structural cell communication in healthy lungs to a T2-dominated interactome in asthmatic lungs.

An Integrated Gene Expression Landscape Profiling

Approach to Identify Lung Tumor Endothelial Cell

Heterogeneity and Angiogenic Candidates

Cancer Cell (IF: 30.24), 2020-1, Yonglun Luo et al. DOI: 10.1016/j.ccell.2019.12.001 PubMed: 31935371

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Heterogeneity of lung tumor endothelial cell (TEC) phenotypes across patients, species (human/mouse), and models (in vivo/in vitro) remains poorly inventoried at the single-cell level. We single-cell RNA (scRNA)-sequenced 56,771 endothelial cells from human/mouse (peri)-tumoral lung and cultured human lung TECs, and detected 17 known and 16 previously unrecognized phenotypes, including TECs putatively regulating immune surveillance. We resolved the canonical tip TECs into a known migratory tip and a putative basement-membrane remodeling breach phenotype. Tip TEC signatures correlated with patient survival, and tip/breach TECs were most sensitive to vascular endothelial growth factor blockade. Only tip TECs were congruent across species models and shared conserved markers. Integrated analysis of the scRNA-sequenced data with orthogonal multi-omics and meta-analysis data across different human tumors, validated by functional analysis, identified collagen modification as a candidate angiogenic pathway.

Single-cell transcriptome and antigen-immunoglobulin analysis reveals the diversity of B cells in non-small cell lung cancer

Genome Biology (IF: 19.04), 2020-6, Gening Jiang et al. DOI: 10.1186/s13059-020-02064-6 PubMed: 32580738
Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Malignant transformation and progression of cancer are driven by the co-evolution of cancer cells and their dysregulated tumor microenvironment (TME). Recent studies on immunotherapy demonstrate the efficacy in reverting the anti-tumoral function of T cells, highlighting the therapeutic potential in targeting certain cell types in TME. However, the functions of other immune cell types remain largely unexplored. We conduct a single-cell RNA-seq analysis of cells isolated from tumor tissue samples of non-small cell lung cancer (NSCLC) patients, and identify subtypes of tumor-infiltrated B cells and their diverse functions in the progression of NSCLC. Flow cytometry and immunohistochemistry experiments on two independent cohorts confirm the co-existence of the two major subtypes of B cells, namely the naïve-like and plasma-like B cells. The naïve-like B cells are decreased in advanced NSCLC, and their lower level is associated with poor prognosis. Co-culture of isolated naïve-like B cells from NSCLC patients with two lung cancer cell lines demonstrate that the naïve-like B cells suppress the growth of lung cancer cells by secreting four factors negatively regulating the cell growth. We also demonstrate that the plasma-like B cells inhibit cancer cell growth in the early stage of NSCLC, but promote cell growth in the advanced stage of NSCLC. The roles of the plasma-like B cell produced immunoglobulins, and their interacting proteins in the progression of NSCLC are further validated by proteomics data. Our analysis reveals versatile functions of tumor-infiltrating B cells and their potential clinical implications in NSCLC.

Single-cell connectomic analysis of adult mammalian lungs

Science Advances (IF: 14.09), 2019-12, Naftali Kaminski et al. DOI: 10.1126/sciadv.aaw3851 PubMed: 31840053
Single Cell Gene Expression, Rat, Other - Mammal, Cell Atlas.

Abstract

Efforts to decipher chronic lung disease and to reconstitute functional lung tissue through regenerative medicine have been hampered by an incomplete understanding of cell-cell interactions governing tissue homeostasis. Because the structure of mammalian lungs is highly conserved at the histologic level, we hypothesized that there are evolutionarily conserved homeostatic mechanisms that keep the fine architecture of the lung in balance. We have leveraged single-cell RNA sequencing techniques to identify conserved patterns of cell-cell cross-talk in adult mammalian lungs, analyzing mouse, rat, pig, and human pulmonary tissues. Specific stereotyped functional roles for each cell type in the distal lung are observed, with alveolar type I cells having a major role in the regulation of tissue homeostasis. This paper provides a systems-level portrait of signaling between alveolar cell populations. These methods may be applicable to other organs, providing a roadmap for identifying key pathways governing pathophysiology and informing regenerative efforts.

scRNA-seq assessment of the human lung, spleen, and esophagus tissue stability after cold preservation

Genome Biology (IF: 19.04), 2019-12, O Stegle et al. DOI: 10.1186/s13059-019-1906 PubMed: 31892341

Single Cell Gene Expression, Human, Assay Method.

Abstract

The Human Cell Atlas is a large international collaborative effort to map all cell types of the human body. Single-cell RNA sequencing can generate high-quality data for the delivery of such an atlas. However, delays between fresh sample collection and processing may lead to poor data and difficulties in experimental design. This study assesses the effect of cold storage on fresh healthy spleen, esophagus, and lung from ≥ 5 donors over 72 h. We collect 240,000 high-quality single-cell transcriptomes with detailed cell type annotations and whole genome sequences of donors, enabling future eQTL studies. Our data provide a valuable resource for the study of these 3 organs and will allow cross-organ comparison of cell types. We see little effect of cold ischemic time on cell yield, total number of reads per cell, and other quality control metrics in any of the tissues within the first 24 h. However, we observe a decrease in the proportions of lung T cells at 72 h, higher percentage of mitochondrial reads, and increased contamination by background ambient RNA reads in the 72-h samples in the spleen, which is cell type specific. In conclusion, we present robust protocols for tissue preservation for up to 24 h prior to scRNA-seq analysis. This greatly facilitates the logistics of sample collection for Human Cell Atlas or clinical studies since it increases the time frames for sample processing.

Single-Cell Reconstruction of Human Basal Cell Diversity in Normal and Idiopathic Pulmonary Fibrosis Lungs

American Journal of Respiratory and Critical Care Medicine (IF: 15.3), 2020-12, Jonathan L McQualter et al. DOI: 10.1164/rccm.201904-0792OC PubMed: 32692579

Single Cell Gene Expression, Human, Cell Atlas.

Abstract

Declining lung function in patients with interstitial lung disease is accompanied by epithelial remodeling and progressive scarring of the gas-exchange region. There is a need to better understand the contribution of basal cell hyperplasia and associated mucosecretory dysfunction to the development of idiopathic pulmonary fibrosis (IPF). We sought to decipher the transcriptome of freshly isolated epithelial cells from normal and IPF lungs to discern disease-dependent changes within basal stem cells. Single-cell RNA sequencing was used to map epithelial cell types of the normal and IPF human airways. Organoid and air-liquid interface cultures were used to investigate functional properties of basal cell subtypes. We found that basal cells included multipotent and secretory primed subsets in control adult lung tissue. Secretory primed basal cells include an overlapping molecular signature with basal cells obtained from the distal lung tissue of IPF lungs. We confirmed that NOTCH2 maintains undifferentiated basal cells and restricts basal-to-ciliated differentiation, and we present evidence that NOTCH3 functions to restrain secretory differentiation. Basal cells are dynamically regulated in disease and are specifically biased toward the expansion of the secretory primed basal cell subset in IPF. Modulation of basal cell plasticity may represent a relevant target for therapeutic intervention in IPF.

Expression of the SARS-CoV-2 ACE2 Receptor in the Human Airway Epithelium

American Journal of Respiratory and Critical Care Medicine (IF: 15.3), 2020-7, Yael Strulovici-Barel et al. DOI: 10.1164/rccm.202003-0541OC PubMed: 32432483

Single Cell Gene Expression, Human, Infectious Disease.

Abstract

Infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease (COVID-19), a predominantly respiratory illness. The first step in SARS-CoV-2 infection is binding of the virus to (angiotensin-converting enzyme 2) on the airway epithelium. The objective was to gain insight into the expression of the human airway epithelium. Airway epithelia sampled by fiberoptic bronchoscopy of trachea, large airway epithelia (LAE), and small airway epithelia (SAE) of nonsmokers and smokers were analyzed for expression of and other coronavirus infection-related genes using microarray, RNA sequencing, and 10x single-cell transcriptome analysis, with associated examination of related microRNA. It is expressed similarly in the trachea and LAE, with lower expression in the SAE; in the SAE, it is expressed in basal, intermediate, club, mucus, and ciliated cells; it is upregulated in the SAE by smoking, significantly in men; levels of miR-1246 expression could play a role in upregulation in the SAE of smokers; and it is expressed in airway epithelium differentiated on air-liquid interface cultures from primary airway basal stem/progenitor cells; this can be replicated using LAE and SAE immortalized basal cell lines derived from healthy nonsmokers. The gene encoding the receptor for SARS-CoV-2, is expressed in the human airway epithelium, with variations in expression relevant to the biology of initial steps in SARS-CoV-2 infection.

Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis

Science Advances (IF: 14.09), 2020-7, Ivan O Rosas et al. DOI: 10.1126/sciadv.aba1983

PubMed: 32937511

Single Cell Gene Expression, Human, Cell Atlas.

Abstract

We provide a single-cell atlas of idiopathic pulmonary fibrosis (IPF), a fatal interstitial lung disease, by profiling 312,928 cells from 32 IPF, 28 smoker and nonsmoker controls, and 18 chronic obstructive pulmonary disease (COPD) lungs. Among epithelial cells enriched in IPF, we identify a previously unidentified population of aberrant basaloid cells that coexpress basal epithelial, mesenchymal, senescence, and developmental markers and are located at the edge of myofibroblast foci in the IPF lung. Among vascular endothelial cells, we identify an ectopically expanded cell population transcriptomically identical to bronchial restricted vascular endothelial cells in IPF. We confirm the presence of both populations by immunohistochemistry and independent datasets. Among stromal cells, we identify IPF myofibroblasts and invasive fibroblasts with partially overlapping cells in control and COPD lungs. Last, we confirm previous findings of profibrotic macrophage populations in the IPF lung. Our comprehensive catalog reveals the complexity and diversity of aberrant cellular populations in IPF.

Single-Cell Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary Fibrosis

American Journal of Respiratory and Critical Care Medicine (IF: 15.3), 2019-6, G R Scott Budinger Alexander V Misharin et al. DOI: 10.1164/rccm.201712-2410OC PubMed: 30554520

Single Cell Gene Expression, Human, Cell Atlas.

Abstract

The contributions of diverse cell populations in the human lung to pulmonary fibrosis pathogenesis are poorly understood. Single-cell RNA sequencing can reveal changes within individual cell populations during pulmonary fibrosis that are important for disease pathogenesis. To determine whether single-cell RNA sequencing can reveal disease-related heterogeneity within alveolar macrophages, epithelial cells, or other cell types in lung tissue from subjects with pulmonary fibrosis compared with control subjects. We performed single-cell RNA sequencing on lung tissue obtained from eight transplant donors and eight recipients with pulmonary fibrosis and on one bronchoscopic cryobiospy sample from a patient with idiopathic pulmonary fibrosis. We validated these data using RNA hybridization, immunohistochemistry, and bulk RNA-sequencing on flow-sorted cells from 22 additional subjects. We identified a distinct, novel population of profibrotic alveolar macrophages exclusively in patients with fibrosis. Within epithelial cells, the expression of genes involved in Wnt secretion and response was restricted to nonoverlapping cells. We identified rare cell populations including airway stem cells and senescent cells emerging during pulmonary fibrosis. We developed a web-based tool to explore these data. We generated a single-cell atlas of pulmonary fibrosis. Using this atlas, we demonstrated heterogeneity within alveolar macrophages and epithelial cells from subjects with pulmonary fibrosis. These results support the feasibility of discovery-based approaches using next-generation sequencing technologies to identify signaling pathways for targeting in the development of personalized therapies for patients with pulmonary fibrosis.

Metastatic-niche labelling reveals parenchymal cells with stem features

Nature (IF: 46.49), 2019-8, Joo-Hyeon Lee et al. DOI: 10.1038/s41586-019-1487-6 PubMed: 31462798

Single Cell Gene Expression, Mouse, Assay Method, Cancer Research.

Abstract

Direct investigation of the early cellular changes induced by metastatic cells within the surrounding tissue remains a challenge. Here we present a system in which metastatic cancer cells release a cell-penetrating fluorescent protein, which is taken up by neighbouring cells and enables spatial identification of the local metastatic cellular environment. Using this system, tissue cells with low representation in the metastatic niche can be identified and characterized within the bulk tissue. To highlight its potential, we applied this strategy to study the cellular environment of metastatic breast cancer cells in the lung. We report the presence of cancer-associated parenchymal cells, which exhibit stem-cell-like features, expression of lung progenitor markers, multi-lineage differentiation potential and self-renewal activity. In ex vivo assays, lung epithelial cells acquire a cancer-associated parenchymal-cell-like phenotype when co-cultured with cancer cells and support their growth. These results highlight the potential of this method as a platform for new discoveries.

Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis

Nature Communications (IF: 13.61), 2020-4, Paul J Wolters et al. DOI: 10.1038/s41467-020-15647-5

PubMed: 32317643

Single Cell Gene Expression, Human, Mouse, Cell Atlas.

Abstract

Collagen-producing cells maintain the complex architecture of the lung and drive pathologic scarring in pulmonary fibrosis. Here we perform single-cell RNA-sequencing to identify all collagen-producing cells in normal and fibrotic lungs. We characterize multiple collagen-producing subpopulations with distinct anatomical localizations in different compartments of murine lungs. One subpopulation, characterized by expression of *Cthrc1* (collagen triple helix repeat containing 1), emerges in fibrotic lungs and expresses the highest levels of collagens. Single-cell RNA-sequencing of human lungs, including those from idiopathic pulmonary fibrosis and scleroderma patients, demonstrate similar heterogeneity and *CTHRC1*-expressing fibroblasts present uniquely in fibrotic lungs. Immunostaining and in situ hybridization show that these cells are concentrated within fibroblastic foci. We purify collagen-producing subpopulations and find disease-relevant phenotypes of *Cthrc1*-expressing fibroblasts in in vitro and adoptive transfer experiments. Our atlas of collagen-producing cells provides a roadmap for studying the roles of these unique populations in homeostasis and pathologic fibrosis.

Expansion of hedgehog disrupts mesenchymal identity and induces emphysema phenotype

Journal of clinical investigation (IF: 12.81), 2018-10, Harold A Chapman et al. DOI: 10.1172/JCI99435

PubMed: 29999500

Single Cell Gene Expression, Human & Mouse, Developmental Biology.

Abstract

GWAS have repeatedly mapped susceptibility loci for emphysema to genes that modify hedgehog signaling, but the functional relevance of hedgehog signaling to this morbid disease remains unclear. In the current study, we identified a broad population of mesenchymal cells in the adult murine lung receptive to hedgehog signaling, characterized by higher activation of hedgehog surrounding the proximal airway relative to the distal alveoli. Single-cell RNA-sequencing showed that the hedgehog-receptive mesenchyme is composed of mostly fibroblasts with distinct proximal and distal subsets with discrete identities. Ectopic hedgehog activation in the distal fibroblasts promoted expression of proximal fibroblast markers and loss of distal alveoli and airspace enlargement of over 20% compared with controls. We found that hedgehog suppressed mesenchymal-derived mitogens enriched in distal fibroblasts that regulate alveolar stem cell regeneration and airspace size. Finally, single-cell analysis of the human lung mesenchyme showed that segregated proximal-distal identity with preferential hedgehog activation in the proximal fibroblasts was conserved between mice and humans. In conclusion, we showed that differential hedgehog activation segregates mesenchymal identities of distinct fibroblast subsets and that disruption of fibroblast identity can alter the alveolar stem cell niche, leading to emphysematous changes in the murine lung.

Single-cell analysis reveals fibroblast heterogeneity and myofibroblasts in systemic sclerosis-associated interstitial lung disease

Annals of the Rheumatic Diseases (IF: 13.49), 2019-10, Mauricio Rojas et al. DOI: 10.1136/annrheumdis-2018-214865 PubMed: 31405848

Single Cell Gene Expression, Human, Cell Atlas, Immunology.

Abstract

Myofibroblasts are key effector cells in the extracellular matrix remodelling of systemic sclerosis-associated interstitial lung disease (SSc-ILD); however, the diversity of fibroblast populations present in the healthy and SSc-ILD lung is unknown and has prevented the specific study of the myofibroblast transcriptome. We sought to identify and define the transcriptomes of myofibroblasts and other mesenchymal cell populations in human healthy and SSc-ILD lungs to understand how alterations in fibroblast phenotypes lead to SSc-ILD fibrosis. We performed droplet-based, single-cell RNA-sequencing with integrated canonical correlation analysis of 13 explanted lung tissue specimens (56 196 cells) from four healthy control and four patients with SSc-ILD, with findings confirmed by cellular indexing of transcriptomes and epitopes by sequencing in additional samples. Examination of gene expression in mesenchymal cells identified two major, SPINT2 and MFAP5, and one minor, WIF1, fibroblast populations in the healthy control lung. Combined analysis of control and SSc-ILD mesenchymal cells identified SPINT2, MFAP5, few WIF1 fibroblasts and a new large myofibroblast population with evidence of actively proliferating myofibroblasts. We compared differential gene expression between all SSc-ILD and control mesenchymal cell populations, as well as among the fibroblast subpopulations, showing that myofibroblasts undergo the greatest phenotypic changes in SSc-ILD and strongly upregulate expression of collagens and other profibrotic genes. Our results demonstrate previously unrecognised fibroblast heterogeneity in SSc-ILD and healthy lungs, and define multimodal transcriptome-phenotypes associated with these populations. Our data indicate that myofibroblast differentiation and proliferation are key pathological mechanisms driving fibrosis in SSc-ILD.

In Vitro and In Vivo Development of the Human Airway at Single-Cell Resolution

Developmental Cell (IF: 10.13), 2020-4, J Gray Camp et al. DOI: 10.1016/j.devcel.2020.01.033 PubMed: 32109386
Single Cell Gene Expression, Human, Cell Atlas, Developmental Biology.

Abstract

Bud tip progenitor cells give rise to all murine lung epithelial lineages and have been described in the developing human lung; however, the mechanisms controlling human bud tip differentiation into specific lineages are unclear. Here, we used homogeneous human bud tip organoid cultures and identified SMAD signaling as a key regulator of the bud tip-to-airway transition. SMAD induction led to the differentiation of airway-like organoids possessing functional basal cells capable of clonal expansion and multilineage differentiation. To benchmark in vitro-derived organoids, we developed a single-cell mRNA sequencing atlas of the human lung from 11.5 to 21 weeks of development, which revealed high degrees of similarity between the in vitro-derived and in vivo airway. Together, this work sheds light on human airway differentiation in vitro and provides a single-cell atlas of the developing human lung.

Dynamics of human monocytes and airway macrophages during healthy aging and after transplant

Journal of experimental medicine (IF: 11.32), 2020-3, Toby M Maher et al. DOI: 10.1084/jem.20191236
PubMed: 31917836

Single Cell Gene Expression, Human, Immunology.

Abstract

The ontogeny of airway macrophages (AMs) in human lung and their contribution to disease are poorly mapped out. In mice, aging is associated with an increasing proportion of peripherally, as opposed to perinatally derived AMs. We sought to understand AM ontogeny in human lung during healthy aging and after transplant. We characterized monocyte/macrophage populations from the peripheral blood and airways of healthy volunteers across infancy/childhood (2-12 yr), maturity (20-50 yr), and older adulthood (>50 yr). Single-cell RNA sequencing (scRNA-seq) was performed on airway inflammatory cells isolated from sex-mismatched lung transplant recipients. During healthy aging, the proportions of blood bronchoalveolar lavage (BAL) classical monocytes peak in adulthood and decline in older adults. scRNA-seq of BAL cells from lung transplant recipients indicates that after transplant, the majority of AMs are recipient derived. These data show that during aging, the peripheral monocyte phenotype is consistent with that found in the airways and, furthermore, that the majority of human AMs after transplant are derived from circulating monocytes.

The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation

Nature (IF: 46.49), 2017-9, Aviv Regev et al. DOI: 10.1038/nature24029 PubMed: 28902842

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Type 2 innate lymphoid cells (ILC2s) both contribute to mucosal homeostasis and initiate pathologic inflammation in allergic asthma. However, the signals that direct ILC2s to promote homeostasis versus inflammation are unclear. To identify such molecular cues, we profiled mouse lung-resident ILCs using single-cell RNA sequencing at steady state and after in vivo stimulation with the alarmin cytokines IL-25 and IL-33. ILC2s were transcriptionally heterogeneous after activation, with subpopulations distinguished by expression of proliferative, homeostatic and effector genes. The neuropeptide receptor *Nmur1* was preferentially expressed by ILC2s at steady state and after IL-25 stimulation. Neuromedin U (NMU), the ligand of NMUR1, activated ILC2s in vitro, and in vivo co-administration of NMU with IL-25 strongly amplified allergic inflammation. Loss of NMU-NMUR1 signalling reduced ILC2 frequency and effector function, and altered transcriptional programs following allergen challenge in vivo. Thus, NMUR1 signalling promotes inflammatory ILC2 responses, highlighting the importance of neuro-immune crosstalk in allergic inflammation at mucosal surfaces.

Chromatin Potential Identified by Shared Single-Cell Profiling of RNA and Chromatin

Cell (IF: 38.62), 2020-11, Aviv Regev et al. DOI: 10.1016/j.cell.2020.09.056 PubMed: 33098772

Single Cell Gene Expression & Single Cell ATAC, Mouse, Method Comparison, Single Cell Multiomics Method.

Abstract

Cell differentiation and function are regulated across multiple layers of gene regulation, including modulation of gene expression by changes in chromatin accessibility. However, differentiation is an asynchronous process precluding a temporal understanding of regulatory events leading to cell fate commitment. Here we developed simultaneous high-throughput ATAC and RNA expression with sequencing (SHARE-seq), a highly scalable approach for measurement of chromatin accessibility and gene expression in the same single cell, applicable to different tissues. Using 34,774 joint profiles from mouse skin, we develop a computational strategy to identify cis-regulatory interactions and define domains of regulatory chromatin (DORCs) that significantly overlap with super-enhancers. During lineage commitment, chromatin accessibility at DORCs precedes gene expression, suggesting that changes in chromatin accessibility may prime cells for lineage commitment. We computationally infer chromatin potential as a quantitative measure of chromatin lineage-priming and use it to predict cell fate outcomes. SHARE-seq is an extensible platform to study regulatory circuitry across diverse cells in tissues.

Co-option of Neutrophil Fates by Tissue Environments

Cell (IF: 38.62), 2020-11, Renato Ostuni et al. DOI: 10.1016/j.cell.2020.10.003 PubMed: 33098771

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Classically considered short-lived and purely defensive leukocytes, neutrophils are unique in their fast and moldable response to stimulation. This plastic behavior may underlie variable and even antagonistic functions during inflammation or cancer, yet the full spectrum of neutrophil properties as they enter healthy tissues remains unexplored. Using a new model to track neutrophil fates, we found short but variable lifetimes across multiple tissues. Through analysis of the receptor, transcriptional, and chromatin accessibility landscapes, we identify varying neutrophil states and assign non-canonical functions, including vascular repair and hematopoietic homeostasis. Accordingly, depletion of neutrophils compromised angiogenesis during early age, genotoxic injury, and viral infection, and impaired hematopoietic recovery after irradiation. Neutrophils acquired these properties in target tissues, a process that, in the lungs, occurred in CXCL12-rich areas and relied on CXCR4. Our results reveal that tissues co-opt neutrophils en route for elimination to induce programs that support their physiological demands.

Progressive Pulmonary Fibrosis Is Caused by Elevated Mechanical Tension on Alveolar Stem Cells

Cell (IF: 38.62), 2020-1, Jingyu Chen et al. DOI: 10.1016/j.cell.2019.11.027 PubMed: 31866069

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Fibrosis can develop in most organs and causes organ failure. The most common type of lung fibrosis is known as idiopathic pulmonary fibrosis, in which fibrosis starts at the lung periphery and then progresses toward the lung center, eventually causing respiratory failure. Little is known about the mechanisms underlying the pathogenesis and periphery-to-center progression of the disease. Here we discovered that loss of Cdc42 function in alveolar stem cells (AT2 cells) causes periphery-to-center progressive lung fibrosis. We further show that Cdc42-null AT2 cells in both post-pneumonectomy and untreated aged mice cannot regenerate new alveoli, resulting in sustained exposure of AT2 cells to elevated mechanical tension. We demonstrate that elevated mechanical tension activates a TGF- β signaling loop in AT2 cells, which drives the periphery-to-center progression of lung fibrosis. Our study establishes a direct mechanistic link between impaired alveolar regeneration, mechanical tension, and progressive lung fibrosis.

BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis

Cell (IF: 38.62), 2018-1, Luis B Barreiro et al. DOI: 10.1016/j.cell.2017.12.031 PubMed: 29328912

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

The dogma that adaptive immunity is the only arm of the immune response with memory capacity has been recently challenged by several studies demonstrating evidence for memory-like innate immune training. However, the underlying mechanisms and location for generating such innate memory responses in vivo remain unknown. Here, we show that access of Bacillus Calmette-Guérin (BCG) to the bone marrow (BM) changes the transcriptional landscape of hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs), leading to local cell expansion and enhanced myelopoiesis at the expense of lymphopoiesis. Importantly, BCG-educated HSCs generate epigenetically modified macrophages that provide significantly better protection against virulent *M. tuberculosis* infection than naïve macrophages. By using parabiotic and chimeric mice, as well as adoptive transfer approaches, we demonstrate that training of the monocyte/macrophage lineage via BCG-induced HSC reprogramming is sustainable in vivo. Our results indicate that targeting the HSC compartment provides a novel approach for vaccine development.

Distinct Mesenchymal Lineages and Niches Promote Epithelial Self-Renewal and Myofibrogenesis in the Lung

Cell (IF: 38.62), 2017-9, Michael P Morley et al. DOI: 10.1016/j.cell.2017.07.034 PubMed: 28886382

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The lung is an architecturally complex organ comprising a heterogeneous mixture of various epithelial and mesenchymal lineages. We use single-cell RNA sequencing and signaling lineage reporters to generate a spatial and transcriptional map of the lung mesenchyme. We find that each mesenchymal lineage has a distinct spatial address and transcriptional profile leading to unique niche regulatory functions. The mesenchymal alveolar niche cell is Wnt responsive, expresses *Pdgfr α* , and is critical for alveolar epithelial cell growth and self-renewal. In contrast, the Axin2+ myofibrogenic progenitor cell preferentially generates pathologically deleterious myofibroblasts after injury. Analysis of the secretome and receptome of the alveolar niche reveals functional pathways that mediate growth and self-renewal of alveolar type 2 progenitor cells, including IL-6/Stat3, Bmp, and Fgf signaling. These studies define the cellular and molecular framework of lung mesenchymal niches and reveal the functional importance of developmental pathways in promoting self-renewal versus a pathological response to tissue injury.

Emergence of a High-Plasticity Cell State during Lung Cancer Evolution

Cancer Cell (IF: 30.24), 2020-8, Aviv Regev et al. DOI: 10.1016/j.ccell.2020.06.012 PubMed: 32707077

Single Cell Gene Expression & Single Cell ATAC, Mouse, Cancer Research.

Abstract

Tumor evolution from a single cell into a malignant, heterogeneous tissue remains poorly understood. Here, we profile single-cell transcriptomes of genetically engineered mouse lung tumors at seven stages, from pre-neoplastic hyperplasia to adenocarcinoma. The diversity of transcriptional states increases over time and is reproducible across tumors and mice. Cancer cells progressively adopt alternate lineage identities, computationally predicted to be mediated through a common transitional, high-plasticity cell state (HPCS). Accordingly, HPCS cells prospectively isolated from mouse tumors and human patient-derived xenografts display high capacity for differentiation and proliferation. The HPCS program is associated with poor survival across human cancers and demonstrates chemoresistance in mice. Our study reveals a central principle underpinning intra-tumoral heterogeneity and motivates therapeutic targeting of the HPCS.

CDK7 Inhibition Potentiates Genome Instability Triggering Anti-tumor Immunity in Small Cell Lung Cancer

Cancer Cell (IF: 30.24), 2020-1, Nathanael S Gray et al. DOI: 10.1016/j.ccell.2019.11.003 PubMed: 31883968

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

Cyclin-dependent kinase 7 (CDK7) is a central regulator of the cell cycle and gene transcription. However, little is known about its impact on genomic instability and cancer immunity. Using a selective CDK7 inhibitor, YKL-5-124, we demonstrated that CDK7 inhibition predominately disrupts cell-cycle progression and induces DNA replication stress and genome instability in small cell lung cancer (SCLC) while simultaneously triggering immune-response signaling. These tumor-intrinsic events provoke a robust immune surveillance program elicited by T cells, which is further enhanced by the addition of immune-checkpoint blockade. Combining YKL-5-124 with anti-PD-1 offers significant survival benefit in multiple highly aggressive murine models of SCLC, providing a rationale for new combination regimens consisting of CDK7 inhibitors and immunotherapies.

In Vivo Epigenetic CRISPR Screen Identifies Asf1a as an Immunotherapeutic Target in Kras-Mutant Lung Adenocarcinoma

Cancer Discovery (IF: 28.3), 2020-2, George Miller et al. DOI: 10.1158/2159-8290.CD-19-0780

PubMed: 31744829

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

Despite substantial progress in lung cancer immunotherapy, the overall response rate in patients with mutant lung adenocarcinoma (LUAD) remains low. Combining standard immunotherapy with adjuvant approaches that enhance adaptive immune responses—such as epigenetic modulation of antitumor immunity—is therefore an attractive strategy. To identify epigenetic regulators of tumor immunity, we constructed an epigenetic-focused single guide RNA library and performed anCRISPR screen in a LUAD model. Our data showed that loss of the histone chaperone in tumor cells sensitizes tumors to anti-PD-1 treatment. Mechanistic studies revealed that tumor cell-intrinsic deficiency induced immunogenic macrophage differentiation in the tumor microenvironment by upregulating GM-CSF expression and potentiated T-cell activation in combination with anti-PD-1. Our results provide a rationale for a novel combination therapy consisting of ASF1A inhibition and anti-PD-1 immunotherapy. **SIGNIFICANCE:** Using an epigenetic CRISPR screen, we identified ASF1A as a critical regulator of LUAD sensitivity to anti-PD-1 therapy. Deficiency synergized with anti-PD-1 immunotherapy by promoting M1-like macrophage polarization and T-cell activation. Thus, we provide a new immunotherapeutic strategy for this subtype of patients with LUAD.

Calcitonin Gene-Related Peptide Negatively Regulates Alarmin-Driven Type 2 Innate Lymphoid Cell Responses

Immunity (IF: 25.73), 2019-10, Aviv Regev et al. DOI: 10.1016/j.immuni.2019.09.005 PubMed: 31604686

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Neuroimmune interactions have emerged as critical modulators of allergic inflammation, and type 2 innate lymphoid cells (ILC2s) are an important cell type for mediating these interactions. Here, we show that ILC2s expressed both the neuropeptide calcitonin gene-related peptide (CGRP) and its receptor. CGRP potently inhibited alarmin-driven type 2 cytokine production and proliferation by lung ILC2s both in vitro and in vivo. CGRP induced marked changes in ILC2 expression programs in vivo and in vitro, attenuating alarmin-driven proliferative and effector responses. A distinct subset of ILCs scored highly for a CGRP-specific gene signature after in vivo alarmin stimulation, suggesting CGRP regulated this response. Finally, we observed increased ILC2 proliferation and type 2 cytokine production as well as exaggerated responses to alarmins in mice lacking the CGRP receptor. Together, these data indicate that endogenous CGRP is a critical negative regulator of ILC2 responses in vivo.

Inflammatory Type 2 cDCs Acquire Features of cDC1s and Macrophages to Orchestrate Immunity to Respiratory Virus Infection

Immunity (IF: 25.73), 2020-6, Martin Williams et al. DOI: 10.1016/j.immuni.2020.04.005 PubMed: 32392463
Single Cell Gene Expression, Mouse, Immunology.

Abstract

The phenotypic and functional dichotomy between IRF8type 1 and IRF4type 2 conventional dendritic cells (cDC1s and cDC2s, respectively) is well accepted; it is unknown how robust this dichotomy is under inflammatory conditions, when additionally monocyte-derived cells (MCs) become competent antigen-presenting cells (APCs). Using single-cell technologies in models of respiratory viral infection, we found that lung cDC2s acquired expression of the Fc receptor CD64 shared with MCs and of IRF8 shared with cDC1s. These inflammatory cDC2s (inf-cDC2s) were superior in inducing CD4T helper (Th) cell polarization while simultaneously presenting antigen to CD8T cells. When carefully separated from inf-cDC2s, MCs lacked APC function. Inf-cDC2s matured in response to cell-intrinsic Toll-like receptor and type 1 interferon receptor signaling, upregulated an IRF8-dependent maturation module, and acquired antigens via convalescent serum and Fc receptors. Because hybrid inf-cDC2s are easily confused with monocyte-derived cells, their existence could explain why APC functions have been attributed to MCs.

Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation

Immunity (IF: 25.73), 2019-10, David Artis et al. DOI: 10.1016/j.immuni.2019.06.009 PubMed: 31353223
Single Cell Gene Expression, Mouse, Immunology.

Abstract

Innate lymphocytes maintain tissue homeostasis at mucosal barriers, with group 2 innate lymphoid cells (ILC2s) producing type 2 cytokines and controlling helminth infection. While the molecular understanding of ILC2 responses has advanced, the complexity of microenvironmental factors impacting ILC2s is becoming increasingly apparent. Herein, we used single-cell analysis to explore the diversity of gene expression among lung lymphocytes during helminth infection. Following infection, we identified a subset of ILC2s that preferentially expressed IL5-encoding interleukin (IL)-5, together with Calca-encoding calcitonin gene-related peptide (CGRP) and its cognate receptor components. CGRP in concert with IL-33 and neuromedin U (NMU) supported IL-5 but constrained IL-13 expression and ILC2 proliferation. Without CGRP signaling, ILC2 responses and worm expulsion were enhanced. Collectively, these data point to CGRP as a context-dependent negative regulatory factor that shapes innate lymphocyte responses to alarmins and neuropeptides during type 2 innate immune responses.

The Lineage-Defining Transcription Factors SOX2 and NKX2-1 Determine Lung Cancer Cell Fate and Shape the Tumor Immune Microenvironment

Immunity (IF: 25.73), 2018-10, Eric L Snyder et al. DOI: 10.1016/j.immuni.2018.09.020 PubMed: 30332632

Single Cell Gene Expression, Mouse, Cancer Research, Immuno-oncology.

Abstract

The major types of non-small-cell lung cancer (NSCLC)-squamous cell carcinoma and adenocarcinoma-have distinct immune microenvironments. We developed a genetic model of squamous NSCLC on the basis of overexpression of the transcription factor Sox2, which specifies lung basal cell fate, and loss of the tumor suppressor Lkb1 (SL mice). SL tumors recapitulated gene-expression and immune-infiltrate features of human squamous NSCLC; such features included enrichment of tumor-associated neutrophils (TANs) and decreased expression of NKX2-1, a transcriptional regulator that specifies alveolar cell fate. In Kras-driven adenocarcinomas, mis-expression of Sox2 or loss of Nkx2-1 led to TAN recruitment. TAN recruitment involved SOX2-mediated production of the chemokine CXCL5. Deletion of Nkx2-1 in SL mice (SNL) revealed that NKX2-1 suppresses SOX2-driven squamous tumorigenesis by repressing adeno-to-squamous transdifferentiation. Depletion of TANs in SNL mice reduced squamous tumors, suggesting that TANs foster squamous cell fate. Thus, lineage-defining transcription factors determine the tumor immune microenvironment, which in turn might impact the nature of the tumor.

Inflammatory Signals Induce AT2 Cell-Derived Damage-Associated Transient Progenitors that Mediate Alveolar Regeneration

Cell Stem Cell (IF: 23.45), 2020-9, Namshik Han et al. DOI: 10.1016/j.stem.2020.06.020 PubMed: 32750316

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Tissue regeneration is a multi-step process mediated by diverse cellular hierarchies and states that are also implicated in tissue dysfunction and pathogenesis. Here we leveraged single-cell RNA sequencing in combination with in vivo lineage tracing and organoid models to finely map the trajectories of alveolar-lineage cells during injury repair and lung regeneration. We identified a distinct AT2-lineage population, damage-associated transient progenitors (DATPs), that arises during alveolar regeneration. We found that interstitial macrophage-derived IL-1 β primes a subset of AT2 cells expressing Il1r1 for conversion into DATPs via a HIF1 α -mediated glycolysis pathway, which is required for mature AT1 cell differentiation. Importantly, chronic inflammation mediated by IL-1 β prevents AT1 differentiation, leading to aberrant accumulation of DATPs and impaired alveolar regeneration. Together, this stepwise mapping to cell fate transitions shows how an inflammatory niche controls alveolar regeneration by controlling stem cell fate and behavior.

Distinct Airway Epithelial Stem Cells Hide among Club Cells but Mobilize to Promote Alveolar Regeneration

Cell Stem Cell (IF: 23.45), 2020-3, Xiaodan Tang et al. DOI: 10.1016/j.stem.2019.12.014 PubMed: 31978363

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Lung injury activates specialized adult epithelial progenitors to regenerate the epithelium. Depending on the extent of injury, both remaining alveolar type II cells (AEC2s) and distal airway stem/progenitors mobilize to cover denuded alveoli and restore normal barriers. The major source of airway stem/progenitors other than basal-like cells remains uncertain. Here, we define a distinct subpopulation (~5%) of club-like lineage-negative epithelial progenitors (LNEPs) marked by high H2-K1 expression critical for alveolar repair. Quiescent H2-K1 cells account for virtually all in vitro regenerative activity of airway lineages. After bleomycin injury, H2-K1 cells expand and differentiate in vivo to alveolar lineages. However, injured H2-K1 cells eventually develop impaired self-renewal with features of senescence, limiting complete repair. Normal H2-K1 cells transplanted into injured lungs differentiate into alveolar cells and rescue lung function. These findings indicate that small subpopulations of specialized stem/progenitors are required for effective lung regeneration and are a potential therapeutic adjunct after major lung injury.

Basophils prime group 2 innate lymphoid cells for neuropeptide-mediated inhibition

Nature Immunology (IF: 22.3), 2020-10, Aimee M Beaulieu et al. DOI: 10.1038/s41590-020-0753

PubMed: 32807943

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Type 2 cytokine responses promote parasitic immunity and initiate tissue repair; however, they can also result in immunopathologies when not properly restricted. Although basophilia is recognized as a common feature of type 2 inflammation, the roles basophils play in regulating these responses are unknown. Here, we demonstrate that helminth-induced group 2 innate lymphoid cell (ILC2) responses are exaggerated in the absence of basophils, resulting in increased inflammation and diminished lung function. Additionally, we show that ILC2s from basophil-depleted mice express reduced amounts of the receptor for the neuropeptide neuromedin B (NMB). Critically, NMB stimulation inhibited ILC2 responses from control but not basophil-depleted mice, and basophils were sufficient to directly enhance NMB receptor expression on ILC2s. These studies suggest that basophils prime ILC2s to respond to neuron-derived signals necessary to maintain tissue integrity. Further, these data provide mechanistic insight into the functions of basophils and identify NMB as a potent inhibitor of type 2 inflammation.

Locally instructed CXCR4hi neutrophils trigger environment-driven allergic asthma through the release of neutrophil extracellular traps

Nature Immunology (IF: 22.3), 2019-10, Fabrice Bureau et al. DOI: 10.1038/s41590-019-0496-9

PubMed: 31591573

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Low exposure to microbial products, respiratory viral infections and air pollution are major risk factors for allergic asthma, yet the mechanistic links between such conditions and host susceptibility to type 2 allergic disorders remain unclear. Through the use of single-cell RNA sequencing, we characterized lung neutrophils in mice exposed to a pro-allergic low dose of lipopolysaccharide (LPS) or a protective high dose of LPS before exposure to house dust mites. Unlike exposure to a high dose of LPS, exposure to a low dose of LPS instructed recruited neutrophils to upregulate their expression of the chemokine receptor CXCR4 and to release neutrophil extracellular traps. Low-dose LPS-induced neutrophils and neutrophil extracellular traps potentiated the uptake of house dust mites by CD11bLy-6Cdendritic cells and type 2 allergic airway inflammation in response to house dust mites. Neutrophil extracellular traps derived from CXCR4neutrophils were also needed to mediate allergic asthma triggered by infection with influenza virus or exposure to ozone. Our study indicates that apparently unrelated environmental risk factors can shape recruited lung neutrophils to promote the initiation of allergic asthma.

STAT3-BDNF-TrkB signalling promotes alveolar epithelial regeneration after lung injury

Nature cell biology (IF: 20.96), 2020-10, Edward E Morrissey et al. DOI: 10.1038/s41556-020-0569

PubMed: 32989251

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Alveolar epithelial regeneration is essential for recovery from devastating lung diseases. This process occurs when type II alveolar pneumocytes (AT2 cells) proliferate and transdifferentiate into type I alveolar pneumocytes (AT1 cells). We used genome-wide analysis of chromatin accessibility and gene expression following acute lung injury to elucidate repair mechanisms. AT2 chromatin accessibility changed substantially following injury to reveal STAT3 binding motifs adjacent to genes that regulate essential regenerative pathways. Single-cell transcriptome analysis identified brain-derived neurotrophic factor (Bdnf) as a STAT3 target gene with newly accessible chromatin in a unique population of regenerating AT2 cells. Furthermore, the BDNF receptor tropomyosin receptor kinase B (TrkB) was enriched on mesenchymal alveolar niche cells (MANCs). Loss or blockade of AT2-specific Stat3, Bdnf or mesenchyme-specific TrkB compromised repair and reduced Fgf7 expression by niche cells. A TrkB agonist improved outcomes in vivo following lung injury. These data highlight the biological and therapeutic importance of the STAT3-BDNF-TrkB axis in orchestrating alveolar epithelial regeneration.

ILC2-driven innate immune checkpoint mechanism antagonizes NK cell antimetastatic function in the lung

Nature Immunology (IF: 22.3), 2020-9, John C Marioni et al. DOI: 10.1038/s41590-020-0745 PubMed: 32747815
Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Metastasis constitutes the primary cause of cancer-related deaths, with the lung being a commonly affected organ. We found that activation of lung-resident group 2 innate lymphoid cells (ILC2s) orchestrated suppression of natural killer (NK) cell-mediated innate antitumor immunity, leading to increased lung metastases and mortality. Using multiple models of lung metastasis, we show that interleukin (IL)-33-dependent ILC2 activation in the lung is involved centrally in promoting tumor burden. ILC2-driven innate type 2 inflammation is accompanied by profound local suppression of interferon- γ production and cytotoxic function of lung NK cells. ILC2-dependent suppression of NK cells is elaborated via an innate regulatory mechanism, which is reliant on IL-5-induced lung eosinophilia, ultimately limiting the metabolic fitness of NK cells. Therapeutic targeting of IL-33 or IL-5 reversed NK cell suppression and alleviated cancer burden. Thus, we reveal an important function of IL-33 and ILC2s in promoting tumor metastasis via their capacity to suppress innate type 1 immunity.

Select autophagy genes maintain quiescence of tissue-resident macrophages and increase susceptibility to *Listeria monocytogenes*

Nature Microbiology (IF: 16.29), 2020-1, Christina L Stallings et al. DOI: 10.1038/s41564-019-0633-0
PubMed: 31959973

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Innate and adaptive immune responses that prime myeloid cells, such as macrophages, protect against pathogens. However, if left uncontrolled, these responses may lead to detrimental inflammation. Macrophages, particularly those resident in tissues, must therefore remain quiescent between infections despite chronic stimulation by commensal microorganisms. The genes required for quiescence of tissue-resident macrophages are not well understood. Autophagy, an evolutionarily conserved cellular process by which cytoplasmic contents are targeted for lysosomal digestion, has homeostatic functions including maintenance of protein and organelle integrity and regulation of metabolism. Recent research has shown that degradative autophagy, as well as various combinations of autophagy genes, regulate immunity and inflammation. Here, we delineate a function of the autophagy proteins Beclin 1 and FIP200-but not of other essential autophagy components ATG5, ATG16L1 or ATG7-in mediating quiescence of tissue-resident macrophages by limiting the effects of systemic interferon- γ . The perturbation of quiescence in mice that lack Beclin 1 or FIP200 in myeloid cells results in spontaneous immune activation and resistance to *Listeria monocytogenes* infection. While antibiotic-treated wild-type mice display diminished macrophage responses to inflammatory stimuli, this is not observed in mice that lack Beclin 1 in myeloid cells, establishing the dominance of this gene over effects of the bacterial microbiota. Thus, select autophagy genes, but not all genes essential for degradative autophagy, have a key function in maintaining immune quiescence of tissue-resident macrophages, resulting in genetically programmed susceptibility to bacterial infection.

Vascular endothelium-targeted Sirt7 gene therapy rejuvenates blood vessels and extends life span in a Hutchinson-Gilford progeria model

Science Advances (IF: 14.09), 2020-2, Zhongjun Zhou et al. DOI: 10.1126/sciadv.aay5556 PubMed: 32128409
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Vascular dysfunction is a typical characteristic of aging, but its contributing roles to systemic aging and the therapeutic potential are lacking experimental evidence. Here, we generated a knock-in mouse model with the causative Hutchinson-Gilford progeria syndrome (HGPS) mutation, called progerin. The TC mice with progerin expression induced by exhibit defective microvasculature and neovascularization, accelerated aging, and shortened life span. Single-cell transcriptomic analysis of murine lung endothelial cells revealed a substantial up-regulation of inflammatory response. Molecularly, progerin interacts and destabilizes deacylase Sirt7; ectopic expression of alleviates the inflammatory response caused by progerin in endothelial cells. Vascular endothelium-targeted gene therapy, driven by an promoter, improves neovascularization, ameliorates aging features, and extends life span in TC mice. These data support endothelial dysfunction as a primary trigger of systemic aging and highlight gene therapy as a potential strategy for the clinical treatment of HGPS and age-related vascular dysfunction.

IL-33-ST2 axis regulates myeloid cell differentiation and activation enabling effective club cell regeneration

Nature Communications (IF: 13.61), 2020-9, Alison A Humbles et al. DOI: 10.1038/s41467-020-18466
PubMed: 32963227
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Evidence points to an indispensable function of macrophages in tissue regeneration, yet the underlying molecular mechanisms remain elusive. Here we demonstrate a protective function for the IL-33-ST2 axis in bronchial epithelial repair, and implicate ST2 in myeloid cell differentiation. ST2 deficiency in mice leads to reduced lung myeloid cell infiltration, abnormal alternatively activated macrophage (AAM) function, and impaired epithelial repair post naphthalene-induced injury. Reconstitution of wild type (WT) AAMs to ST2-deficient mice completely restores bronchial re-epithelialization. Central to this mechanism is the direct effect of IL-33-ST2 signaling on monocyte/macrophage differentiation, self-renewal and repairing ability, as evidenced by the downregulation of key pathways regulating myeloid cell cycle, maturation and regenerative function of the epithelial niche in ST2 mice. Thus, the IL-33-ST2 axis controls epithelial niche regeneration by activating a large multi-cellular circuit, including monocyte differentiation into competent repairing AAMs, as well as group-2 innate lymphoid cell (ILC2)-mediated AAM activation.

The in vivo genetic program of murine primordial lung epithelial progenitors

Nature Communications (IF: 13.61), 2020-1, Pankaj Mehta et al. DOI: 10.1038/s41467-020-14348-3

PubMed: 32005814

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Multipotent Nkx2-1-positive lung epithelial primordial progenitors of the foregut endoderm are thought to be the developmental precursors to all adult lung epithelial lineages. However, little is known about the global transcriptomic programs or gene networks that regulate these gateway progenitors in vivo. Here we use bulk RNA-sequencing to describe the unique genetic program of in vivo murine lung primordial progenitors and computationally identify signaling pathways, such as Wnt and Tgf- β superfamily pathways, that are involved in their cell-fate determination from pre-specified embryonic foregut. We integrate this information in computational models to generate in vitro engineered lung primordial progenitors from mouse pluripotent stem cells, improving the fidelity of the resulting cells through unbiased, easy-to-interpret similarity scores and modulation of cell culture conditions, including substratum elastic modulus and extracellular matrix composition. The methodology proposed here can have wide applicability to the in vitro derivation of bona fide tissue progenitors of all germ layers.

Non-classical tissue monocytes and two functionally distinct populations of interstitial macrophages populate the mouse lung

Nature Communications (IF: 13.61), 2019-9, Fabrice Bureau et al. DOI: 10.1038/s41467-019-11843-0

PubMed: 31481690

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Resident tissue macrophages (RTM) can fulfill various tasks during development, homeostasis, inflammation and repair. In the lung, non-alveolar RTM, called interstitial macrophages (IM), importantly contribute to tissue homeostasis but remain little characterized. Here we show, using single-cell RNA-sequencing (scRNA-seq), two phenotypically distinct subpopulations of long-lived monocyte-derived IM, i.e. CD206⁺ and CD206⁺IM, as well as a discrete population of extravasating CD64⁺CD16.2⁺ monocytes. CD206⁺IM are peribronchial self-maintaining RTM that constitutively produce high levels of chemokines and immunosuppressive cytokines. Conversely, CD206⁺IM preferentially populate the alveolar interstitium and exhibit features of antigen-presenting cells. In addition, our data support that CD64⁺CD16.2⁺ monocytes arise from intravascular Ly-6C⁺ patrolling monocytes that enter the tissue at steady-state to become putative precursors of CD206⁺IM. This study expands our knowledge about the complexity of lung IM and reveals an ontogenic pathway for one IM subset, an important step for elaborating future macrophage-targeted therapies.

Murine single-cell RNA-seq reveals cell-identity- and tissue-specific trajectories of aging

Genome research (IF: 12.01), 2019-12, David R Kelley et al. DOI: 10.1101/gr.253880.119 PubMed: 31754020
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Aging is a pleiotropic process affecting many aspects of mammalian physiology. Mammals are composed of distinct cell type identities and tissue environments, but the influence of these cell identities and environments on the trajectory of aging in individual cells remains unclear. Here, we performed single-cell RNA-seq on >50,000 individual cells across three tissues in young and old mice to allow for direct comparison of aging phenotypes across cell types. We found transcriptional features of aging common across many cell types, as well as features of aging unique to each type. Leveraging matrix factorization and optimal transport methods, we found that both cell identities and tissue environments exert influence on the trajectory and magnitude of aging, with cell identity influence predominating. These results suggest that aging manifests with unique directionality and magnitude across the diverse cell identities in mammals.

Identification of a nerve-associated, lung-resident interstitial macrophage subset with distinct localization and immune-regulatory properties

Science Immunology (IF: 13.41), 2020-3, Boris Reizis et al. DOI: 10.1126/sciimmunol.aax8756 PubMed: 32220976
Single Cell Gene Expression, Mouse, Immunology.

Abstract

Tissue-resident macrophages are a diverse population of cells that perform specialized functions including sustaining tissue homeostasis and tissue surveillance. Here, we report an interstitial subset of CD169lung-resident macrophages that are transcriptionally and developmentally distinct from alveolar macrophages (AMs). They are primarily localized around the airways and are found in close proximity to the sympathetic nerves in the bronchovascular bundle. These nerve- and airway-associated macrophages (NAMs) are tissue resident, yolk sac derived, self-renewing, and do not require CCR2monocytes for development or maintenance. Unlike AMs, the development of NAMs requires CSF1 but not GM-CSF. Bulk population and single-cell transcriptome analysis indicated that NAMs are distinct from other lung-resident macrophage subsets and highly express immunoregulatory genes under steady-state and inflammatory conditions. NAMs proliferated robustly after influenza infection and activation with the TLR3 ligand poly(I:C), and in their absence, the inflammatory response was augmented, resulting in excessive production of inflammatory cytokines and innate immune cell infiltration. Overall, our study provides insights into a distinct subset of airway-associated pulmonary macrophages that function to maintain immune and tissue homeostasis.

Autophagic protein ATG5 controls antiviral immunity via glycolytic reprogramming of dendritic cells against respiratory syncytial virus infection

Autophagy (IF: 11.97), 2020-8, Hyun-Jin Kim et al. DOI: 10.1080/15548627.2020.1812218 PubMed: 32816604
Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

Respiratory syncytial virus (RSV) is a leading cause of respiratory tract infections in infants. Macroautophagy autophagy is a catalytic metabolic process required for cellular homeostasis. Although intracellular metabolism is important for immune responses in dendritic cells, the link between autophagy and immunometabolism remains unknown. Here, we show that the autophagy-related protein ATG5 regulates immunometabolism. -deficient mouse dendritic cells showed increased CD8AT-cell response and increased secretion of proinflammatory cytokines upon RSV infection. Transcriptome analysis showed that deficiency alters the expression of metabolism-related genes. -deficient dendritic cells also showed increased activation of glycolysis and the AKT-MTOR-RPS6KB1 pathway and decreased mitochondrial activity, all of which are cellular signatures for metabolic activation. These cells also showed elevated CD8AT-cell priming and surface major histocompatibility complex (MHC) class I expression. Our results suggested that ATG5 regulated host immune responses by modulating dendritic cell metabolism. These findings may help develop potential antiviral therapies that alter host immunity by regulating autophagy and immunometabolism.

2-DG: 2-deoxyglucose; AAK1: AP2 associated kinase 1; AKT: AKT serine/threonine kinase; AM: alveolar macrophage; ATG: autophagy; ATP: adenosine triphosphate; BAL: bronchoalveolar lavage; BMDC: bone marrow dendritic cell; CSF2/GM-CSF: colony-stimulating factor 2 (granulocyte-macrophage); CTL: cytotoxic T lymphocyte; ELISA: enzyme-linked immunosorbent assay; GFP: green fluorescent protein; GSEA: gene-set enrichment analysis; H-2D: H-2 class I histocompatibility antigen, D-B alpha chain; H-2K: MHC class I H2-K-b; HIF1A: hypoxia-inducible factor 1 alpha; IFNG: interferon-gamma; IL: interleukin; ITGAX: integrin alpha X; MAP1LC3/LC3: microtubule-associated protein 1 light chain 3; MAP1LC3B/LC3B: microtubule-associated protein 1 light chain 3 beta; MHC: major histocompatibility complex; MTORC1: mammalian target of rapamycin kinase complex 1; PBS: phosphate-buffered saline; PFU: plaque-forming unit; RLR: retinoic acid-inducible-I-like receptor; ROS: reactive oxygen species; RPMI: Roswell Park Memorial Institute; RPS6KB1/S6K: ribosomal protein S6 kinase, polypeptide 1; RSV: respiratory syncytial virus; Th: T helper; TLR: toll-like receptor; Treg: regulatory T cells; UMAP: uniform manifold approximation and projection.

A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signalling in monocyte-derived alveolar macrophages

European Respiratory Journal (IF: 11.71), 2020-1, G R Scott Budinger et al. DOI: 10.1183/13993003.00646-2019
PubMed: 31601718

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Ontologically distinct populations of macrophages differentially contribute to organ fibrosis through unknown mechanisms. We applied lineage tracing, single-cell RNA sequencing and single-molecule fluorescence hybridisation to a spatially restricted model of asbestos-induced pulmonary fibrosis. We demonstrate that tissue-resident alveolar macrophages, tissue-resident peribronchial and perivascular interstitial macrophages, and monocyte-derived alveolar macrophages are present in the fibrotic niche. Deletion of monocyte-derived alveolar macrophages but not tissue-resident alveolar macrophages ameliorated asbestos-induced lung fibrosis. Monocyte-derived alveolar macrophages were specifically localised to fibrotic regions in the proximity of fibroblasts where they expressed molecules known to drive fibroblast proliferation, including platelet-derived growth factor subunit A. Using single-cell RNA sequencing and spatial transcriptomics in both humans and mice, we identified macrophage colony-stimulating factor receptor (M-CSFR) signalling as one of the novel druggable targets controlling self-maintenance and persistence of these pathogenic monocyte-derived alveolar macrophages. Pharmacological blockade of M-CSFR signalling led to the disappearance of monocyte-derived alveolar macrophages and ameliorated fibrosis. Our findings suggest that inhibition of M-CSFR signalling during fibrosis disrupts an essential fibrotic niche that includes monocyte-derived alveolar macrophages and fibroblasts during asbestos-induced fibrosis.

Single-cell analysis of ROR α tracer mouse lung reveals ILC progenitors and effector ILC2 subsets

Journal of experimental medicine (IF: 11.32), 2020-3, Avinash Bhandoola et al. DOI: 10.1084/jem.20182293
PubMed: 31816636

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Lung group 2 innate lymphoid cells (ILC2s) drive allergic inflammation and promote tissue repair. ILC2 development is dependent on the transcription factor retinoic acid receptor-related orphan receptor (ROR α), which is also expressed in common ILC progenitors. To elucidate the developmental pathways of lung ILC2s, we generated ROR α lineage tracer mice and performed single-cell RNA sequencing, flow cytometry, and functional analyses. In adult mouse lungs, we found an IL-18R α +ST2- population different from conventional IL-18R α -ST2+ ILC2s. The former was GATA-3^{int}Tcf7EGFP+Kit+, produced few cytokines, and differentiated into multiple ILC lineages in vivo and in vitro. In neonatal mouse lungs, three ILC populations were identified, namely an ILC progenitor population similar to that in adult lungs and two distinct effector ILC2 subsets that differentially produced type 2 cytokines and amphiregulin. Lung ILC progenitors might actively contribute to ILC-poiesis in neonatal and inflamed adult lungs. In addition, neonatal lung ILC2s include distinct proinflammatory and tissue-repairing subsets.

β 2 Integrins differentially regulate $\gamma\delta$ T cell subset thymic development and peripheral maintenance

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-9, Adrian C Hayday et al. DOI: 10.1073/pnas.1921930117 PubMed: 32848068

Single Cell Gene Expression, Mouse, Immunology.

Abstract

The $\gamma\delta$ T cells reside predominantly at barrier sites and play essential roles in immune protection against infection and cancer. Despite recent advances in the development of $\gamma\delta$ T cell immunotherapy, our understanding of the basic biology of these cells, including how their numbers are regulated in vivo, remains poor. This is particularly true for tissue-resident $\gamma\delta$ T cells. We have identified the β family of integrins as regulators of $\gamma\delta$ T cells. β -integrin-deficient mice displayed a striking increase in numbers of IL-17-producing V γ 6V δ 1 $\gamma\delta$ T cells in the lungs, uterus, and circulation. Thymic development of this population was normal. However, single-cell RNA sequencing revealed the enrichment of genes associated with T cell survival and proliferation specifically in β -integrin-deficient IL-17 cells compared to their wild-type counterparts. Indeed, β -integrin-deficient V γ 6 cells from the lungs showed reduced apoptosis ex vivo, suggesting that increased survival contributes to the accumulation of these cells in β -integrin-deficient tissues. Furthermore, our data revealed an unexpected role for β integrins in promoting the thymic development of the IFN γ -producing CD27V γ 4 $\gamma\delta$ T cell subset. Together, our data reveal that β integrins are important regulators of $\gamma\delta$ T cell homeostasis, inhibiting the survival of IL-17-producing V γ 6V δ 1 cells and promoting the thymic development of the IFN γ -producing V γ 4 subset. Our study introduces unprecedented mechanisms of control for $\gamma\delta$ T cell subsets.

Low ambient humidity impairs barrier function and innate resistance against influenza infection

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-5, Robert J Homer et al. DOI: 10.1073/pnas.1902840116 PubMed: 31085641

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

In the temperate regions, seasonal influenza virus outbreaks correlate closely with decreases in humidity. While low ambient humidity is known to enhance viral transmission, its impact on host response to influenza virus infection and disease outcome remains unclear. Here, we showed that housing Mx1 congenic mice in low relative humidity makes mice more susceptible to severe disease following respiratory challenge with influenza A virus. We find that inhalation of dry air impairs mucociliary clearance, innate antiviral defense, and tissue repair. Moreover, disease exacerbated by low relative humidity was ameliorated in caspase-1/11-deficient Mx1 mice, independent of viral burden. Single-cell RNA sequencing revealed that induction of IFN-stimulated genes in response to viral infection was diminished in multiple cell types in the lung of mice housed in low humidity condition. These results indicate that exposure to dry air impairs host defense against influenza infection, reduces tissue repair, and inflicts caspase-dependent disease pathology.

Redundant and additive functions of the four Lef/Tcf transcription factors in lung epithelial progenitors

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, Haruhiko Akiyama et al. DOI: 10.1073/pnas.2002082117 PubMed: 32414917

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

In multicellular organisms, paralogs from gene duplication survive purifying selection by evolving tissue-specific expression and function. Whether this genetic redundancy is also selected for within a single cell type is unclear for multimember paralogs, as exemplified by the four obligatory Lef/Tcf transcription factors of canonical Wnt signaling, mainly due to the complex genetics involved. Using the developing mouse lung as a model system, we generate two quadruple conditional knockouts, four triple mutants, and various combinations of double mutants, showing that the four Lef/Tcf genes function redundantly in the presence of at least two Lef/Tcf paralogs, but additively upon losing additional paralogs to specify and maintain lung epithelial progenitors. Prelung-specification, pan-epithelial double knockouts have no lung phenotype; triple knockouts have varying phenotypes, including defective branching and tracheoesophageal fistulas; and the quadruple knockout barely forms a lung, resembling the mutant. Postlung-specification deletion of all four Lef/Tcf genes leads to branching defects, down-regulation of progenitor genes, premature alveolar differentiation, and derepression of gastrointestinal genes, again phenocopying the corresponding mutant. Our study supports a monotonic, positive signaling relationship between CTNNB1 and Lef/Tcf in lung epithelial progenitors as opposed to reported repressor functions of Lef/Tcf, and represents a thorough in vivo analysis of cell-type-specific genetic redundancy among the four Lef/Tcf paralogs.

Epithelial Vegfa Specifies a Distinct Endothelial Population in the Mouse Lung

Developmental Cell (IF: 10.13), 2020-3, Joshua D Wythe et al. DOI: 10.1016/j.devcel.2020.01.009 PubMed: 32059772

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The lung microvasculature is essential for gas exchange and commonly considered homogeneous. We show that VEGFA from the epithelium is required for a distinct endothelial cell (EC) population in the mouse lung. Vegfa is predominantly expressed by alveolar type 1 (AT1) cells and locally required to specify a subset of ECs. Single-cell RNA sequencing (scRNA-seq) reveals that ~15% of lung ECs are transcriptionally distinct—marked by Carbonic anhydrase 4 (Car4)—and arise from bulk ECs, as suggested by trajectory analysis. Car4 ECs have extensive cellular projections and are separated from AT1 cells by a limited basement membrane without intervening pericytes. Car4 ECs are specifically lost upon epithelial Vegfa deletion; without Car4 ECs, the alveolar space is aberrantly enlarged despite the normal appearance of myofibroblasts. Lung Car4 ECs and retina tip ECs have common and distinct features. These findings support a signaling role of AT1 cells and shed light on alveologenesis.

FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence

Clinical cancer research (IF: 10.12), 2020-3, Arturo Londoño-Vallejo et al. DOI: 10.1158/1078-0432.CCR-19-1440
PubMed: 31796518

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

One of the main limitations to anticancer radiotherapy lies in irreversible damage to healthy tissues located within the radiation field. FLASH irradiation at very high dose-rate is a new treatment modality that has been reported to specifically spare normal tissue from late radiation-induced toxicity in animal models and therefore could be a promising strategy to reduce treatment toxicity. Lung responses to FLASH irradiation were investigated by qPCR, single-cell RNA sequencing (sc-RNA-Seq), and histologic methods during the acute wound healing phase as well as at late stages using C57BL/6J wild-type and Terc mice exposed to bilateral thorax irradiation as well as human lung cells grown. Studies gave evidence of a reduced level of DNA damage and induced lethality at the advantage of FLASH. In mouse lung, sc-RNA-seq and the monitoring of proliferating cells revealed that FLASH minimized the induction of proinflammatory genes and reduced the proliferation of progenitor cells after injury. At late stages, FLASH-irradiated lungs presented less persistent DNA damage and senescent cells than after CONV exposure, suggesting a higher potential for lung regeneration with FLASH. Consistent with this hypothesis, the beneficial effect of FLASH was lost in Terc mice harboring critically short telomeres and lack of telomerase activity. The results suggest that, compared with conventional radiotherapy, FLASH minimizes DNA damage in normal cells, spares lung progenitor cells from excessive damage, and reduces the risk of replicative senescence.

Single-cell connectomic analysis of adult mammalian lungs

Science Advances (IF: 14.09), 2019-12, Naftali Kaminski et al. DOI: 10.1126/sciadv.aaw3851 PubMed: 31840053
Single Cell Gene Expression, Rat, Other - Mammal, Cell Atlas.

Abstract

Efforts to decipher chronic lung disease and to reconstitute functional lung tissue through regenerative medicine have been hampered by an incomplete understanding of cell-cell interactions governing tissue homeostasis. Because the structure of mammalian lungs is highly conserved at the histologic level, we hypothesized that there are evolutionarily conserved homeostatic mechanisms that keep the fine architecture of the lung in balance. We have leveraged single-cell RNA sequencing techniques to identify conserved patterns of cell-cell cross-talk in adult mammalian lungs, analyzing mouse, rat, pig, and human pulmonary tissues. Specific stereotyped functional roles for each cell type in the distal lung are observed, with alveolar type I cells having a major role in the regulation of tissue homeostasis. This paper provides a systems-level portrait of signaling between alveolar cell populations. These methods may be applicable to other organs, providing a roadmap for identifying key pathways governing pathophysiology and informing regenerative efforts.

Inherited myeloproliferative neoplasm risk affects haematopoietic stem cells

Nature (IF: 46.49), 2020-10, Sekar Kathiresan et al. DOI: 10.1038/s41586-020-2786-7 PubMed: 33057200

Single Cell Gene Expression, Human, Cancer Research, Population Genetics.

Abstract

Myeloproliferative neoplasms (MPNs) are blood cancers that are characterized by the excessive production of mature myeloid cells and arise from the acquisition of somatic driver mutations in haematopoietic stem cells (HSCs). Epidemiological studies indicate a substantial heritable component of MPNs that is among the highest known for cancers. However, only a limited number of genetic risk loci have been identified, and the underlying biological mechanisms that lead to the acquisition of MPNs remain unclear. Here, by conducting a large-scale genome-wide association study (3,797 cases and 1,152,977 controls), we identify 17 MPN risk loci ($P < 5.0 \times 10^{-8}$), 7 of which have not been previously reported. We find that there is a shared genetic architecture between MPN risk and several haematopoietic traits from distinct lineages; that there is an enrichment for MPN risk variants within accessible chromatin of HSCs; and that increased MPN risk is associated with longer telomere length in leukocytes and other clonal haematopoietic states—collectively suggesting that MPN risk is associated with the function and self-renewal of HSCs. We use gene mapping to identify modulators of HSC biology linked to MPN risk, and show through targeted variant-to-function assays that CHEK2 and GFI1B have roles in altering the function of HSCs to confer disease risk. Overall, our results reveal a previously unappreciated mechanism for inherited MPN risk through the modulation of HSC function.

Characterization of cell fate probabilities in single-cell data with Palantir

Nature Biotechnology (IF: 42.3), 2019-3, Linas Mazutis Dana Pe'er DOI: 10.1038/s41587-019-0068-4

PubMed: 30899105

Single Cell Gene Expression, Human, Computational Method.

Abstract

Single-cell RNA sequencing studies of differentiating systems have raised fundamental questions regarding the discrete versus continuous nature of both differentiation and cell fate. Here we present Palantir, an algorithm that models trajectories of differentiating cells by treating cell fate as a probabilistic process and leverages entropy to measure cell plasticity along the trajectory. Palantir generates a high-resolution pseudo-time ordering of cells and, for each cell state, assigns a probability of differentiating into each terminal state. We apply our algorithm to human bone marrow single-cell RNA sequencing data and detect important landmarks of hematopoietic differentiation. Palantir's resolution enables the identification of key transcription factors that drive lineage fate choice and closely track when cells lose plasticity. We show that Palantir outperforms existing algorithms in identifying cell lineages and recapitulating gene expression trends during differentiation, is generalizable to diverse tissue types, and is well-suited to resolving less-studied differentiating systems.

A Multiplexed Single-Cell CRISPR Screening Platform Enables Systematic Dissection of the Unfolded Protein Response

Cell (IF: 38.62), 2016-12, Aviv Regev, Jonathan S Weissman et al. DOI: 10.1016/j.cell.2016.11.048 PubMed: 27984733
Single Cell Gene Expression, Human, Functional Genomics, Single Cell Multiomics Method.

Abstract

Functional genomics efforts face tradeoffs between number of perturbations examined and complexity of phenotypes measured. We bridge this gap with Perturb-seq, which combines droplet-based single-cell RNA-seq with a strategy for barcoding CRISPR-mediated perturbations, allowing many perturbations to be profiled in pooled format. We applied Perturb-seq to dissect the mammalian unfolded protein response (UPR) using single and combinatorial CRISPR perturbations. Two genome-scale CRISPR interference (CRISPRi) screens identified genes whose repression perturbs ER homeostasis. Subjecting ~100 hits to Perturb-seq enabled high-precision functional clustering of genes. Single-cell analyses decoupled the three UPR branches, revealed bifurcated UPR branch activation among cells subject to the same perturbation, and uncovered differential activation of the branches across hits, including an isolated feedback loop between the translocon and IRE1 α . These studies provide insight into how the three sensors of ER homeostasis monitor distinct types of stress and highlight the ability of Perturb-seq to dissect complex cellular responses.

Extensive Remodeling of the Immune Microenvironment in B Cell Acute Lymphoblastic Leukemia

Cancer Cell (IF: 30.24), 2020-6, William L Carroll et al. DOI: 10.1016/j.ccell.2020.04.015 PubMed: 32470390
Single Cell Gene Expression, Human, Cancer Research.

Abstract

A subset of B cell acute lymphoblastic leukemia (B-ALL) patients will relapse and succumb to therapy-resistant disease. The bone marrow microenvironment may support B-ALL progression and treatment evasion. Utilizing single-cell approaches, we demonstrate B-ALL bone marrow immune microenvironment remodeling upon disease initiation and subsequent re-emergence during conventional chemotherapy. We uncover a role for non-classical monocytes in B-ALL survival, and demonstrate monocyte abundance at B-ALL diagnosis is predictive of pediatric and adult B-ALL patient survival. We show that human B-ALL blasts alter a vascularized microenvironment promoting monocytic differentiation, while depleting leukemia-associated monocytes in B-ALL animal models prolongs disease remission in vivo. Our profiling of the B-ALL immune microenvironment identifies extrinsic regulators of B-ALL survival supporting new immune-based therapeutic approaches for high-risk B-ALL treatment.

Osteogenesis depends on commissioning of a network of stem cell transcription factors that act as repressors of adipogenesis

Nature Genetics (IF: 30.33), 2019-3, Moustapha KassemSusanne Mandrup et al. DOI: 10.1038/s41588-019-0359-1 PubMed: 30833796

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Mesenchymal (stromal) stem cells (MSCs) constitute populations of mesodermal multipotent cells involved in tissue regeneration and homeostasis in many different organs. Here we performed comprehensive characterization of the transcriptional and epigenomic changes associated with osteoblast and adipocyte differentiation of human MSCs. We demonstrate that adipogenesis is driven by considerable remodeling of the chromatin landscape and de novo activation of enhancers, whereas osteogenesis involves activation of preestablished enhancers. Using machine learning algorithms for in silico modeling of transcriptional regulation, we identify a large and diverse transcriptional network of pro-osteogenic and antiadipogenic transcription factors. Intriguingly, binding motifs for these factors overlap with SNPs related to bone and fat formation in humans, and knockdown of single members of this network is sufficient to modulate differentiation in both directions, thus indicating that lineage determination is a delicate balance between the activities of many different transcription factors.

Immunogenomic Landscape of Hematological Malignancies

Cancer Cell (IF: 30.24), 2020-9, Merja Heinäniemi et al. DOI: 10.1016/j.ccell.2020.06.002 PubMed: 32649887

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Understanding factors that shape the immune landscape across hematological malignancies is essential for immunotherapy development. We integrated over 8,000 transcriptomes and 2,000 samples with multilevel genomics of hematological cancers to investigate how immunological features are linked to cancer subtypes, genetic and epigenetic alterations, and patient survival, and validated key findings experimentally. Infiltration of cytotoxic lymphocytes was associated with TP53 and myelodysplasia-related changes in acute myeloid leukemia, and activated B cell-like phenotype and interferon- γ response in lymphoma. CIITA methylation regulating antigen presentation, cancer type-specific immune checkpoints, such as VISTA in myeloid malignancies, and variation in cancer antigen expression further contributed to immune heterogeneity and predicted survival. Our study provides a resource linking immunology with cancer subtypes and genomics in hematological malignancies.

Targeting CD70 with cusatuzumab eliminates acute myeloid leukemia stem cells in patients treated with hypomethylating agents

Nature Medicine (IF: 36.23), 2020-9, Nicolas Leupin et al. DOI: 10.1038/s41591-020-0910-8 PubMed: 32601337
Single Cell Gene Expression, Human, Cancer Research.

Abstract

Acute myeloid leukemia (AML) is driven by leukemia stem cells (LSCs) that resist conventional chemotherapy and are the major cause of relapse. Hypomethylating agents (HMAs) are the standard of care in the treatment of older or unfit patients with AML, but responses are modest and not durable. Here we demonstrate that LSCs upregulate the tumor necrosis factor family ligand CD70 in response to HMA treatment resulting in increased CD70/CD27 signaling. Blocking CD70/CD27 signaling and targeting CD70-expressing LSCs with cusatuzumab, a human α CD70 monoclonal antibody with enhanced antibody-dependent cellular cytotoxicity activity, eliminated LSCs in vitro and in xenotransplantation experiments. Based on these preclinical results, we performed a phase 1/2 trial in previously untreated older patients with AML with a single dose of cusatuzumab monotherapy followed by a combination therapy with the HMA azacitidine (NCT03030612). We report results from the phase 1 dose escalation part of the clinical trial. Hematological responses in the 12 patients enrolled included 8 complete remission, 2 complete remission with incomplete blood count recovery and 2 partial remission with 4 patients achieving minimal residual disease negativity by flow cytometry at $\leq 10^{-4}$. Median time to response was 3.3 months. Median progression-free survival was not reached yet at the time of the data cutoff. No dose-limiting toxicities were reported and the maximum tolerated dose of cusatuzumab was not reached. Importantly, cusatuzumab treatment substantially reduced LSCs and triggered gene signatures related to myeloid differentiation and apoptosis.

dropEst: pipeline for accurate estimation of molecular counts in droplet-based single-cell RNA-seq experiments

Genome Biology (IF: 19.04), 2018-12, Maria G SamsonovaPeter V Kharchenko DOI: 10.1186/s13059-018-1449-6 PubMed: 29921301

Single Cell Gene Expression, Human, Computational Method.

Abstract

Recent single-cell RNA-seq protocols based on droplet microfluidics use massively multiplexed barcoding to enable simultaneous measurements of transcriptomes for thousands of individual cells. The increasing complexity of such data creates challenges for subsequent computational processing and troubleshooting of these experiments, with few software options currently available. Here, we describe a flexible pipeline for processing droplet-based transcriptome data that implements barcode corrections, classification of cell quality, and diagnostic information about the droplet libraries. We introduce advanced methods for correcting composition bias and sequencing errors affecting cellular and molecular barcodes to provide more accurate estimates of molecular counts in individual cells.

Single-cell analysis of bone marrow-derived CD34+ cells from children with sickle cell disease and thalassemia

Blood (IF: 14.82), 2019-12, Irene Roberts et al. DOI: 10.1182/blood.2019002301 PubMed: 31697810

Single Cell Gene Expression, Human, Developmental Biology, Genetic Health.

Abstract

Sickle cell disease (SCD) and thalassaemia, the most common inherited anaemias, affect ~300,000 births p.a. world-wide. They result in progressive end organ damage mainly due to recurrent vasoocclusive crises (in SCD) and iron overload. Allogeneic haemopoietic stem cell transplantation (HSCT) has only been a curative option for a minority of young patients with severe disease because of transplant-related mortality. Recently, gene therapy and genome editing to modify HSCs for autologous haemopoietic stem cell transplantation (HSCT) have become possible. Genome editing strategies include inducing fetal haemoglobin, reducing alpha globin expression and correcting the HBB gene using homology directed repair. Our hypothesis is that a chronic inflammatory state in the bone marrow of these haemoglobinopathy patients perturbs haemopoietic stem/progenitor cell (HSPC) function, a process that will significantly impact on genome editing and HSCT outcomes.

The myeloid type I interferon response to myocardial infarction begins in bone marrow and is regulated by Nrf2-activated macrophages

Science Immunology (IF: 13.41), 2020-9, Zhenxing Fu et al. DOI: 10.1126/sciimmunol.aaz1974 PubMed: 32978242

Single Cell Gene Expression, Human & Mouse, Immunology.

Abstract

Sterile tissue injury is thought to locally activate innate immune responses via damage-associated molecular patterns (DAMPs). Whether innate immune pathways are remotely activated remains relatively unexplored. Here, by analyzing ~145,000 single-cell transcriptomes at steady state and after myocardial infarction (MI) in mice and humans, we show that the type I interferon (IFN) response, characterized by expression of IFN-stimulated genes (ISGs), begins far from the site of injury, in neutrophil and monocyte progenitors within the bone marrow. In the peripheral blood of patients, we observed defined subsets of ISG-expressing neutrophils and monocytes. In the bone marrow and blood of mice, ISG expression was detected in neutrophils and monocytes and their progenitors, intensified with maturation at steady-state and after MI, and was controlled by Tet2 and Irf3 transcriptional regulators. Within the infarcted heart, ISG-expressing cells were negatively regulated by Nrf2 activation in Ccr2steady-state cardiac macrophages. Our results show that IFN signaling begins in the bone marrow, implicate multiple transcriptional regulators (Tet2, Irf3, and Nrf2) in governing ISG expression, and provide a clinical biomarker (ISG score) for studying IFN signaling in patients.

Evolution and structure of clinically relevant gene fusions in multiple myeloma

Nature Communications (IF: 13.61), 2020-5, Li Ding et al. DOI: 10.1038/s41467-020-16434 PubMed: 32471990
Single Cell Gene Expression & Single Cell Immune Profiling, Human, Cancer Research, Genetic Health.

Abstract

Multiple myeloma is a plasma cell blood cancer with frequent chromosomal translocations leading to gene fusions. To determine the clinical relevance of fusion events, we detect gene fusions from a cohort of 742 patients from the Multiple Myeloma Research Foundation CoMMpass Study. Patients with multiple clinic visits enable us to track tumor and fusion evolution, and cases with matching peripheral blood and bone marrow samples allow us to evaluate the concordance of fusion calls in patients with high tumor burden. We examine the joint upregulation of WHSC1 and FGFR3 in samples with t(4;14)-related fusions, and we illustrate a method for detecting fusions from single cell RNA-seq. We report fusions at MYC and a neighboring gene, PVT1, which are related to MYC translocations and associated with divergent progression-free survival patterns. Finally, we find that 4% of patients may be eligible for targeted fusion therapies, including three with an NTRK1 fusion.

Targeting enhancer switching overcomes non-genetic drug resistance in acute myeloid leukaemia

Nature Communications (IF: 13.61), 2019-6, Rab K Prinjha et al. DOI: 10.1038/s41467-019-10652-9
PubMed: 31222014

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Non-genetic drug resistance is increasingly recognised in various cancers. Molecular insights into this process are lacking and it is unknown whether stable non-genetic resistance can be overcome. Using single cell RNA-sequencing of paired drug naïve and resistant AML patient samples and cellular barcoding in a unique mouse model of non-genetic resistance, here we demonstrate that transcriptional plasticity drives stable epigenetic resistance. With a CRISPR-Cas9 screen we identify regulators of enhancer function as important modulators of the resistant cell state. We show that inhibition of Lsd1 (Kdm1a) is able to overcome stable epigenetic resistance by facilitating the binding of the pioneer factor, Pu.1 and cofactor, Irf8, to nucleate new enhancers that regulate the expression of key survival genes. This enhancer switching results in the re-distribution of transcriptional co-activators, including Brd4, and provides the opportunity to disable their activity and overcome epigenetic resistance. Together these findings highlight key principles to help counteract non-genetic drug resistance.

Heterogeneity of human bone marrow and blood natural killer cells defined by single-cell transcriptome

Nature Communications (IF: 13.61), 2019-9, Monica S Thakar et al. DOI: 10.1038/s41467-019-11947-7

PubMed: 31477722

Single Cell Gene Expression, Human, Immunology.

Abstract

Natural killer (NK) cells are critical to both innate and adaptive immunity. However, the development and heterogeneity of human NK cells are yet to be fully defined. Using single-cell RNA-sequencing technology, here we identify distinct NK populations in human bone marrow and blood, including one population expressing higher levels of immediate early genes indicative of a homeostatic activation. Functionally matured NK cells with high expression of CX3CR1, HAVCR2 (TIM-3), and ZEB2 represents terminally differentiated status with the unique transcriptional profile. Transcriptomic and pseudotime analyses identify a transitional population between CD56^{low} and CD56^{high} NK cells. Finally, a donor with GATA2 mutation exhibits reduced percentage of CD56^{high} NK cells with altered transcriptome and elevated cell death. These data expand our understanding of the heterogeneity and development of human NK cells.

Global prediction of chromatin accessibility using small-cell-number and single-cell RNA-seq

Nucleic Acids Research (IF: 11.8), 2019-11, Weixiang Fang et al. DOI: 10.1093/nar/gkz716 PubMed: 31428792

Single Cell Gene Expression, Human, Computational Method.

Abstract

Conventional high-throughput genomic technologies for mapping regulatory element activities in bulk samples such as ChIP-seq, DNase-seq and FAIRE-seq cannot analyze samples with small numbers of cells. The recently developed low-input and single-cell regulome mapping technologies such as ATAC-seq and single-cell ATAC-seq (scATAC-seq) allow analyses of small-cell-number and single-cell samples, but their signals remain highly discrete or noisy. Compared to these regulome mapping technologies, transcriptome profiling by RNA-seq is more widely used. Transcriptome data in single-cell and small-cell-number samples are more continuous and often less noisy. Here, we show that one can globally predict chromatin accessibility and infer regulatory element activities using RNA-seq. Genome-wide chromatin accessibility predicted by RNA-seq from 30 cells can offer better accuracy than ATAC-seq from 500 cells. Predictions based on single-cell RNA-seq (scRNA-seq) can more accurately reconstruct bulk chromatin accessibility than using scATAC-seq. Integrating ATAC-seq with predictions from RNA-seq increases the power and value of both methods. Thus, transcriptome-based prediction provides a new tool for decoding gene regulatory circuitry in samples with limited cell numbers.

Putative regulators for the continuum of erythroid differentiation revealed by single-cell transcriptome of human BM and UCB cells

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, Xinghua Pan et al. DOI: 10.1073/pnas.1915085117 PubMed: 32457162
Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Fine-resolution differentiation trajectories of adult human hematopoietic stem cells (HSCs) involved in the generation of red cells is critical for understanding dynamic developmental changes that accompany human erythropoiesis. Using single-cell RNA sequencing (scRNA-seq) of primary human terminal erythroid cells (CD34CD235a) isolated directly from adult bone marrow (BM) and umbilical cord blood (UCB), we documented the transcriptome of terminally differentiated human erythroblasts at unprecedented resolution. The insights enabled us to distinguish polychromatic erythroblasts (PolyEs) at the early and late stages of development as well as the different development stages of orthochromatic erythroblasts (OrthoEs). We further identified a set of putative regulators of terminal erythroid differentiation and functionally validated three of the identified genes and by monitoring cell differentiation and apoptosis. We documented that knockdown of suppressed the commitment of HSCs to erythroid lineage and cell proliferation and delayed differentiation of colony-forming unit-erythroid (CFU-E) to the proerythroblast stage (ProE). In contrast, the knockdown of and delayed differentiation of PolyE to OrthoE stage. Taken together, the convergence and divergence of the transcriptional continuums at single-cell resolution underscore the transcriptional regulatory networks that underlie human fetal and adult terminal erythroid differentiation.

Mouse models of neutropenia reveal progenitor-stage-specific defects

Nature (IF: 46.49), 2020-6, Nathan Salomonis et al. DOI: 10.1038/s41586-020-2227-7 PubMed: 32494068
Single Cell Gene Expression & Single Cell ATAC, Mouse, Genetic Health, Immunology.

Abstract

Advances in genetics and sequencing have identified a plethora of disease-associated and disease-causing genetic alterations. To determine causality between genetics and disease, accurate models for molecular dissection are required; however, the rapid expansion of transcriptional populations identified through single-cell analyses presents a major challenge for accurate comparisons between mutant and wild-type cells. Here we generate mouse models of human severe congenital neutropenia (SCN) using patient-derived mutations in the GFI1 transcription factor. To determine the effects of SCN mutations, we generated single-cell references for granulopoietic genomic states with linked epitopes, aligned mutant cells to their wild-type equivalents and identified differentially expressed genes and epigenetic loci. We find that GFI1-target genes are altered sequentially, as cells go through successive states of differentiation. These insights facilitated the genetic rescue of granulocytic specification but not post-commitment defects in innate immune effector function, and underscore the importance of evaluating the effects of mutations and therapy within each relevant cell state.

Alterations in the Transcriptional Programs of Myeloma Cells and the Microenvironment during Extramedullary Progression Affect Proliferation and Immune Evasion

Clinical cancer research (IF: 10.12), 2020-2, Kihyun Kim et al. DOI: 10.1158/1078-0432.CCR-19-0694

PubMed: 31558476

Single Cell Gene Expression, Human, Cancer Research.

Abstract

In multiple myeloma, extramedullary progression is associated with treatment resistance and a high mortality rate. To understand the molecular mechanisms controlling the devastating progression of myeloma, we applied single-cell RNA-sequencing (RNA-seq) to myeloma in the bone marrow and myelomatous pleural effusions or ascites. Bone marrow or extramedullary myeloma samples were collected from 15 patients and subjected to single-cell RNA-seq. The single-cell transcriptome data of malignant plasma cells and the surrounding immune microenvironment were analyzed. Comparisons of single-cell transcriptomes revealed the systematic activation of proliferation, antigen presentation, proteasomes, glycolysis, and oxidative phosphorylation pathways in extramedullary myeloma cells. The myeloma cells expressed multiple combinations of growth factors and receptors, suggesting autonomous and pleiotropic growth potential at the single-cell level. Comparisons of the tumor microenvironment revealed the presence of cytotoxic T lymphocytes and natural killer (NK) cells in both the bone marrow and extramedullary ascites, demonstrating a gene-expression phenotype indicative of functional compromise. In parallel, isolated myeloma cells persistently expressed class I MHC molecules and upregulated inhibitory molecules for cytotoxic T and NK cells. These data suggest that myeloma cells are equipped with specialized immune evasion mechanisms in cytotoxic microenvironments. Taken together, single-cell transcriptome analysis revealed transcriptional programs associated with aggressive myeloma progression that support autonomous cell proliferation and immune evasion.

A Cellular Taxonomy of the Bone Marrow Stroma in Homeostasis and Leukemia

Cell (IF: 38.62), 2019-6, Aviv Regev et al. DOI: 10.1016/j.cell.2019.04.040 PubMed: 31130381

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Stroma is a poorly defined non-parenchymal component of virtually every organ with key roles in organ development, homeostasis, and repair. Studies of the bone marrow stroma have defined individual populations in the stem cell niche regulating hematopoietic regeneration and capable of initiating leukemia. Here, we use single-cell RNA sequencing (scRNA-seq) to define a cellular taxonomy of the mouse bone marrow stroma and its perturbation by malignancy. We identified seventeen stromal subsets expressing distinct hematopoietic regulatory genes spanning new fibroblastic and osteoblastic subpopulations including distinct osteoblast differentiation trajectories. Emerging acute myeloid leukemia impaired mesenchymal osteogenic differentiation and reduced regulatory molecules necessary for normal hematopoiesis. These data suggest that tissue stroma responds to malignant cells by disadvantaging normal parenchymal cells. Our taxonomy of the stromal compartment provides a comprehensive bone marrow cell census and experimental support for cancer cell crosstalk with specific stromal elements to impair normal tissue function and thereby enable emergent cancer.

A single-cell molecular map of mouse gastrulation and early organogenesis

Nature (IF: 46.49), 2019-2, John C Marioni et al. DOI: 10.1038/s41586-019-0933-9 PubMed: 30787436

Single Cell Gene Expression, Mouse, Cell Atlas, Developmental Biology.

Abstract

Across the animal kingdom, gastrulation represents a key developmental event during which embryonic pluripotent cells diversify into lineage-specific precursors that will generate the adult organism. Here we report the transcriptional profiles of 116,312 single cells from mouse embryos collected at nine sequential time points ranging from 6.5 to 8.5 days post-fertilization. We construct a molecular map of cellular differentiation from pluripotency towards all major embryonic lineages, and explore the complex events involved in the convergence of visceral and primitive streak-derived endoderm. Furthermore, we use single-cell profiling to show that *Tal1* chimeric embryos display defects in early mesoderm diversification, and we thus demonstrate how combining temporal and transcriptional information can illuminate gene function. Together, this comprehensive delineation of mammalian cell differentiation trajectories in vivo represents a baseline for understanding the effects of gene mutations during development, as well as a roadmap for the optimization of in vitro differentiation protocols for regenerative medicine.

The bone marrow microenvironment at single-cell resolution

Nature (IF: 46.49), 2019-4, Aristotelis Tsirigos et al. DOI: 10.1038/s41586-019-1104-8 PubMed: 30971824

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

The bone marrow microenvironment has a key role in regulating haematopoiesis, but its molecular complexity and response to stress are incompletely understood. Here we map the transcriptional landscape of mouse bone marrow vascular, perivascular and osteoblast cell populations at single-cell resolution, both at homeostasis and under conditions of stress-induced haematopoiesis. This analysis revealed previously unappreciated levels of cellular heterogeneity within the bone marrow niche and resolved cellular sources of pro-haematopoietic growth factors, chemokines and membrane-bound ligands. Our studies demonstrate a considerable transcriptional remodelling of niche elements under stress conditions, including an adipocytic skewing of perivascular cells. Among the stress-induced changes, we observed that vascular Notch delta-like ligands (encoded by *Dll1* and *Dll4*) were downregulated. In the absence of vascular *Dll4*, haematopoietic stem cells prematurely induced a myeloid transcriptional program. These findings refine our understanding of the cellular architecture of the bone marrow niche, reveal a dynamic and heterogeneous molecular landscape that is highly sensitive to stress and illustrate the utility of single-cell transcriptomic data in evaluating the regulation of haematopoiesis by discrete niche populations.

M. tuberculosis Reprograms Hematopoietic Stem Cells to Limit Myelopoiesis and Impair Trained Immunity

Cell (IF: 38.62), 2020-10, Luis B Barreiro et al. DOI: 10.1016/j.cell.2020.09.062 PubMed: 33125891

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

A greater understanding of hematopoietic stem cell (HSC) regulation is required for dissecting protective versus detrimental immunity to pathogens that cause chronic infections such as *Mycobacterium tuberculosis* (Mtb). We have shown that systemic administration of Bacille Calmette-Guérin (BCG) or β -glucan reprograms HSCs in the bone marrow (BM) via a type II interferon (IFN-II) or interleukin-1 (IL1) response, respectively, which confers protective trained immunity against Mtb. Here, we demonstrate that, unlike BCG or β -glucan, Mtb reprograms HSCs via an IFN-I response that suppresses myelopoiesis and impairs development of protective trained immunity to Mtb. Mechanistically, IFN-I signaling dysregulates iron metabolism, depolarizes mitochondrial membrane potential, and induces cell death specifically in myeloid progenitors. Additionally, activation of the IFN-I/iron axis in HSCs impairs trained immunity to Mtb infection. These results identify an unanticipated immune evasion strategy of Mtb in the BM that controls the magnitude and intrinsic anti-microbial capacity of innate immunity to infection.

Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens

Cell (IF: 38.62), 2016-12, Nir Friedman et al. DOI: 10.1016/j.cell.2016.11.038 PubMed: 27984732

Single Cell Gene Expression, Mouse, Functional Genomics, Single Cell Multiomics Method.

Abstract

Genetic screens help infer gene function in mammalian cells, but it has remained difficult to assay complex phenotypes—such as transcriptional profiles—at scale. Here, we develop Perturb-seq, combining single-cell RNA sequencing (RNA-seq) and clustered regularly interspaced short palindromic repeats (CRISPR)-based perturbations to perform many such assays in a pool. We demonstrate Perturb-seq by analyzing 200,000 cells in immune cells and cell lines, focusing on transcription factors regulating the response of dendritic cells to lipopolysaccharide (LPS). Perturb-seq accurately identifies individual gene targets, gene signatures, and cell states affected by individual perturbations and their genetic interactions. We posit new functions for regulators of differentiation, the anti-viral response, and mitochondrial function during immune activation. By decomposing many high content measurements into the effects of perturbations, their interactions, and diverse cell metadata, Perturb-seq dramatically increases the scope of pooled genomic assays.

DNA methylation disruption reshapes the hematopoietic differentiation landscape

Nature Genetics (IF: 30.33), 2020-4, Omar Abdel-Wahab et al. DOI: 10.1038/s41588-020-0595-4

PubMed: 32203468

Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

Mutations in genes involved in DNA methylation (DNAm; for example, TET2 and DNMT3A) are frequently observed in hematological malignancies and clonal hematopoiesis. Applying single-cell sequencing to murine hematopoietic stem and progenitor cells, we observed that these mutations disrupt hematopoietic differentiation, causing opposite shifts in the frequencies of erythroid versus myelomonocytic progenitors following Tet2 or Dnmt3a loss. Notably, these shifts trace back to transcriptional priming skews in uncommitted hematopoietic stem cells. To reconcile genome-wide DNAm changes with specific erythroid versus myelomonocytic skews, we provide evidence in support of differential sensitivity of transcription factors due to biases in CpG enrichment in their binding motif. Single-cell transcriptomes with targeted genotyping showed similar skews in transcriptional priming of DNMT3A-mutated human clonal hematopoiesis bone marrow progenitors. These data show that DNAm shapes the topography of hematopoietic differentiation, and support a model in which genome-wide methylation changes are transduced to differentiation skews through biases in CpG enrichment of the transcription factor binding motif.

Affinity-Restricted Memory B Cells Dominate Recall Responses to Heterologous Flaviviruses

Immunity (IF: 25.73), 2020-11, Michael S Diamond et al. DOI: 10.1016/j.immuni.2020.09.001 PubMed: 33010224

Single Cell Immune Profiling, Mouse, Immuno-oncology, Immunology.

Abstract

Memory B cells (MBCs) can respond to heterologous antigens either by molding new specificities through secondary germinal centers (GCs) or by selecting preexisting clones without further affinity maturation. To distinguish these mechanisms in flavivirus infections and immunizations, we studied recall responses to envelope protein domain III (DIII). Conditional deletion of activation-induced cytidine deaminase (AID) between heterologous challenges of West Nile, Japanese encephalitis, Zika, and dengue viruses did not affect recall responses. DIII-specific MBCs were contained mostly within the plasma-cell-biased CD80 subset, and few GCs arose following heterologous boosters, demonstrating that recall responses are confined by preexisting clonal diversity. Measurement of monoclonal antibody (mAb) binding affinity to DIII proteins, timed AID deletion, single-cell RNA sequencing, and lineage tracing experiments point to selection of relatively low-affinity MBCs as a mechanism to promote diversity. Engineering immunogens to avoid this MBC diversity may facilitate flavivirus-type-specific vaccines with minimized potential for infection enhancement.

Resolving Fates and Single-Cell Transcriptomes of Hematopoietic Stem Cell Clones by PolyloxExpress Barcoding

Cell Stem Cell (IF: 23.45), 2020-9, Thomas Höfer et al. DOI: 10.1016/j.stem.2020.07.018 PubMed: 32783885
Single Cell Gene Expression, Mouse, Single Cell Multiomics Method.

Abstract

Lineage tracing reveals hematopoietic stem cell (HSC) fates, while single-cell RNA sequencing identifies snapshots of HSC transcriptomes. To obtain information on fate plus transcriptome in the same cell, we developed the PolyloxExpress allele, enabling Cre-recombinase-dependent RNA barcoding in situ. Linking fates to single HSC transcriptomes provided the information required to identify transcriptional signatures of HSC fates, which were not apparent in single-HSC transcriptomes alone. We find that differentiation-inactive, multilineage, and lineage-restricted HSC clones reside in distinct regions of the transcriptional landscape of hematopoiesis. Differentiation-inactive HSC clones are closer to the origin of the transcriptional trajectory, yet they are not characterized by a quiescent gene signature. Fate-specific gene signatures imply coherence of clonal HSC fates, and HSC output toward short-lived lineage progenitors indicates stability of HSC fates over time. These combined analyses of fate and transcriptome under physiological conditions may pave the way toward identifying molecular determinants of HSC fates.

Cohesin Members Stag1 and Stag2 Display Distinct Roles in Chromatin Accessibility and Topological Control of HSC Self-Renewal and Differentiation

Cell Stem Cell (IF: 23.45), 2019-11, Richard Koche et al. DOI: 10.1016/j.stem.2019.08.003 PubMed: 31495782
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Transcriptional regulators, including the cohesin complex member STAG2, are recurrently mutated in cancer. The role of STAG2 in gene regulation, hematopoiesis, and tumor suppression remains unresolved. We show that Stag2 deletion in hematopoietic stem and progenitor cells (HSPCs) results in altered hematopoietic function, increased self-renewal, and impaired differentiation. Chromatin immunoprecipitation (ChIP) sequencing revealed that, although Stag2 and Stag1 bind a shared set of genomic loci, a component of Stag2 binding sites is unoccupied by Stag1, even in Stag2-deficient HSPCs. Although concurrent loss of Stag2 and Stag1 abrogated hematopoiesis, Stag2 loss alone decreased chromatin accessibility and transcription of lineage-specification genes, including Ebf1 and Pax5, leading to increased self-renewal and reduced HSPC commitment to the B cell lineage. Our data illustrate a role for Stag2 in transformation and transcriptional dysregulation distinct from its shared role with Stag1 in chromosomal segregation.

RBPJ-dependent Notch signaling initiates the T cell program in a subset of thymus-seeding progenitors

Nature Immunology (IF: 22.3), 2019-10, Patrycja K Thompson et al. DOI: 10.1038/s41590-019-0518-7

PubMed: 31636466

Single Cell Gene Expression, Mouse, Functional Genomics, Immunology.

Abstract

T cell specification and commitment require Notch signaling. Although the requirement for Notch signaling during intrathymic T cell development is known, it is still unclear whether the onset of T cell priming can occur in a prethymic niche and whether RBPJ-dependent Notch signaling has a role during this event. Here, we established an Rbpj-inducible system that allowed temporal and tissue-specific control of the responsiveness to Notch in all hematopoietic cells. Using this system, we found that Notch signaling was required before the early T cell progenitor stage in the thymus. Lymphoid-primed multipotent progenitors in the bone marrow underwent Notch signaling with Rbpj induction, which inhibited development towards the myeloid lineage in thymus-seeding progenitors. Thus, our results indicated that the onset of T cell differentiation occurred in a prethymic setting, and that Notch played an important role during this event.

The E3 ubiquitin ligase SPOP controls resolution of systemic inflammation by triggering MYD88 degradation

Nature Immunology (IF: 22.3), 2019-8, Luca Busino et al. DOI: 10.1038/s41590-019-0454-6 PubMed: 31406379

Single Cell Gene Expression, Mouse, Immunology.

Abstract

The response to systemic infection and injury requires the rapid adaptation of hematopoietic stem cells (HSCs), which proliferate and divert their differentiation toward the myeloid lineage. Significant interest has emerged in understanding the signals that trigger the emergency hematopoietic program. However, the mechanisms that halt this response of HSCs, which is critical to restore homeostasis, remain unknown. Here we reveal that the E3 ubiquitin ligase Speckle-type BTB-POZ protein (SPOP) restrains the inflammatory activation of HSCs. In the absence of SPOP, systemic inflammation proceeded in an unresolved manner, and the sustained response in the HSCs resulted in a lethal phenotype reminiscent of hyper-inflammatory syndrome or sepsis. Our proteomic studies decipher that SPOP restricted inflammation by ubiquitinating the innate signal transducer myeloid differentiation primary response protein 88 (MYD88). These findings unearth an HSC-intrinsic post-translational mechanism that is essential for reestablishing homeostasis after emergency hematopoiesis.

An Nfil3–Zeb2–Id2 pathway imposes Irf8 enhancer switching during cDC1 development

Nature Immunology (IF: 22.3), 2019-8, Theresa L Murphy et al. DOI: 10.1038/s41590-019-0449-3

PubMed: 31406377

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Classical type 1 dendritic cells (cDC1s) are required for antiviral and antitumor immunity, which necessitates an understanding of their development. Development of the cDC1 progenitor requires an E-protein-dependent enhancer located 41 kilobases downstream of the transcription start site of the transcription factor Irf8 (+41-kb Irf8 enhancer), but its maturation instead requires the Batf3-dependent +32-kb Irf8 enhancer. To understand this switch, we performed single-cell RNA sequencing of the common dendritic cell progenitor (CDP) and identified a cluster of cells that expressed transcription factors that influence cDC1 development, such as Nfil3, Id2 and Zeb2. Genetic epistasis among these factors revealed that Nfil3 expression is required for the transition from Zeb2 and Id2 CDPs to Zeb2 and Id2 CDPs, which represent the earliest committed cDC1 progenitors. This genetic circuit blocks E-protein activity to exclude plasmacytoid dendritic cell potential and explains the switch in Irf8 enhancer usage during cDC1 development.

The transcription factor TCF-1 enforces commitment to the innate lymphoid cell lineage

Nature Immunology (IF: 22.3), 2019-7, Keji Zhao et al. DOI: 10.1038/s41590-019-0445-7 PubMed: 31358996

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Innate lymphoid cells (ILCs) play important functions in immunity and tissue homeostasis, but their development is poorly understood. Through the use of single-cell approaches, we examined the transcriptional and functional heterogeneity of ILC progenitors, and studied the precursor-product relationships that link the subsets identified. This analysis identified two successive stages of ILC development within T cell factor 1-positive (TCF-1) early innate lymphoid progenitors (EILPs), which we named 'specified EILPs' and 'committed EILPs'. Specified EILPs generated dendritic cells, whereas this potential was greatly decreased in committed EILPs. TCF-1 was dispensable for the generation of specified EILPs, but required for the generation of committed EILPs. TCF-1 used a pre-existing regulatory landscape established in upstream lymphoid precursors to bind chromatin in EILPs. Our results provide insight into the mechanisms by which TCF-1 promotes developmental progression of ILC precursors, while constraining their dendritic cell lineage potential and enforcing commitment to ILC fate.

Erythromyeloid progenitors give rise to a population of osteoclasts that contribute to bone homeostasis and repair

Nature cell biology (IF: 20.96), 2020-1, Mari L Shinohara et al. DOI: 10.1038/s41556-019-0437-8

PubMed: 31907410

Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

Osteoclasts are multinucleated cells of the monocyte/macrophage lineage that degrade bone. Here, we used lineage tracing studies-labelling cells expressing Cx3cr1, Csf1r or Flt3-to identify the precursors of osteoclasts in mice. We identified an erythromyeloid progenitor (EMP)-derived osteoclast precursor population. Yolk-sac macrophages of EMP origin produced neonatal osteoclasts that can create a space for postnatal bone marrow haematopoiesis. Furthermore, EMPs gave rise to long-lasting osteoclast precursors that contributed to postnatal bone remodelling in both physiological and pathological settings. Our single-cell RNA-sequencing data showed that EMP-derived osteoclast precursors arose independently of the haematopoietic stem cell (HSC) lineage and the data from fate tracking of EMP and HSC lineages indicated the possibility of cell-cell fusion between these two lineages. Cx3cr1yolk-sac macrophage descendants resided in the adult spleen, and parabiosis experiments showed that these cells migrated through the bloodstream to the remodelled bone after injury.

Skewing of the population balance of lymphoid and myeloid cells by secreted and intracellular osteopontin

Nature Immunology (IF: 22.3), 2017-9, Simon G Gregory et al. DOI: 10.1038/ni.3791 PubMed: 28671690

Single Cell Gene Expression, Mouse, Immunology.

Abstract

The balance of myeloid populations and lymphoid populations must be well controlled. Here we found that osteopontin (OPN) skewed this balance during pathogenic conditions such as infection and autoimmunity. Notably, two isoforms of OPN exerted distinct effects in shifting this balance through cell-type-specific regulation of apoptosis. Intracellular OPN (iOPN) diminished the population size of myeloid progenitor cells and myeloid cells, and secreted OPN (sOPN) increase the population size of lymphoid cells. The total effect of OPN on skewing the leukocyte population balance was observed as host sensitivity to early systemic infection with *Candida albicans* and T cell-mediated colitis. Our study suggests previously unknown detrimental roles for two OPN isoforms in causing the imbalance of leukocyte populations.

Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization

Nature cell biology (IF: 20.96), 2019-12, Andreas Trumpp et al. DOI: 10.1038/s41556-019-0439-6

PubMed: 31871321

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

The bone marrow constitutes the primary site for life-long blood production and skeletal regeneration. However, its cellular and spatial organization remains controversial. Here, we combine single-cell and spatially resolved transcriptomics to systematically map the molecular, cellular and spatial composition of distinct bone marrow niches. This allowed us to transcriptionally profile all major bone-marrow-resident cell types, determine their localization and clarify sources of pro-haematopoietic factors. Our data demonstrate that Cxcl12-abundant-reticular (CAR) cell subsets (Adipo-CAR and Osteo-CAR) differentially localize to sinusoidal and arteriolar surfaces, act locally as 'professional cytokine-secreting cells' and thereby establish peri-vascular micro-niches. Importantly, the three-dimensional bone-marrow organization can be accurately inferred from single-cell transcriptome data using the RNA-Magnet algorithm described here. Together, our study reveals the cellular and spatial organization of bone marrow niches and offers a systematic approach to dissect the complex organization of whole organs.

Two Aldehyde Clearance Systems Are Essential to Prevent Lethal Formaldehyde Accumulation in Mice and Humans

Molecular Cell (IF: 16.13), 2020-10, Minoru Takata et al. DOI: 10.1016/j.molcel.2020.10.012 PubMed: 33147438

Single Cell Gene Expression, Mouse, Cancer Research, Genetic Health.

Abstract

Reactive aldehydes arise as by-products of metabolism and are normally cleared by multiple families of enzymes. We find that mice lacking two aldehyde detoxifying enzymes, mitochondrial ALDH2 and cytoplasmic ADH5, have greatly shortened lifespans and develop leukemia. Hematopoiesis is disrupted profoundly, with a reduction of hematopoietic stem cells and common lymphoid progenitors causing a severely depleted acquired immune system. We show that formaldehyde is a common substrate of ALDH2 and ADH5 and establish methods to quantify elevated blood formaldehyde and formaldehyde-DNA adducts in tissues. Bone-marrow-derived progenitors actively engage DNA repair but also imprint a formaldehyde-driven mutation signature similar to aging-associated human cancer mutation signatures. Furthermore, we identify analogous genetic defects in children causing a previously uncharacterized inherited bone marrow failure and pre-leukemic syndrome. Endogenous formaldehyde clearance alone is therefore critical for hematopoiesis and in limiting mutagenesis in somatic tissues.

VEGF-C protects the integrity of the bone marrow perivascular niche in mice

Blood (IF: 14.82), 2020-10, Hanna Mikkola et al. DOI: 10.1182/blood.2020005699 PubMed: 32842144

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Hematopoietic stem cells (HSCs) reside in the bone marrow (BM) stem cell niche, which provides a vital source of HSC regulatory signals. Radiation and chemotherapy disrupt the HSC niche, including its sinusoidal vessels and perivascular cells, contributing to delayed hematopoietic recovery. Thus, identification of factors that can protect the HSC niche during an injury could offer a significant therapeutic opportunity to improve hematopoietic regeneration. In this study, we identified a critical function for vascular endothelial growth factor-C (VEGF-C), that of maintaining the integrity of the BM perivascular niche and improving BM niche recovery after irradiation-induced injury. Both global and conditional deletion of *Vegfc* in endothelial or leptin receptor-positive (LepR+) cells led to a disruption of the BM perivascular niche. Furthermore, deletion of *Vegfc* from the microenvironment delayed hematopoietic recovery after transplantation by decreasing endothelial proliferation and LepR+ cell regeneration. Exogenous administration of VEGF-C via an adenoassociated viral vector improved hematopoietic recovery after irradiation by accelerating endothelial and LepR+ cell regeneration and by increasing the expression of hematopoietic regenerative factors. Our results suggest that preservation of the integrity of the perivascular niche via VEGF-C signaling could be exploited therapeutically to enhance hematopoietic regeneration.

A single-cell hematopoietic landscape resolves 8 lineage trajectories and defects in Kit mutant mice

Blood (IF: 14.82), 2018-5, Berthold Göttgens et al. DOI: 10.1182/blood-2017-12-821413 PubMed: 29588278

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Hematopoietic stem and progenitor cells (HSPCs) maintain the adult blood system, and their dysregulation causes a multitude of diseases. However, the differentiation journeys toward specific hematopoietic lineages remain ill defined, and system-wide disease interpretation remains challenging. Here, we have profiled 44 802 mouse bone marrow HSPCs using single-cell RNA sequencing to provide a comprehensive transcriptional landscape with entry points to 8 different blood lineages (lymphoid, megakaryocyte, erythroid, neutrophil, monocyte, eosinophil, mast cell, and basophil progenitors). We identified a common basophil/mast cell bone marrow progenitor and characterized its molecular profile at the single-cell level. Transcriptional profiling of 13 815 HSPCs from the c-Kit mutant (W/W) mouse model revealed the absence of a distinct mast cell lineage entry point, together with global shifts in cell type abundance. Proliferative defects were accompanied by reduced expression. Potential compensatory processes included upregulation of the integrated stress response pathway and downregulation of proapoptotic gene expression in erythroid progenitors, thus providing a template of how large-scale single-cell transcriptomic studies can bridge between molecular phenotypes and quantitative population changes.

Discrete populations of isotype-switched memory B lymphocytes are maintained in murine spleen and bone marrow

Nature Communications (IF: 13.61), 2020-5, Mir-Farzin Mashreghi et al. DOI: 10.1038/s41467-020-16464-6

PubMed: 32444631

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

At present, it is not clear how memory B lymphocytes are maintained over time, and whether only as circulating cells or also residing in particular tissues. Here we describe distinct populations of isotype-switched memory B lymphocytes (Bsm) of murine spleen and bone marrow, identified according to individual transcriptional signature and B cell receptor repertoire. A population of marginal zone-like cells is located exclusively in the spleen, while a population of quiescent Bsm is found only in the bone marrow. Three further resident populations, present in spleen and bone marrow, represent transitional and follicular B cells and B1 cells, respectively. A population representing 10-20% of spleen and bone marrow memory B cells is the only one qualifying as circulating. In the bone marrow, all cells individually dock onto VCAM1 stromal cells and, reminiscent of resident memory T and plasma cells, are void of activation, proliferation and mobility.

A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration

Nature Communications (IF: 13.61), 2020-1, Wanida Ono et al. DOI: 10.1038/s41467-019-14029

PubMed: 31949165

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Bone marrow stromal cells (BMSCs) are versatile mesenchymal cell populations underpinning the major functions of the skeleton, a majority of which adjoin sinusoidal blood vessels and express C-X-C motif chemokine ligand 12 (CXCL12). However, how these cells are activated during regeneration and facilitate osteogenesis remains largely unknown. Cell-lineage analysis using Cxcl12-creER mice reveals that quiescent Cxcl12-creER perisinusoidal BMSCs differentiate into cortical bone osteoblasts solely during regeneration. A combined single cell RNA-seq analysis demonstrate that these cells convert their identity into a skeletal stem cell-like state in response to injury, associated with upregulation of osteoblast-signature genes and activation of canonical Wnt signaling components along the single-cell trajectory. β -catenin deficiency in these cells indeed causes insufficiency in cortical bone regeneration. Therefore, quiescent Cxcl12-creER BMSCs transform into osteoblast precursor cells in a manner mediated by canonical Wnt signaling, highlighting a unique mechanism by which dormant stromal cells are enlisted for skeletal regeneration.

MLL-AF9 initiates transformation from fast-proliferating myeloid progenitors

Nature Communications (IF: 13.61), 2019-12, Smita Krishnaswamy et al. DOI: 10.1038/s41467-019-13666-5
PubMed: 31852898

Single Cell Gene Expression, Mouse, Cancer Research, Genetic Health.

Abstract

Cancer is a hyper-proliferative disease. Whether the proliferative state originates from the cell-of-origin or emerges later remains difficult to resolve. By tracking de novo transformation from normal hematopoietic progenitors expressing an acute myeloid leukemia (AML) oncogene MLL-AF9, we reveal that the cell cycle rate heterogeneity among granulocyte-macrophage progenitors (GMPs) determines their probability of transformation. A fast cell cycle intrinsic to these progenitors provide permissiveness for transformation, with the fastest cycling 3% GMPs acquiring malignancy with near certainty. Molecularly, we propose that MLL-AF9 preserves gene expression of the cellular states in which it is expressed. As such, when expressed in the naturally-existing, rapidly-cycling immature myeloid progenitors, this cell state becomes perpetuated, yielding malignancy. In humans, high CCND1 expression predicts worse prognosis for MLL fusion AMLs. Our work elucidates one of the earliest steps toward malignancy and suggests that modifying the cycling state of the cell-of-origin could be a preventative approach against malignancy.

B1 oligomerization regulates PML nuclear body biogenesis and leukemogenesis

Nature Communications (IF: 13.61), 2019-8, Zhu Chen et al. DOI: 10.1038/s41467-019-11746-0

PubMed: 31439836

Single Cell Gene Expression, Mouse, Cancer Research, Functional Genomics.

Abstract

ProMyelocyticLeukemia (PML) protein can polymerize into a mega-Dalton nuclear assembly of 0.1-2 μm in diameter. The mechanism of PML nuclear body biogenesis remains elusive. Here, PML is successfully purified. The gel filtration and ultracentrifugation analysis suggest a previously unrecognized sequential oligomerization mechanism via PML monomer, dimer, tetramer and N-mer. Consistently, PML B1-box structure (2.0 Å) and SAXS characterization reveal an unexpected networking by W157-, F158- and SD1-interfaces. Structure-based perturbations in these B1 interfaces not only impair oligomerization in vitro but also abolish PML sumoylation and nuclear body biogenesis in HeLa cell. More importantly, as demonstrated by in vivo study using transgenic mice, PML-RAR α (PR) F158E precludes leukemogenesis. In addition, single cell RNA sequencing analysis shows that B1 oligomerization is an important regulator in PML-RAR α -driven transactivation. Altogether, these results not only define a previously unrecognized B1-box oligomerization in PML, but also highlight oligomerization as an important factor in carcinogenesis.

Haploinsufficiency for DNA methyltransferase 3A predisposes hematopoietic cells to myeloid malignancies

Journal of clinical investigation (IF: 12.81), 2017-9, Christopher A Miller et al. DOI: 10.1172/JCI93041

PubMed: 28872462

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

The gene that encodes de novo DNA methyltransferase 3A (DNMT3A) is frequently mutated in acute myeloid leukemia genomes. Point mutations at position R882 have been shown to cause a dominant negative loss of DNMT3A methylation activity, but 15% of DNMT3A mutations are predicted to produce truncated proteins that could either have dominant negative activities or cause loss of function and haploinsufficiency. Here, we demonstrate that 3 of these mutants produce truncated, inactive proteins that do not dimerize with WT DNMT3A, strongly supporting the haploinsufficiency hypothesis. We therefore evaluated hematopoiesis in mice heterozygous for a constitutive null *Dnmt3a* mutation. With no other manipulations, *Dnmt3a*^{+/-} mice developed myeloid skewing over time, and their hematopoietic stem/progenitor cells exhibited a long-term competitive transplantation advantage. *Dnmt3a*^{+/-} mice also spontaneously developed transplantable myeloid malignancies after a long latent period, and 3 of 12 tumors tested had cooperating mutations in the Ras/MAPK pathway. The residual *Dnmt3a* allele was neither mutated nor downregulated in these tumors. The bone marrow cells of *Dnmt3a*^{+/-} mice had a subtle but statistically significant DNA hypomethylation phenotype that was not associated with gene dysregulation. These data demonstrate that haploinsufficiency for *Dnmt3a* alters hematopoiesis and predisposes mice (and probably humans) to myeloid malignancies by a mechanism that is not yet clear.

Membrane budding is a major mechanism of in vivo platelet biogenesis

Journal of experimental medicine (IF: 11.32), 2020-9, Edwin D Hawkins et al. DOI: 10.1084/jem.20191206

PubMed: 32706855

Single Cell Gene Expression, Mouse, Immunology.

Abstract

How platelets are produced by megakaryocytes in vivo remains controversial despite more than a century of investigation. Megakaryocytes readily produce proplatelet structures in vitro; however, visualization of platelet release from proplatelets in vivo has remained elusive. We show that within the native prenatal and adult environments, the frequency and rate of proplatelet formation is incompatible with the physiological demands of platelet replacement. We resolve this inconsistency by performing in-depth analysis of plasma membrane budding, a cellular process that has previously been dismissed as a source of platelet production. Our studies demonstrate that membrane budding results in the sustained release of platelets directly into the peripheral circulation during both fetal and adult life without induction of cell death or proplatelet formation. In support of this model, we demonstrate that in mice deficient for NF-E2 (the thrombopoietic master regulator), the absence of membrane budding correlates with failure of in vivo platelet production. Accordingly, we propose that membrane budding, rather than proplatelet formation, supplies the majority of the platelet biomass.

Focal adhesion protein Kindlin-2 regulates bone homeostasis in mice

Bone Research (IF: 10.64), 2020-1, Xu Cao et al. DOI: 10.1038/s41413-019-0073-8 PubMed: 31934494

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Our recent studies demonstrate that the focal adhesion protein Kindlin-2 is critical for chondrogenesis and early skeletal development. Here, we show that deleting Kindlin-2 from osteoblasts using the 2.3-kb mouse transgene minimally impacts bone mass in mice, but deleting Kindlin-2 using the 10-kb mouse transgene, which targets osteocytes and mature osteoblasts, results in striking osteopenia in mice. Kindlin-2 loss reduces the osteoblastic population but increases the osteoclastic and adipocytic populations in the bone microenvironment. Kindlin-2 loss upregulates sclerostin in osteocytes, downregulates β -catenin in osteoblasts, and inhibits osteoblast formation and differentiation in vitro and in vivo. Upregulation of β -catenin in the mutant cells reverses the osteopenia induced by Kindlin-2 deficiency. Kindlin-2 loss additionally increases the expression of RANKL in osteocytes and increases osteoclast formation and bone resorption. Kindlin-2 deletion in osteocytes promotes osteoclast formation in osteocyte/bone marrow monocyte cocultures, which is significantly blocked by an anti-RANKL-neutralizing antibody. Finally, Kindlin-2 loss increases osteocyte apoptosis and impairs osteocyte spreading and dendrite formation. Thus, we demonstrate an important role of Kindlin-2 in the regulation of bone homeostasis and provide a potential target for the treatment of metabolic bone diseases.

Dissection of progenitor compartments resolves developmental trajectories in B-lymphopoiesis

Journal of experimental medicine, (IF: 11.32), 2018-7, Göran Karlsson et al. DOI: 10.1084/jem.20171384

PubMed: 29899037

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

To understand the developmental trajectories in early lymphocyte differentiation, we identified differentially expressed surface markers on lineage-negative lymphoid progenitors (LPs). Single-cell polymerase chain reaction experiments allowed us to link surface marker expression to that of lineage-associated transcription factors (TFs) and identify GFRA2 and BST1 as markers of early B cells. Functional analyses in vitro and in vivo as well as single-cell gene expression analyses supported that surface expression of these proteins defined distinct subpopulations that include cells from both the classical common LPs (CLPs) and Fraction A compartments. The formation of the GFRA2-expressing stages of development depended on the TF EBF1, critical both for the activation of stage-specific target genes and modulation of the epigenetic landscape. Our data show that consecutive expression of Ly6D, GFRA2, and BST1 defines a developmental trajectory linking the CLP to the CD19 progenitor compartment.

Remethylation of Dnmt3a hematopoietic cells is associated with partial correction of gene dysregulation and reduced myeloid skewing

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-2, Christopher A Miller et al. DOI: 10.1073/pnas.1918611117 PubMed: 31996479

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Mutations in the DNA methyltransferase 3A (DNMT3A) gene are the most common cause of age-related clonal hematopoiesis (ARCH) in older individuals, and are among the most common initiating events for acute myeloid leukemia (AML). The most frequent mutation in AML patients (R882H) encodes a dominant-negative protein that reduces methyltransferase activity by ~80% in cells with heterozygous mutations, causing a focal, canonical DNA hypomethylation phenotype; this phenotype is partially recapitulated in murine bone marrow cells. To determine whether the hypomethylation phenotype of hematopoietic cells is reversible, we developed an inducible transgene to restore expression of DNMT3A in transplanted bone marrow cells from mice. Partial remethylation was detected within 1 wk, but near-complete remethylation required 6 mo. Remethylation was accurate, dynamic, and highly ordered, suggesting that differentially methylated regions have unique properties that may be relevant for their functions. Importantly, 22 wk of addback partially corrected dysregulated gene expression, and mitigated the expansion of myeloid cells. These data show that restoring expression can alter the epigenetic state created by loss of DNMT3A activity; this genetic proof-of-concept experiment suggests that this approach could be relevant for patients with ARCH or AML caused by loss-of-function mutations.

Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3

Genes & development (IF: 10.33), 2019-8, Markus Hafner et al. DOI: 10.1101/gad.325100.119 PubMed: 31221665

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Fetal hematopoietic stem and progenitor cells (HSPCs) hold promise to cure a wide array of hematological diseases, and we previously found a role for the RNA-binding protein (RBP) Lin28b in respecifying adult HSPCs to resemble their fetal counterparts. Here we show by single-cell RNA sequencing that Lin28b alone was insufficient for complete reprogramming of gene expression from the adult toward the fetal pattern. Using proteomics and in situ analyses, we found that Lin28b (and its closely related paralog, Lin28a) directly interacted with Igf2bp3, another RBP, and their enforced co-expression in adult HSPCs reactivated fetal-like B-cell development in vivo more efficiently than either factor alone. In B-cell progenitors, Lin28b and Igf2bp3 jointly stabilized thousands of mRNAs by binding at the same sites, including those of the B-cell regulators and as well as mRNA itself, forming an autoregulatory loop. Our results suggest that Lin28b and Igf2bp3 are at the center of a gene regulatory network that mediates the fetal-adult hematopoietic switch. A method to efficiently generate induced fetal-like hematopoietic stem cells (ifHSCs) will facilitate basic studies of their biology and possibly pave a path toward their clinical application.

Three-step transcriptional priming that drives the commitment of multipotent progenitors toward B cells

Genes & development (IF: 10.33), 2018-1, Hiroshi Kawamoto et al. DOI: 10.1101/gad.309575.117

PubMed: 29440259

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Stem cell fate is orchestrated by core transcription factors (TFs) and epigenetic modifications. Although regulatory genes that control cell type specification are identified, the transcriptional circuit and the cross-talk among regulatory factors during cell fate decisions remain poorly understood. To identify the time-lapse TF networks during B-lineage commitment, we used multipotent progenitors harboring a tamoxifen-inducible form of Id3, an in vitro system in which virtually all cells became B cells within 6 d by simply withdrawing 4-hydroxytamoxifen (4-OHT). Transcriptome and epigenome analysis at multiple time points revealed that ~10%-30% of differentially expressed genes were virtually controlled by the core TFs, including E2A, EBF1, and PAX5. Strikingly, we found unexpected transcriptional priming before the onset of the key TF program. Inhibition of the immediate early genes such as, and severely impaired the generation of B cells. Integration of multiple data sets, including transcriptome, protein interactome, and epigenome profiles, identified three representative transcriptional circuits. Single-cell RNA sequencing (RNA-seq) analysis of lymphoid progenitors in bone marrow strongly supported the three-step TF network model during specification of multipotent progenitors toward B-cell lineage in vivo. Thus, our findings will provide a blueprint for studying the normal and neoplastic development of B lymphocytes.

m6A RNA Methylation Maintains Hematopoietic Stem Cell Identity and Symmetric Commitment

Cell Reports (IF: 8.82), 2019-8, Dan A Landau et al. DOI: 10.1016/j.celrep.2019.07.032 PubMed: 31412241

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Stem cells balance cellular fates through asymmetric and symmetric divisions in order to self-renew or to generate downstream progenitors. Symmetric commitment divisions in stem cells are required for rapid regeneration during tissue damage and stress. The control of symmetric commitment remains poorly defined. Using single-cell RNA sequencing (scRNA-seq) in combination with transcriptomic profiling of HSPCs (hematopoietic stem and progenitor cells) from control and m⁶A methyltransferase Mett13 conditional knockout mice, we found that m⁶A-deficient hematopoietic stem cells (HSCs) fail to symmetrically differentiate. Dividing HSCs are expanded and are blocked in an intermediate state that molecularly and functionally resembles multipotent progenitors. Mechanistically, RNA methylation controls Myc mRNA abundance in differentiating HSCs. We identified MYC as a marker for HSC asymmetric and symmetric commitment. Overall, our results indicate that RNA methylation controls symmetric commitment and cell identity of HSCs and may provide a general mechanism for how stem cells regulate differentiation fate choice.

Reticular Fibroblasts Expressing the Transcription Factor WT1 Define a Stromal Niche that Maintains and Replenishes Splenic Red Pulp Macrophages

Immunity (IF: 25.73), 2020-7, Menna R Clatworthy et al. DOI: 10.1016/j.immuni.2020.06.008 PubMed: 32562599
Single Cell Gene Expression, Human & Mouse, Cancer Research, Immunology.

Abstract

Located within red pulp cords, splenic red pulp macrophages (RPMs) are constantly exposed to the blood flow, clearing senescent red blood cells (RBCs) and recycling iron from hemoglobin. Here, we studied the mechanisms underlying RPM homeostasis, focusing on the involvement of stromal cells as these cells perform anchoring and nurturing macrophage niche functions in lymph nodes and liver. Microscopy revealed that RPMs are embedded in a reticular meshwork of red pulp fibroblasts characterized by the expression of the transcription factor Wilms' Tumor 1 (WT1) and colony stimulating factor 1 (CSF1). Conditional deletion of *Csf1* in WT1 red pulp fibroblasts, but not white pulp fibroblasts, drastically altered the RPM network without altering circulating CSF1 levels. Upon RPM depletion, red pulp fibroblasts transiently produced the monocyte chemoattractants CCL2 and CCL7, thereby contributing to the replenishment of the RPM network. Thus, red pulp fibroblasts anchor and nurture RPM, a function likely conserved in humans.

Single-Cell Transcriptomics of Regulatory T Cells Reveals Trajectories of Tissue Adaptation

Immunity (IF: 25.73), 2019-2, Fiona Powrie et al. DOI: 10.1016/j.immuni.2019.01.001 PubMed: 30737144
Single Cell Gene Expression, Human, Mouse, Immunology.

Abstract

Non-lymphoid tissues (NLTs) harbor a pool of adaptive immune cells with largely unexplored phenotype and development. We used single-cell RNA-seq to characterize 35,000 CD4 regulatory (Treg) and memory (Tmem) T cells in mouse skin and colon, their respective draining lymph nodes (LNs) and spleen. In these tissues, we identified Treg cell subpopulations with distinct degrees of NLT phenotype. Subpopulation pseudotime ordering and gene kinetics were consistent in recruitment to skin and colon, yet the initial NLT-priming in LNs and the final stages of NLT functional adaptation reflected tissue-specific differences. Predicted kinetics were recapitulated using an in vivo melanoma-induction model, validating key regulators and receptors. Finally, we profiled human blood and NLT Treg and Tmem cells, and identified cross-mammalian conserved tissue signatures. In summary, we describe the relationship between Treg cell heterogeneity and recruitment to NLTs through the combined use of computational prediction and in vivo validation.

High-Dimensional Single-Cell Analysis Identifies Organ-Specific Signatures and Conserved NK Cell Subsets in Humans and Mice

Immunity (IF: 25.73), 2018-11, Emilie Narni-MancinelliEric Vivier DOI: 10.1016/j.immuni.2018.09.009

PubMed: 30413361

Single Cell Gene Expression, Human, Immunology.

Abstract

Natural killer (NK) cells are innate lymphoid cells (ILCs) involved in antimicrobial and antitumoral responses. Several NK cell subsets have been reported in humans and mice, but their heterogeneity across organs and species remains poorly characterized. We assessed the diversity of human and mouse NK cells by single-cell RNA sequencing on thousands of individual cells isolated from spleen and blood. Unbiased transcriptional clustering revealed two distinct signatures differentiating between splenic and blood NK cells. This analysis at single-cell resolution identified three subpopulations in mouse spleen and four in human spleen, and two subsets each in mouse and human blood. A comparison of transcriptomic profiles within and between species highlighted the similarity of the two major subsets, NK1 and NK2, across organs and species. This unbiased approach provides insight into the biology of NK cells and establishes a rationale for the translation of mouse studies to human physiology and disease.

scRNA-seq assessment of the human lung, spleen, and esophagus tissue stability after cold preservation

Genome Biology (IF: 19.04), 2019-12, O Stegle et al. DOI: 10.1186/s13059-019-1906 PubMed: 31892341

Single Cell Gene Expression, Human, Assay Method.

Abstract

The Human Cell Atlas is a large international collaborative effort to map all cell types of the human body. Single-cell RNA sequencing can generate high-quality data for the delivery of such an atlas. However, delays between fresh sample collection and processing may lead to poor data and difficulties in experimental design. This study assesses the effect of cold storage on fresh healthy spleen, esophagus, and lung from ≥ 5 donors over 72 h. We collect 240,000 high-quality single-cell transcriptomes with detailed cell type annotations and whole genome sequences of donors, enabling future eQTL studies. Our data provide a valuable resource for the study of these 3 organs and will allow cross-organ comparison of cell types. We see little effect of cold ischemic time on cell yield, total number of reads per cell, and other quality control metrics in any of the tissues within the first 24 h. However, we observe a decrease in the proportions of lung T cells at 72 h, higher percentage of mitochondrial reads, and increased contamination by background ambient RNA reads in the 72-h samples in the spleen, which is cell type specific. In conclusion, we present robust protocols for tissue preservation for up to 24 h prior to scRNA-seq analysis. This greatly facilitates the logistics of sample collection for Human Cell Atlas or clinical studies since it increases the time frames for sample processing.

Comprehensive Profiling of an Aging Immune System Reveals Clonal GZMK⁺ CD8⁺ T Cells as Conserved Hallmark of Inflammaging

Immunity (IF: 25.73), 2020-11, Marco Colonna et al. DOI: 10.1016/j.immuni.2020.11.005 PubMed: 33271118
Single Cell Immune Profiling & Single Cell ATAC, Human & Mouse, Immunology.

Abstract

Systematic understanding of immune aging on a whole-body scale is currently lacking. We characterized age-associated alterations in immune cells across multiple mouse organs using single-cell RNA and antigen receptor sequencing and flow cytometry-based validation. We defined organ-specific and common immune alterations and identified a subpopulation of age-associated granzyme K (GZMK)-expressing CD8T (Taa) cells that are distinct from T effector memory (Tem) cells. Taa cells were highly clonal, had specific epigenetic and transcriptional signatures, developed in response to an aged host environment, and expressed markers of exhaustion and tissue homing. Activated Taa cells were the primary source of GZMK, which enhanced inflammatory functions of non-immune cells. In humans, proportions of the circulating GZMKCD8T cell population that shares transcriptional and epigenetic signatures with mouse Taa cells increased during healthy aging. These results identify GZMKTaa cells as a potential target to address age-associated dysfunctions of the immune system.

MYC-induced human acute myeloid leukemia requires a continuing IL-3/GM-CSF costimulus

Blood (IF: 14.82), 2020-12, Martin Hirst et al. DOI: 10.1182/blood.2020006374 PubMed: 33301029
Single Cell Gene Expression, Human, Mouse, Cancer Research.

Abstract

Hematopoietic clones with leukemogenic mutations arise in healthy people as they age, but progression to acute myeloid leukemia (AML) is rare. Recent evidence suggests that the microenvironment may play an important role in modulating human AML population dynamics. To investigate this concept further, we examined the combined and separate effects of an oncogene (c-MYC) and exposure to interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and stem cell factor (SCF) on the experimental genesis of a human AML in xenografted immunodeficient mice. Initial experiments showed that normal human CD34⁺ blood cells transduced with a lentiviral MYC vector and then transplanted into immunodeficient mice produced a hierarchically organized, rapidly fatal, and serially transplantable blast population, phenotypically and transcriptionally similar to human AML cells, but only in mice producing IL-3, GM-CSF, and SCF transgenically or in regular mice in which the cells were exposed to IL-3 or GM-CSF delivered using a cotransduction strategy. In their absence, the MYC⁺ human cells produced a normal repertoire of lymphoid and myeloid progeny in transplanted mice for many months, but, on transfer to secondary mice producing the human cytokines, the MYC⁺ cells rapidly generated AML. Indistinguishable diseases were also obtained efficiently from both primitive (CD34⁺CD38⁻) and late granulocyte-macrophage progenitor (GMP) cells. These findings underscore the critical role that these cytokines can play in activating a malignant state in normally differentiating human hematopoietic cells in which MYC expression has been deregulated. They also introduce a robust experimental model of human leukemogenesis to further elucidate key mechanisms involved and test strategies to suppress them.

High throughput pMHC-I tetramer library production using chaperone-mediated peptide exchange

Nature Communications (IF: 13.61), 2020-4, Peter Smibert et al. DOI: 10.1038/s41467-020-15710-1

PubMed: 32312993

Single Cell Immune Profiling, Human, Assay Method, Immunology.

Abstract

Peptide exchange technologies are essential for the generation of pMHC-multimer libraries used to probe diverse, polyclonal TCR repertoires in various settings. Here, using the molecular chaperone TAPBPR, we develop a robust method for the capture of stable, empty MHC-I molecules comprising murine H2 and human HLA alleles, which can be readily tetramerized and loaded with peptides of choice in a high-throughput manner. Alternatively, catalytic amounts of TAPBPR can be used to exchange placeholder peptides with high affinity peptides of interest. Using the same system, we describe high throughput assays to validate binding of multiple candidate peptides on empty MHC-I/TAPBPR complexes. Combined with tetramer-barcoding via a multi-modal cellular indexing technology, ECCITE-seq, our approach allows a combined analysis of TCR repertoires and other T cell transcription profiles together with their cognate antigen specificities in a single experiment. The new approach allows TCR/pMHC interactions to be interrogated easily at large scale.

Interferon lambda promotes immune dysregulation and tissue inflammation in TLR7-induced lupus

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-3, Carmelo Carmona-Rivera et al. DOI: 10.1073/pnas.1916897117 PubMed: 32094169

Single Cell Gene Expression, Human, Mouse, Immunology.

Abstract

Type III IFN lambdas (IFN- λ) have recently been described as important mediators of immune responses at barrier surfaces. However, their role in autoimmune diseases such as systemic lupus erythematosus (SLE), a condition characterized by aberrant type I IFN signaling, has not been determined. Here, we identify a nonredundant role for IFN- λ in immune dysregulation and tissue inflammation in a model of TLR7-induced lupus. IFN- λ protein is increased in murine lupus and IFN- λ receptor (Ifn1r1) deficiency significantly reduces immune cell activation and associated organ damage in the skin and kidneys without effects on autoantibody production. Single-cell RNA sequencing in mouse spleen and human peripheral blood revealed that only mouse neutrophils and human B cells are directly responsive to this cytokine. Rather, IFN- λ activates keratinocytes and mesangial cells to produce chemokines that induce immune cell recruitment and promote tissue inflammation. These data provide insights into the immunobiology of SLE and identify type III IFNs as important factors for tissue-specific pathology in this disease.

Mucosal or systemic microbiota exposures shape the B cell repertoire

Nature (IF: 46.49), 2020-8, Stephanie C Ganal-Vonarburg et al. DOI: 10.1038/s41586-020-2564-6

PubMed: 32760003

Single Cell Immune Profiling, Mouse, Immunology, Microbiology.

Abstract

Colonization by the microbiota causes a marked stimulation of B cells and induction of immunoglobulin, but mammals colonized with many taxa have highly complex and individualized immunoglobulin repertoires. Here we use a simplified model of defined transient exposures to different microbial taxa in germ-free mice to deconstruct how the microbiota shapes the B cell pool and its functional responsiveness. We followed the development of the immunoglobulin repertoire in B cell populations, as well as single cells by deep sequencing. Microbial exposures at the intestinal mucosa generated oligoclonal responses that differed from those of germ-free mice, and from the diverse repertoire that was generated after intravenous systemic exposure to microbiota. The IgA repertoire—predominantly to cell-surface antigens—did not expand after dose escalation, whereas increased systemic exposure broadened the IgG repertoire to both microbial cytoplasmic and cell-surface antigens. These microbial exposures induced characteristic immunoglobulin heavy-chain repertoires in B cells, mainly at memory and plasma cell stages. Whereas sequential systemic exposure to different microbial taxa diversified the IgG repertoire and facilitated alternative specific responses, sequential mucosal exposure produced limited overlapping repertoires and the attrition of initial IgA binding specificities. This shows a contrast between a flexible response to systemic exposure with the need to avoid fatal sepsis, and a restricted response to mucosal exposure that reflects the generic nature of host-microbial mutualism in the mucosa.

MAIT cells are imprinted by the microbiota in early life and promote tissue repair

Science (IF: 44.37), 2019-10, Michael A Fischbach et al. DOI: 10.1126/science.aax6624 PubMed: 31649166

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

How early-life colonization and subsequent exposure to the microbiota affect long-term tissue immunity remains poorly understood. Here, we show that the development of mucosal-associated invariant T (MAIT) cells relies on a specific temporal window, after which MAIT cell development is permanently impaired. This imprinting depends on early-life exposure to defined microbes that synthesize riboflavin-derived antigens. In adults, cutaneous MAIT cells are a dominant population of interleukin-17A (IL-17A)-producing lymphocytes, which display a distinct transcriptional signature and can subsequently respond to skin commensals in an IL-1-, IL-18-, and antigen-dependent manner. Consequently, local activation of cutaneous MAIT cells promotes wound healing. Together, our work uncovers a privileged interaction between defined members of the microbiota and MAIT cells, which sequentially controls both tissue-imprinting and subsequent responses to injury.

Migrant memory B cells secrete luminal antibody in the vagina

Nature (IF: 46.49), 2019-6, Steven H Kleinstein et al. DOI: 10.1038/s41586-019-1285-1 PubMed: 31189952

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Antibodies secreted into mucosal barriers serve to protect the host from a variety of pathogens, and are the basis for successful vaccines. In type I mucosa (such as the intestinal tract), dimeric IgA secreted by local plasma cells is transported through polymeric immunoglobulin receptors and mediates robust protection against viruses. However, owing to the paucity of polymeric immunoglobulin receptors and plasma cells, how and whether antibodies are delivered to the type II mucosa represented by the lumen of the lower female reproductive tract remains unclear. Here, using genital herpes infection in mice, we show that primary infection does not establish plasma cells in the lamina propria of the female reproductive tract. Instead, upon secondary challenge with herpes simplex virus 2, circulating memory B cells that enter the female reproductive tract serve as the source of rapid and robust antibody secretion into the lumen of this tract. CD4 tissue-resident memory T cells secrete interferon- γ , which induces expression of chemokines, including CXCL9 and CXCL10. Circulating memory B cells are recruited to the vaginal mucosa in a CXCR3-dependent manner, and secrete virus-specific IgG2b, IgG2c and IgA into the lumen. These results reveal that circulating memory B cells act as a rapidly inducible source of mucosal antibodies in the female reproductive tract.

Identification of a T follicular helper cell subset that drives anaphylactic IgE

Science (IF: 44.37), 2019-8, Adam Williams et al. DOI: 10.1126/science.aaw6433 PubMed: 31371561

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Cross-linking of high-affinity immunoglobulin E (IgE) results in the life-threatening allergic reaction anaphylaxis. Yet the cellular mechanisms that induce B cells to produce IgE in response to allergens remain poorly understood. T follicular helper (T) cells direct the affinity and isotype of antibodies produced by B cells. Although Tcell-derived interleukin-4 (IL-4) is necessary for IgE production, it is not sufficient. We report a rare population of IL-13-producing Tcells present in mice and humans with IgE to allergens, but not when allergen-specific IgE was absent or only low-affinity. These T13 cells have an unusual cytokine profile (IL-13IL-4IL-5IL-21) and coexpress the transcription factors BCL6 and GATA3. T13 cells are required for production of high- but not low-affinity IgE and subsequent allergen-induced anaphylaxis. Blocking T13 cells may represent an alternative therapeutic target to ameliorate anaphylaxis.

In vivo CRISPR screening in CD8 T cells with AAV–Sleeping Beauty hybrid vectors identifies membrane targets for improving immunotherapy for glioblastoma

Nature Biotechnology (IF: 42.3), 2019-9, Youssef Errami et al. DOI: 10.1038/s41587-019-0246-4

PubMed: 31548728

Single Cell Gene Expression, Mouse, Functional Genomics, Immuno-oncology.

Abstract

Targeting membrane proteins could improve the efficacy of T cell-based immunotherapies. To facilitate the identification of T cell targets, we developed a hybrid genetic screening system where the Sleeping Beauty (SB) transposon and single guide RNA cassette are nested in an adeno-associated virus (AAV). SB-mediated genomic integration of the single guide RNA cassette enables efficient gene editing in primary murine T cells as well as a screen readout. We performed in vivo AAV-SB-CRISPR screens for membrane protein targets in CD8T cells in mouse models of glioblastoma (GBM). We validated screen hits by demonstrating that adoptive transfer of CD8T cells with *Pdia3*, *Mgat5*, *Emp1* or *Lag3* gene editing enhances the survival of GBM-bearing mice in both syngeneic and T-cell receptor transgenic models. Transcriptome profiling, single cell sequencing, cytokine assays and T cell signaling analysis showed that *Pdia3* editing in T cells enhances effector functions. Engineered PDIA3 mutant EGFRvIII chimeric antigen T cells are more potent in antigen-specific killing of human GBM cells.

The epigenetic control of stemness in CD8+ T cell fate commitment

Science (IF: 44.37), 2018-1, Geneviève Almouzni et al. DOI: 10.1126/science.aah6499 PubMed: 29326266

Single Cell Gene Expression, Mouse, Immunology.

Abstract

After priming, naïve CD8T lymphocytes establish specific heritable transcription programs that define progression to long-lasting memory cells or to short-lived effector cells. Although lineage specification is critical for protection, it remains unclear how chromatin dynamics contributes to the control of gene expression programs. We explored the role of gene silencing by the histone methyltransferase Suv39h1. In murine CD8T cells activated after infection, Suv39h1-dependent trimethylation of histone H3 lysine 9 controls the expression of a set of stem cell-related memory genes. Single-cell RNA sequencing revealed a defect in silencing of stem/memory genes selectively in defective T cell effectors. As a result, defective CD8T cells show sustained survival and increased long-term memory reprogramming capacity. Thus, Suv39h1 plays a critical role in marking chromatin to silence stem/memory genes during CD8T effector terminal differentiation.

Co-option of Neutrophil Fates by Tissue Environments

Cell (IF: 38.62), 2020-11, Renato Ostuni et al. DOI: 10.1016/j.cell.2020.10.003 PubMed: 33098771

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Classically considered short-lived and purely defensive leukocytes, neutrophils are unique in their fast and moldable response to stimulation. This plastic behavior may underlie variable and even antagonistic functions during inflammation or cancer, yet the full spectrum of neutrophil properties as they enter healthy tissues remains unexplored. Using a new model to track neutrophil fates, we found short but variable lifetimes across multiple tissues. Through analysis of the receptor, transcriptional, and chromatin accessibility landscapes, we identify varying neutrophil states and assign non-canonical functions, including vascular repair and hematopoietic homeostasis. Accordingly, depletion of neutrophils compromised angiogenesis during early age, genotoxic injury, and viral infection, and impaired hematopoietic recovery after irradiation. Neutrophils acquired these properties in target tissues, a process that, in the lungs, occurred in CXCL12-rich areas and relied on CXCR4. Our results reveal that tissues co-opt neutrophils en route for elimination to induce programs that support their physiological demands.

Single-Cell Transcriptome Atlas of Murine Endothelial Cells

Cell (IF: 38.62), 2020-2, Yonglun Luo et al. DOI: 10.1016/j.cell.2020.01.015 PubMed: 32059779

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

The heterogeneity of endothelial cells (ECs) across tissues remains incompletely inventoried. We constructed an atlas of >32,000 single-EC transcriptomes from 11 mouse tissues and identified 78 EC subclusters, including Aqp7 intestinal capillaries and angiogenic ECs in healthy tissues. ECs from brain/testis, liver/spleen, small intestine/colon, and skeletal muscle/heart pairwise expressed partially overlapping marker genes. Arterial, venous, and lymphatic ECs shared more markers in more tissues than did heterogeneous capillary ECs. ECs from different vascular beds (arteries, capillaries, veins, lymphatics) exhibited transcriptome similarity across tissues, but the tissue (rather than the vessel) type contributed to the EC heterogeneity. Metabolic transcriptome analysis revealed a similar tissue-grouping phenomenon of ECs and heterogeneous metabolic gene signatures in ECs between tissues and between vascular beds within a single tissue in a tissue-type-dependent pattern. The EC atlas taxonomy enabled identification of EC subclusters in public scRNA-seq datasets and provides a powerful discovery tool and resource value.

Mutant EZH2 Induces a Pre-malignant Lymphoma Niche by Reprogramming the Immune Response

Cancer Cell (IF: 30.24), 2020-1, Steven H Kleinstein et al. DOI: 10.1016/j.ccell.2020.04.004 PubMed: 32396861

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Follicular lymphomas (FLs) are slow-growing, indolent tumors containing extensive follicular dendritic cell (FDC) networks and recurrent EZH2 gain-of-function mutations. Paradoxically, FLs originate from highly proliferative germinal center (GC) B cells with proliferation strictly dependent on interactions with T follicular helper cells. Herein, we show that EZH2 mutations initiate FL by attenuating GC B cell requirement for T cell help and driving slow expansion of GC centrocytes that become enmeshed with and dependent on FDCs. By impairing T cell help, mutant EZH2 prevents induction of proliferative MYC programs. Thus, EZH2 mutation fosters malignant transformation by epigenetically reprogramming B cells to form an aberrant immunological niche that reflects characteristic features of human FLs, explaining how indolent tumors arise from GC B cells.

Rapid Enhancer Remodeling and Transcription Factor Repurposing Enable High Magnitude Gene Induction upon Acute Activation of NK Cells

Immunity (IF: 25.73), 2020-10, John J O'Shea et al. DOI: 10.1016/j.immuni.2020.09.008 PubMed: 33010223

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Innate immune responses rely on rapid and precise gene regulation mediated by accessibility of regulatory regions to transcription factors (TFs). In natural killer (NK) cells and other innate lymphoid cells, competent enhancers are primed during lineage acquisition, and formation of de novo enhancers characterizes the acquisition of innate memory in activated NK cells and macrophages. Here, we investigated how primed and de novo enhancers coordinate to facilitate high-magnitude gene induction during acute activation. Epigenomic and transcriptomic analyses of regions near highly induced genes (HIGs) in NK cells both in vitro and in a model of *Toxoplasma gondii* infection revealed de novo chromatin accessibility and enhancer remodeling controlled by signal-regulated TFs STATs. Acute NK cell activation redeployed the lineage-determining TF T-bet to de novo enhancers, independent of DNA-sequence-specific motif recognition. Thus, acute stimulation reshapes enhancer function through the combinatorial usage and repurposing of both lineage-determining and signal-regulated TFs to ensure an effective response.

Affinity-Restricted Memory B Cells Dominate Recall Responses to Heterologous Flaviviruses

Immunity (IF: 25.73), 2020-11, Michael S Diamond et al. DOI: 10.1016/j.immuni.2020.09.001 PubMed: 33010224
Single Cell Immune Profiling, Mouse, Immuno-oncology, Immunology.

Abstract

Memory B cells (MBCs) can respond to heterologous antigens either by molding new specificities through secondary germinal centers (GCs) or by selecting preexisting clones without further affinity maturation. To distinguish these mechanisms in flavivirus infections and immunizations, we studied recall responses to envelope protein domain III (DIII). Conditional deletion of activation-induced cytidine deaminase (AID) between heterologous challenges of West Nile, Japanese encephalitis, Zika, and dengue viruses did not affect recall responses. DIII-specific MBCs were contained mostly within the plasma-cell-biased CD80subset, and few GCs arose following heterologous boosters, demonstrating that recall responses are confined by preexisting clonal diversity. Measurement of monoclonal antibody (mAb) binding affinity to DIII proteins, timed AID deletion, single-cell RNA sequencing, and lineage tracing experiments point to selection of relatively low-affinity MBCs as a mechanism to promote diversity. Engineering immunogens to avoid this MBC diversity may facilitate flavivirus-type-specific vaccines with minimized potential for infection enhancement.

Single-Cell RNA Sequencing of Tumor-Infiltrating NK Cells Reveals that Inhibition of Transcription Factor HIF-1 α Unleashes NK Cell Activity

Immunity (IF: 25.73), 2020-6, Thomas Höfer et al. DOI: 10.1016/j.immuni.2020.05.001 PubMed: 32445619
Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Enhancing immune cell functions in tumors remains a major challenge in cancer immunotherapy. Hypoxia is a common feature of solid tumors, and cells adapt by upregulating the transcription factor HIF-1 α . Here, we defined the transcriptional landscape of mouse tumor-infiltrating natural killer (NK) cells by using single-cell RNA sequencing. Conditional deletion of Hif1a in NK cells resulted in reduced tumor growth, elevated expression of activation markers, effector molecules, and an enriched NF- κ B pathway in tumor-infiltrating NK cells. Interleukin-18 (IL-18) from myeloid cells was required for NF- κ B activation and the enhanced anti-tumor activity of Hif1aNK cells. Extended culture with an HIF-1 α inhibitor increased human NK cell responses. Low HIF1A expression was associated with high expression of IFNG in human tumor-infiltrating NK cells, and an enriched NK-IL18-IFNG signature in solid tumors correlated with increased overall patient survival. Thus, inhibition of HIF-1 α unleashes NK cell anti-tumor activity and could be exploited for cancer therapy.

Heterogenous Populations of Tissue-Resident CD8+ T Cells Are Generated in Response to Infection and Malignancy

Immunity (IF: 25.73), 2020-5, Kyla D Omilusik et al. DOI: 10.1016/j.immuni.2020.04.007 PubMed: 32433949

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

Tissue-resident memory CD8T cells (Trm) provide host protection through continuous surveillance of non-lymphoid tissues. Using single-cell RNA-sequencing (scRNA-seq) and genetic reporter mice, we identified discrete lineages of intestinal antigen-specific CD8T cells, including a Blimp1Id3tissue-resident effector cell population most prominent in the early phase of acute viral and bacterial infections and a molecularly distinct Blimp1Id3tissue-resident memory population that subsequently accumulated at later infection time points. These Trm populations exhibited distinct cytokine production, secondary memory potential, and transcriptional programs including differential roles for transcriptional regulators Blimp1, T-bet, Id2, and Id3 in supporting and maintaining intestinal Trm. Extending our analysis to malignant tissue, we also identified discrete populations of effector-like and memory-like CD8T cell populations with tissue-resident gene-expression signatures that shared features of terminally exhausted and progenitor-exhausted T cells, respectively. Our findings provide insight into the development and functional heterogeneity of Trm cells, which has implications for enhancing vaccination and immunotherapy approaches.

Precursors for Nonlymphoid-Tissue Treg Cells Reside in Secondary Lymphoid Organs and Are Programmed by the Transcription Factor BATF

Immunity (IF: 25.73), 2020-2, Christian Schmidl et al. DOI: 10.1016/j.immuni.2019.12.002 PubMed: 31924477

Single Cell Gene Expression & Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Specialized regulatory T (Treg) cells accumulate and perform homeostatic and regenerative functions in nonlymphoid tissues. Whether common precursors for nonlymphoid-tissue Treg cells exist and how they differentiate remain elusive. Using transcription factor nuclear factor, interleukin 3 regulated (Nfil3) reporter mice and single-cell RNA-sequencing (scRNA-seq), we identified two precursor stages of interleukin 33 (IL-33) receptor ST2-expressing nonlymphoid tissue Treg cells, which resided in the spleen and lymph nodes. Global chromatin profiling of nonlymphoid tissue Treg cells and the two precursor stages revealed a stepwise acquisition of chromatin accessibility and reprogramming toward the nonlymphoid-tissue Treg cell phenotype. Mechanistically, we identified and validated the transcription factor Batf as the driver of the molecular tissue program in the precursors. Understanding this tissue development program will help to harness regenerative properties of tissue Treg cells for therapy.

CD4+ T Cell Help Is Required for the Formation of a Cytolytic CD8+ T Cell Subset that Protects against Chronic Infection and Cancer

Immunity (IF: 25.73), 2019-12, Allan Zajac et al. DOI: 10.1016/j.immuni.2019.10.009 PubMed: 31810883

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Although CD4T cell help is crucial to sustain antiviral immunity, the mechanisms by which CD4T cells regulate CD8T cell differentiation during chronic infection remain elusive. Here, using single-cell RNA sequencing, we show that CD8T cells responding to chronic infection were more heterogeneous than previously appreciated. Importantly, our findings uncovered the formation of a CXCR1-expressing CD8T cell subset that exhibited potent cytolytic function and was required for viral control. Notably, our data further demonstrate that formation of this cytotoxic subset was critically dependent on CD4T cell help via interleukin-21 (IL-21) and that exploitation of this developmental pathway could be used therapeutically to enhance the killer function of CD8T cells infiltrated into the tumor. These findings uncover additional molecular mechanisms of how “CD4T cell help regulates CD8T cell differentiation during persistent infection and have implications toward optimizing the generation of protective CD8T cells in immunotherapy.

TCF-1-Centered Transcriptional Network Drives an Effector versus Exhausted CD8 T Cell-Fate Decision

Immunity (IF: 25.73), 2019-11, Hongkai Ji et al. DOI: 10.1016/j.immuni.2019.09.013 PubMed: 31606264

Single Cell Gene Expression, Mouse, Immunology.

Abstract

TCF-1 is a key transcription factor in progenitor exhausted CD8 T cells (Tex). Moreover, this Tex cell subset mediates responses to PD-1 checkpoint pathway blockade. However, the role of the transcription factor TCF-1 in early fate decisions and initial generation of Tex cells is unclear. Single-cell RNA sequencing (scRNA-seq) and lineage tracing identified a TCF-1^{Ly108}PD-1^{CD8} T cell population that seeds development of mature Tex cells early during chronic infection. TCF-1 mediated the bifurcation between divergent fates, repressing development of terminal KLRG1^{eff} while fostering KLRG1^{Tex} precursor cells, and PD-1 stabilized this TCF-1^{Tex} precursor cell pool. TCF-1 mediated a T-bet-to-Eomes transcription factor transition in Tex precursors by promoting Eomes expression and drove c-Myb expression that controlled Bcl-2 and survival. These data define a role for TCF-1 in early-fate-bifurcation-driving Tex precursor cells and also identify PD-1 as a protector of this early TCF-1 subset.

Intravenous nanoparticle vaccination generates stem-like TCF1+ neoantigen-specific CD8+ T cells

Nature Immunology (IF: 22.3), 2020-11, Andrew S Ishizuka et al. DOI: 10.1038/s41590-020-00810-3

PubMed: 33139915

Single Cell Immune Profiling, Mouse, Cancer Research, Immunology.

Abstract

Personalized cancer vaccines are a promising approach for inducing T cell immunity to tumor neoantigens. Using a self-assembling nanoparticle vaccine that links neoantigen peptides to a Toll-like receptor 7/8 agonist (SNP-7/8a), we show how the route and dose alter the magnitude and quality of neoantigen-specific CD8T cells. Intravenous vaccination (SNP-IV) induced a higher proportion of TCF1PD-1CD8T cells as compared to subcutaneous immunization (SNP-SC). Single-cell RNA sequencing showed that SNP-IV induced stem-like genes (Tcf7, Slamf6, Xcl1) whereas SNP-SC enriched for effector genes (Gzmb, Klrg1, Cx3cr1). Stem-like cells generated by SNP-IV proliferated and differentiated into effector cells upon checkpoint blockade, leading to superior antitumor response as compared to SNP-SC in a therapeutic model. The duration of antigen presentation by dendritic cells controlled the magnitude and quality of CD8T cells. These data demonstrate how to optimize antitumor immunity by modulating vaccine parameters for specific generation of effector or stem-like CD8T cells.

Transcriptome dynamics of CD4+ T cells during malaria maps gradual transit from effector to memory

Nature Immunology (IF: 22.3), 2020-12, Sarah A Teichmann et al. DOI: 10.1038/s41590-020-0800-8

PubMed: 33046889

Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

The dynamics of CD4T cell memory development remain to be examined at genome scale. In malaria-endemic regions, antimalarial chemoprevention protects long after its cessation and associates with effects on CD4T cells. We applied single-cell RNA sequencing and computational modelling to track memory development during Plasmodium infection and treatment. In the absence of central memory precursors, two trajectories developed as T helper 1 (T1) and follicular helper T (T) transcriptomes contracted and partially coalesced over three weeks. Progeny of single clones populated T1 and Ttrajectories, and fate-mapping suggested that there was minimal lineage plasticity. Relationships between Tand central memory were revealed, with antimalarials modulating these responses and boosting T1 recall. Finally, single-cell epigenomics confirmed that heterogeneity among effectors was partially reset in memory. Thus, the effector-to-memory transition in CD4T cells is gradual during malaria and is modulated by antiparasitic drugs. Graphical user interfaces are presented for examining gene-expression dynamics and gene-gene correlations (http://haquelab.mdhs.unimelb.edu.au/cd4_memory/).

Meningeal $\gamma\delta$ T cells regulate anxiety-like behavior via IL-17a signaling in neurons

Nature Immunology (IF: 22.3), 2020-11, Jasmin Herz et al. DOI: 10.1038/s41590-020-0776-4 PubMed: 32929273
Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Interleukin (IL)-17a has been highly conserved during evolution of the vertebrate immune system and widely studied in contexts of infection and autoimmunity. Studies suggest that IL-17a promotes behavioral changes in experimental models of autism and aggregation behavior in worms. Here, through a cellular and molecular characterization of meningeal $\gamma\delta$ 17 T cells, we defined the nearest central nervous system-associated source of IL-17a under homeostasis. Meningeal $\gamma\delta$ T cells express high levels of the chemokine receptor CXCR6 and seed meninges shortly after birth. Physiological release of IL-17a by these cells was correlated with anxiety-like behavior in mice and was partially dependent on T cell receptor engagement and commensal-derived signals. IL-17a receptor was expressed in cortical glutamatergic neurons under steady state and its genetic deletion decreased anxiety-like behavior in mice. Our findings suggest that IL-17a production by meningeal $\gamma\delta$ 17 T cells represents an evolutionary bridge between this conserved anti-pathogen molecule and survival behavioral traits in vertebrates.

PTPN2 regulates the generation of exhausted CD8+ T cell subpopulations and restrains tumor immunity

Nature Immunology (IF: 22.3), 2019-9, W Nicholas Haining et al. DOI: 10.1038/s41590-019-0480-4
PubMed: 31527834

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

CD8T cell exhaustion is a state of dysfunction acquired in chronic viral infection and cancer, characterized by the formation of Slamf6progenitor exhausted and Tim-3terminally exhausted subpopulations through unknown mechanisms. Here we establish the phosphatase PTPN2 as a new regulator of the differentiation of the terminally exhausted subpopulation that functions by attenuating type 1 interferon signaling. Deletion of Ptpn2 in CD8T cells increased the generation, proliferative capacity and cytotoxicity of Tim-3cells without altering Slamf6numbers during lymphocytic choriomeningitis virus clone 13 infection. Likewise, Ptpn2 deletion in CD8T cells enhanced Tim-3anti-tumor responses and improved tumor control. Deletion of Ptpn2 throughout the immune system resulted in MC38 tumor clearance and improved programmed cell death-1 checkpoint blockade responses to B16 tumors. Our results indicate that increasing the number of cytotoxic Tim-3CD8T cells can promote effective anti-tumor immunity and implicate PTPN2 in immune cells as an attractive cancer immunotherapy target.

The transcription factor TCF-1 enforces commitment to the innate lymphoid cell lineage

Nature Immunology (IF: 22.3), 2019-7, Keji Zhao et al. DOI: 10.1038/s41590-019-0445-7 PubMed: 31358996

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Innate lymphoid cells (ILCs) play important functions in immunity and tissue homeostasis, but their development is poorly understood. Through the use of single-cell approaches, we examined the transcriptional and functional heterogeneity of ILC progenitors, and studied the precursor-product relationships that link the subsets identified. This analysis identified two successive stages of ILC development within T cell factor 1-positive (TCF-1) early innate lymphoid progenitors (EILPs), which we named “specified EILPs” and “committed EILPs”. Specified EILPs generated dendritic cells, whereas this potential was greatly decreased in committed EILPs. TCF-1 was dispensable for the generation of specified EILPs, but required for the generation of committed EILPs. TCF-1 used a pre-existing regulatory landscape established in upstream lymphoid precursors to bind chromatin in EILPs. Our results provide insight into the mechanisms by which TCF-1 promotes developmental progression of ILC precursors, while constraining their dendritic cell lineage potential and enforcing commitment to ILC fate.

Select autophagy genes maintain quiescence of tissue-resident macrophages and increase susceptibility to *Listeria monocytogenes*

Nature Microbiology (IF: 16.29), 2020-1, Christina L Stallings et al. DOI: 10.1038/s41564-019-0633-0

PubMed: 31959973

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Innate and adaptive immune responses that prime myeloid cells, such as macrophages, protect against pathogens. However, if left uncontrolled, these responses may lead to detrimental inflammation. Macrophages, particularly those resident in tissues, must therefore remain quiescent between infections despite chronic stimulation by commensal microorganisms. The genes required for quiescence of tissue-resident macrophages are not well understood. Autophagy, an evolutionarily conserved cellular process by which cytoplasmic contents are targeted for lysosomal digestion, has homeostatic functions including maintenance of protein and organelle integrity and regulation of metabolism. Recent research has shown that degradative autophagy, as well as various combinations of autophagy genes, regulate immunity and inflammation. Here, we delineate a function of the autophagy proteins Beclin 1 and FIP200-but not of other essential autophagy components ATG5, ATG16L1 or ATG7-in mediating quiescence of tissue-resident macrophages by limiting the effects of systemic interferon- γ . The perturbation of quiescence in mice that lack Beclin 1 or FIP200 in myeloid cells results in spontaneous immune activation and resistance to *Listeria monocytogenes* infection. While antibiotic-treated wild-type mice display diminished macrophage responses to inflammatory stimuli, this is not observed in mice that lack Beclin 1 in myeloid cells, establishing the dominance of this gene over effects of the bacterial microbiota. Thus, select autophagy genes, but not all genes essential for degradative autophagy, have a key function in maintaining immune quiescence of tissue-resident macrophages, resulting in genetically programmed susceptibility to bacterial infection.

Neuroblast senescence in the aged brain augments natural kill cell cytotoxicity leading to impaired neurogenesis and cognition

Nature Neuroscience (IF: 21.5), 2020-11, Fu-Dong Shi et al. DOI: 10.1038/s41593-020-00745 PubMed: 33257875
Single Cell Gene Expression, Mouse, Immunology, Neuroscience.

Abstract

Normal aging is accompanied by escalating systemic inflammation. Yet the potential impact of immune homeostasis on neurogenesis and cognitive decline during brain aging have not been previously addressed. Here we report that natural killer (NK) cells of the innate immune system reside in the dentate gyrus neurogenic niche of aged brains in humans and mice. In situ expansion of these cells contributes to their abundance, which dramatically exceeds that of other immune subsets. Neuroblasts within the aged dentate gyrus display a senescence-associated secretory phenotype and reinforce NK cell activities and surveillance functions, which result in NK cell elimination of aged neuroblasts. Genetic or antibody-mediated depletion of NK cells leads to sustained improvements in neurogenesis and cognitive function during normal aging. These results demonstrate that NK cell accumulation in the aging brain impairs neurogenesis, which may serve as a therapeutic target to improve cognition in the aged population.

Metabolic conditioning of CD8+ effector T cells for adoptive cell therapy

Nature Metabolism (IF: 18.57), 2020-8, Edward J Pearce et al. DOI: 10.1038/s42255-020-0256 PubMed: 32747793
Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

CD8effector T (T) cell proliferation and cytokine production depends on enhanced glucose metabolism. However, circulating T cells continuously adapt to glucose fluctuations caused by diet and inter-organ metabolite exchange. Here we show that transient glucose restriction (TGR) in activated CD8Tcells metabolically primes effector functions and enhances tumour clearance in mice. Tumour-specific TGR CD8Tcells co-cultured with tumour spheroids in replete conditions display enhanced effector molecule expression, and adoptive transfer of these cells in a murine lymphoma model leads to greater numbers of immunologically functional circulating donor cells and complete tumour clearance. Mechanistically, Tcells treated with TGR undergo metabolic remodelling that, after glucose re-exposure, supports enhanced glucose uptake, increased carbon allocation to the pentose phosphate pathway (PPP) and a cellular redox shift towards a more reduced state-all indicators of a more anabolic programme to support their enhanced functionality. Thus, metabolic conditioning could be used to promote efficiency of T-cell products for adoptive cellular therapy.

Aging promotes reorganization of the CD4 T cell landscape toward extreme regulatory and effector phenotypes

Science Advances (IF: 14.09), 2019-8, Esti Yeger-Lotem et al. DOI: 10.1126/sciadv.aaw8330 PubMed: 31457092
Single Cell Gene Expression, Mouse, Immunology.

Abstract

Age-associated changes in CD4 T-cell functionality have been linked to chronic inflammation and decreased immunity. However, a detailed characterization of CD4 T cell phenotypes that could explain these dysregulated functional properties is lacking. We used single-cell RNA sequencing and multidimensional protein analyses to profile thousands of CD4 T cells obtained from young and old mice. We found that the landscape of CD4 T cell subsets differs markedly between young and old mice, such that three cell subsets-exhausted, cytotoxic, and activated regulatory T cells (aT)-appear rarely in young mice but gradually accumulate with age. Most unexpected were the extreme pro- and anti-inflammatory phenotypes of cytotoxic CD4 T cells and aT, respectively. These findings provide a comprehensive view of the dynamic reorganization of the CD4 T cell milieu with age and illuminate dominant subsets associated with chronic inflammation and immunity decline, suggesting new therapeutic avenues for age-related diseases.

Antibody-secreting cell destiny emerges during the initial stages of B-cell activation

Nature Communications (IF: 13.61), 2020-8, Sakeenah L Hicks et al. DOI: 10.1038/s41467-020-17798
PubMed: 32778653
Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Upon stimulation, B cells assume heterogeneous cell fates, with only a fraction differentiating into antibody-secreting cells (ASC). Here we investigate B cell fate programming and heterogeneity during ASC differentiation using T cell-independent models. We find that maximal ASC induction requires at least eight cell divisions in vivo, with BLIMP-1 being required for differentiation at division eight. Single cell RNA-sequencing of activated B cells and construction of differentiation trajectories reveal an early cell fate bifurcation. The ASC-destined branch requires induction of IRF4, MYC-target genes, and oxidative phosphorylation, with the loss of CD62L expression serving as a potential early marker of ASC fate commitment. Meanwhile, the non-ASC branch expresses an inflammatory signature, and maintains B cell fate programming. Finally, ASC can be further subsetted based on their differential responses to ER-stress, indicating multiple development branch points. Our data thus define the cell division kinetics of B cell differentiation in vivo, and identify the molecular trajectories of B cell fate and ASC formation.

Discrete populations of isotype-switched memory B lymphocytes are maintained in murine spleen and bone marrow

Nature Communications (IF: 13.61), 2020-5, Mir-Farzin Mashreghi et al. DOI: 10.1038/s41467-020-16464-6
PubMed: 32444631

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

At present, it is not clear how memory B lymphocytes are maintained over time, and whether only as circulating cells or also residing in particular tissues. Here we describe distinct populations of isotype-switched memory B lymphocytes (Bsm) of murine spleen and bone marrow, identified according to individual transcriptional signature and B cell receptor repertoire. A population of marginal zone-like cells is located exclusively in the spleen, while a population of quiescent Bsm is found only in the bone marrow. Three further resident populations, present in spleen and bone marrow, represent transitional and follicular B cells and B1 cells, respectively. A population representing 10-20% of spleen and bone marrow memory B cells is the only one qualifying as circulating. In the bone marrow, all cells individually dock onto VCAM1⁺ stromal cells and, reminiscent of resident memory T and plasma cells, are void of activation, proliferation and mobility.

Origin and differentiation trajectories of fibroblastic reticular cells in the splenic white pulp

Nature Communications (IF: 13.61), 2019-4, Thomas Hehlhans et al. DOI: 10.1038/s41467-019-09728-3
PubMed: 30988302

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

The splenic white pulp is underpinned by poorly characterized stromal cells that demarcate distinct immune cell microenvironments. Here we establish fibroblastic reticular cell (FRC)-specific fate-mapping in mice to define their embryonic origin and differentiation trajectories. Our data show that all reticular cell subsets descend from multipotent progenitors emerging at embryonic day 19.5 from periaarterial progenitors. Commitment of FRC progenitors is concluded during the first week of postnatal life through occupation of niches along developing central arterioles. Single cell transcriptomic analysis facilitated deconvolution of FRC differentiation trajectories and indicated that perivascular reticular cells function both as adult lymphoid organizer cells and mural cell progenitors. The lymphotoxin- β receptor-independent sustenance of postnatal progenitor stemness unveils that systemic immune surveillance in the splenic white pulp is governed through subset specification of reticular cells from a multipotent periaarterial progenitor cell. In sum, the finding that discrete signaling events in perivascular niches determine the differentiation trajectories of reticular cell networks explains the development of distinct microenvironmental niches in secondary and tertiary lymphoid tissues that are crucial for the induction and regulation of innate and adaptive immune processes.

CXCR5+PD-1+ follicular helper CD8 T cells control B cell tolerance

Nature Communications (IF: 13.61), 2019-9, Renren Wen et al. DOI: 10.1038/s41467-019-12446-5 PubMed: 31562329

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Many autoimmune diseases are characterized by the production of autoantibodies. The current view is that CD4T follicular helper (Tfh) cells are the main subset regulating autoreactive B cells. Here we report a CXCR5PD1Tfh subset of CD8T cells whose development and function are negatively modulated by Stat5. These CD8Tfh cells regulate the germinal center B cell response and control autoantibody production, as deficiency of Stat5 in CD8 T cells leads to an increase of CD8Tfh cells, resulting in the breakdown of B cell tolerance and concomitant autoantibody production. CD8Tfh cells share similar gene signatures with CD4Tfh, and require CD40L/CD40 and TCR/MHCI interactions to deliver help to B cells. Our study thus highlights the diversity of follicular T cell subsets that contribute to the breakdown of B-cell tolerance.

Early precursors and molecular determinants of tissue-resident memory CD8+ T lymphocytes revealed by single-cell RNA sequencing

Science Immunology (IF: 13.41), 2020-5, Gene W Yeo et al. DOI: 10.1126/sciimmunol.aaz6894 PubMed: 32414833

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

During an immune response to microbial infection, CD8T cells give rise to distinct classes of cellular progeny that coordinately mediate clearance of the pathogen and provide long-lasting protection against reinfection, including a subset of noncirculating tissue-resident memory (T) cells that mediate potent protection within nonlymphoid tissues. Here, we used single-cell RNA sequencing to examine the gene expression patterns of individual CD8T cells in the spleen and small intestine intraepithelial lymphocyte (siEL) compartment throughout the course of their differentiation in response to viral infection. These analyses revealed previously unknown transcriptional heterogeneity within the siEL CD8T cell population at several stages of differentiation, representing functionally distinct Tcell subsets and a subset of Tcell precursors within the tissue early in infection. Together, these findings may inform strategies to optimize CD8T cell responses to protect against microbial infection and cancer.

Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics

Science Immunology (IF: 13.41), 2020-2, Craig Walsh et al. DOI: 10.1126/sciimmunol.aay6017 PubMed: 32086381
Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Myeloid-derived suppressor cells (MDSCs) are innate immune cells that acquire the capacity to suppress adaptive immune responses during cancer. It remains elusive how MDSCs differ from their normal myeloid counterparts, which limits our ability to specifically detect and therapeutically target MDSCs during cancer. Here, we sought to determine the molecular features of breast cancer-associated MDSCs using the widely studied mouse model based on the mouse mammary tumor virus (MMTV) promoter-driven expression of the polyomavirus middle T oncoprotein (MMTV-PyMT). To identify MDSCs in an unbiased manner, we used single-cell RNA sequencing to compare MDSC-containing splenic myeloid cells from breast tumor-bearing mice with wild-type controls. Our computational analysis of 14,646 single-cell transcriptomes revealed that MDSCs emerge through an aberrant neutrophil maturation trajectory in the spleen that confers them an immunosuppressive cell state. We establish the MDSC-specific gene signature and identify CD84 as a surface marker for improved detection and enrichment of MDSCs in breast cancers.

Preoperative stimulation of resolution and inflammation blockade eradicates micrometastases

Journal of clinical investigation (IF: 12.81), 2019-6, Charles N Serhan et al. DOI: 10.1172/JCI127282
PubMed: 31205032

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Cancer therapy is a double-edged sword, as surgery and chemotherapy can induce an inflammatory/immunosuppressive injury response that promotes dormancy escape and tumor recurrence. We hypothesized that these events could be altered by early blockade of the inflammatory cascade and/or by accelerating the resolution of inflammation. Preoperative, but not postoperative, administration of the nonsteroidal antiinflammatory drug ketorolac and/or resolvins, a family of specialized proresolving autacoid mediators, eliminated micrometastases in multiple tumor-resection models, resulting in long-term survival. Ketorolac unleashed anticancer T cell immunity that was augmented by immune checkpoint blockade, negated by adjuvant chemotherapy, and dependent on inhibition of the COX-1/thromboxane A₂ (TXA₂) pathway. Preoperative stimulation of inflammation resolution via resolvins (RvD2, RvD3, and RvD4) inhibited metastases and induced T cell responses. Ketorolac and resolvins exhibited synergistic antitumor activity and prevented surgery- or chemotherapy-induced dormancy escape. Thus, simultaneously blocking the ensuing proinflammatory response and activating endogenous resolution programs before surgery may eliminate micrometastases and reduce tumor recurrence.

Murine single-cell RNA-seq reveals cell-identity- and tissue-specific trajectories of aging

Genome research (IF: 12.01), 2019-12, David R Kelley et al. DOI: 10.1101/gr.253880.119 PubMed: 31754020

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Aging is a pleiotropic process affecting many aspects of mammalian physiology. Mammals are composed of distinct cell type identities and tissue environments, but the influence of these cell identities and environments on the trajectory of aging in individual cells remains unclear. Here, we performed single-cell RNA-seq on >50,000 individual cells across three tissues in young and old mice to allow for direct comparison of aging phenotypes across cell types. We found transcriptional features of aging common across many cell types, as well as features of aging unique to each type. Leveraging matrix factorization and optimal transport methods, we found that both cell identities and tissue environments exert influence on the trajectory and magnitude of aging, with cell identity influence predominating. These results suggest that aging manifests with unique directionality and magnitude across the diverse cell identities in mammals.

Crossreactive public TCR sequences undergo positive selection in the human thymic repertoire

Journal of clinical investigation (IF: 12.81), 2019-3, Todd M Brusko et al. DOI: 10.1172/JCI124358

PubMed: 30920391

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

We investigated human T-cell repertoire formation using high throughput TCR β CDR3 sequencing in immunodeficient mice receiving human hematopoietic stem cells (HSCs) and human thymus grafts. Replicate humanized mice generated diverse and highly divergent repertoires. Repertoire narrowing and increased CDR3 β sharing was observed during thymocyte selection. While hydrophobicity analysis implicated self-peptides in positive selection of the overall repertoire, positive selection favored shorter shared sequences that had reduced hydrophobicity at positions 6 and 7 of CDR3 β s, suggesting weaker interactions with self-peptides than unshared sequences, possibly allowing escape from negative selection. Sharing was similar between autologous and allogeneic thymi and occurred between different cell subsets. Shared sequences were enriched for allo-crossreactive CDR3 β s and for Type 1 diabetes-associated autoreactive CDR3 β s. Single-cell TCR-sequencing showed increased sharing of CDR3 α s compared to CDR3 β s between mice. Our data collectively implicate preferential positive selection for shared human CDR3 β s that are highly cross-reactive. While previous studies suggested a role for recombination bias in producing public sequences in mice, our study is the first to demonstrate a role for thymic selection. Our results implicate positive selection for promiscuous TCR β sequences that likely evade negative selection, due to their low affinity for self-ligands, in the abundance of public human TCR β sequences.

BACH2 drives quiescence and maintenance of resting Treg cells to promote homeostasis and cancer immune-suppression

Journal of experimental medicine (IF: 11.32), 2020-9, Klaus Okkenhaug et al. DOI: 10.1084/jem.20190711
PubMed: 32515782

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Regulatory T (Treg) cell populations are composed of functionally quiescent resting Treg (rTreg) cells which differentiate into activated Treg (aTreg) cells upon antigen stimulation. How rTreg cells remain quiescent despite chronic exposure to cognate self- and foreign antigens is unclear. The transcription factor BACH2 is critical for early Treg lineage specification, but its function following lineage commitment is unresolved. Here, we show that BACH2 is repurposed following Treg lineage commitment and promotes the quiescence and long-term maintenance of rTreg cells. Bach2 is highly expressed in rTreg cells but is down-regulated in aTreg cells and during inflammation. In rTreg cells, BACH2 binds to enhancers of genes involved in aTreg differentiation and represses their TCR-driven induction by competing with AP-1 factors for DNA binding. This function promotes rTreg cell quiescence and long-term maintenance and is required for immune homeostasis and durable immunosuppression in cancer. Thus, BACH2 supports a division of labor between quiescent rTreg cells and their activated progeny in Treg maintenance and function, respectively.

Physiological expression and function of the MDR1 transporter in cytotoxic T lymphocytes

Journal of experimental medicine (IF: 11.32), 2020-5, Sergei B Koralov et al. DOI: 10.1084/jem.20191388
PubMed: 32302378

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Multidrug resistance-1 (MDR1) acts as a chemotherapeutic drug efflux pump in tumor cells, although its physiological functions remain enigmatic. Using a recently developed MDR1-knockin reporter allele (Abcb1aAME), we found that constitutive MDR1 expression among hematopoietic cells was observed in cytolytic lymphocytes-including CD8⁺ cytotoxic T lymphocytes (CTLs) and natural killer cells-and regulated by Runt-related (Runx) transcription factors. Whereas MDR1 was dispensable for naive CD8⁺ T cell development, it was required for both the normal accumulation of effector CTLs following acute viral infection and the protective function of memory CTLs following challenge with an intracellular bacterium. MDR1 acted early after naive CD8⁺ T cell activation to suppress oxidative stress, enforce survival, and safeguard mitochondrial function in nascent CTLs. These data highlight an important endogenous function of MDR1 in cell-mediated immune responses and suggest that ongoing efforts to intentionally inhibit MDR1 in cancer patients could be counterproductive.

Trib1 regulates T cell differentiation during chronic infection by restraining the effector program

Journal of experimental medicine (IF: 11.32), 2020-5, Martha S Jordan et al. DOI: 10.1084/jem.20190888

PubMed: 32150623

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

In chronic infections, the immune response fails to control virus, leading to persistent antigen stimulation and the progressive development of T cell exhaustion. T cell effector differentiation is poorly understood in the context of exhaustion, but targeting effector programs may provide new strategies for reinvigorating T cell function. We identified Tribbles pseudokinase 1 (Trib1) as a central regulator of antiviral T cell immunity, where loss of Trib1 led to a sustained enrichment of effector-like KLRG1⁺ T cells, enhanced function, and improved viral control. Single-cell profiling revealed that Trib1 restrains a population of KLRG1⁺ effector CD8 T cells that is transcriptionally distinct from exhausted cells. Mechanistically, we identified an interaction between Trib1 and the T cell receptor (TCR) signaling activator, MALT1, which disrupted MALT1 signaling complexes. These data identify Trib1 as a negative regulator of TCR signaling and downstream function, and reveal a link between Trib1 and effector versus exhausted T cell differentiation that can be targeted to improve antiviral immunity.

IL-2 production by self-reactive CD4 thymocytes scales regulatory T cell generation in the thymus

Journal of experimental medicine (IF: 11.32), 2019-11, Grégoire Altan-Bonnet et al. DOI: 10.1084/jem.20190993

PubMed: 31434685

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Regulatory T (T reg) cells, a specialized subset of CD4⁺T cells, are essential to prevent fatal autoimmunity. Expression of the T reg lineage-defining transcription factor Foxp3, and therefore their differentiation in the thymus, is dependent upon T cell receptor (TCR) and interleukin-2 (IL-2) signaling. Here, we report that the majority of IL-2-producing cells in the thymus are mature CD4 single-positive (CD4SP) thymocytes and that continuous IL-2 production sustained thymic T reg cell generation and control of systemic immune activation. Furthermore, single-cell RNA sequencing analysis of CD4 thymocyte subsets revealed that IL-2 was expressed in self-reactive CD4SP thymocytes, which also contain T reg precursor cells. Thus, our results suggest that the thymic T reg cell pool size is scaled by a key niche factor, IL-2, produced by self-reactive CD4SP thymocytes. This IL-2-dependent scaling of thymic T reg cell generation by overall self-reactivity of a mature post-selection thymic precursor pool may likely ensure adequate control of autoimmunity.

Absence of central tolerance in Aire-deficient mice synergizes with immune-checkpoint inhibition to enhance antitumor responses

Communications Biology (IF: 11.2), 2020-7, Matthew A Sleeman et al. DOI: 10.1038/s42003-020-1083-1

PubMed: 32641748

Single Cell Gene Expression & Single Cell Immune Profiling, Mouse, Cancer Research, Immuno-oncology.

Abstract

The endogenous anti-tumor responses are limited in part by the absence of tumor-reactive T cells, an inevitable consequence of thymic central tolerance mechanisms ensuring prevention of autoimmunity. Here we show that tumor rejection induced by immune checkpoint blockade is significantly enhanced in Aire-deficient mice, the epitome of central tolerance breakdown. The observed synergy in tumor rejection extended to different tumor models, was accompanied by increased numbers of activated T cells expressing high levels of Gzma, Gzmb, Perforin, Cxcr3, and increased intratumoural levels of Cxcl9 and Cxcl10 compared to wild-type mice. Consistent with Aire's central role in T cell repertoire selection, single cell TCR sequencing unveiled expansion of several clones with high tumor reactivity. The data suggest that breakdown in central tolerance synergizes with immune checkpoint blockade in enhancing anti-tumor immunity and may serve as a model to unmask novel anti-tumor therapies including anti-tumor TCRs, normally purged during central tolerance.

CD49b defines functionally mature Treg cells that survey skin and vascular tissues

Journal of experimental medicine (IF: 11.32), 2018-11, Dmitriy M Chudakov et al. DOI: 10.1084/jem.20181442

PubMed: 30355617

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Regulatory T (Treg) cells prevent autoimmunity by limiting immune responses and inflammation in the secondary lymphoid organs and nonlymphoid tissues. While unique subsets of Treg cells have been described in some nonlymphoid tissues, their relationship to Treg cells in secondary lymphoid organs and circulation remains unclear. Furthermore, it is possible that Treg cells from similar tissue types share largely similar properties. We have identified a short-lived effector Treg cell subset that expresses the α integrin, CD49b, and exhibits a unique tissue distribution, being abundant in peripheral blood, vasculature, skin, and skin-draining lymph nodes, but uncommon in the intestines and in viscera-draining lymph nodes. CD49bTreg cells, which display superior functionality revealed by in vitro and in vivo assays, appear to develop after multiple rounds of cell division and TCR-dependent activation. Accordingly, single-cell RNA-seq analysis placed these cells at the apex of the Treg developmental trajectory. These results shed light on the identity and development of a functionally potent subset of mature effector Treg cells that recirculate through and survey peripheral tissues.

Activated CARD11 accelerates germinal center kinetics, promoting mTORC1 and terminal differentiation

Journal of experimental medicine (IF: 11.32), 2018-9, Richard G James et al. DOI: 10.1084/jem.20180230

PubMed: 30127060

Single Cell Gene Expression, Mouse, Genetic Health, Immunology.

Abstract

Activating mutations in the adapter protein CARD11 associated with diffuse large B cell lymphomas (DLBCLs) are predicted to arise during germinal center (GC) responses, leading to inappropriate activation of NF- κ B signaling. Here, we modeled the B cell-intrinsic impact of the L251P activating mutation in CARD11 (aCARD11) on the GC response. Global B cell aCARD11 expression led to a modest increase in splenic B cells and a severe reduction in B1 B cell numbers, respectively. Following T cell-dependent immunization, aCARD11 cells exhibited increased rates of GC formation, resolution, and differentiation. Restriction of aCARD11 to GC B cells similarly altered the GC response and B cell differentiation. In this model, aCARD11 promoted dark zone skewing along with increased cycling, AID levels, and class switch recombination. Furthermore, aCard11 GC B cells displayed increased biomass and mTORC1 signaling, suggesting a novel strategy for targeting aCARD11-driven DLBCL. While aCARD11 potentially impacts GC responses, the rapid GC contraction suggests it requires collaboration with events that limit terminal differentiation to promote lymphoma.

Ly49R activation receptor drives self-MHC-educated NK cell immunity against cytomegalovirus infection

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-12, Wenhao Xu et al. DOI: 10.1073/pnas.1913064117 PubMed: 31843910

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

Natural killer (NK) cells mediate vital control of cancer and viral infection. They rely on MHC class I (MHC I)-specific self-receptors to identify and lyse diseased cells without harming self-MHC I-bearing host cells. NK cells bearing inhibitory self-receptors for host MHC I also undergo education, referred to as licensing, which causes them to become more responsive to stimulation via activation receptor signaling. Previous work has shown that licensed NK cells selectively expand during virus infections and they are associated with improved clinical response in human patients experiencing certain chronic virus infections, including HIV and hepatitis C virus. However, the importance of inhibitory self-receptors in NK-mediated virus immunity is debated as they also limit signals in NK cells emanating from virus-specific activation receptors. Using a mouse model of MHC I-dependent (H-2D) virus immunity, we discovered that NK cells depend on the Ly49G2 inhibitory self-receptor to mediate virus control, which coincided with host survival during murine cytomegalovirus infection. This antiviral effect further requires active signaling in NK cells via the Ly49R activation receptor that also binds H-2D. In tandem, these functionally discordant Ly49 self-receptors increase NK cell proliferation and effector activity during infection, resulting in selective up-regulation of CD25 and KLRG1 in virus-specific Ly49R⁺Ly49G2⁺NK cells. Our findings establish that paired self-receptors act as major determinants of NK cell-mediated virus sensing and immunity.

Single-cell transcriptomics reveal that PD-1 mediates immune tolerance by regulating proliferation of regulatory T cells

Genome Medicine (IF: 10.64), 2018-9, Yuk Ming Dennis Lo et al. DOI: 10.1186/s13073-018-0581

PubMed: 30236153

Single Cell Gene Expression, Mouse, Immunology.

Abstract

We have previously reported an antigen-specific protocol to induce transplant tolerance and linked suppression to human embryonic stem cell (hESC)-derived tissues in immunocompetent mice through coreceptor and costimulation blockade. However, the exact mechanisms of acquired immune tolerance in this model have remained unclear. We utilize the NOD.Foxp3reporter mouse line and an ablative anti-hCD2 antibody to ask if CD4FOXP3regulatory T cells (Treg) are required for coreceptor and costimulation blockade-induced immune tolerance. We also perform genome-wide single-cell RNA-sequencing to interrogate Treg during immune rejection and tolerance and to indicate possible mechanisms involved in sustaining Treg function. We show that Treg are indispensable for tolerance induced by coreceptor and costimulation blockade as depletion of which with an anti-hCD2 antibody resulted in rejection of hESC-derived pancreatic islets in NOD.Foxp3mice. Single-cell transcriptomic profiling of 12,964 intragraft CD4T cells derived from rejecting and tolerated grafts reveals that Treg are heterogeneous and functionally distinct in the two outcomes of transplant rejection and tolerance. Treg appear to mainly promote chemotactic and ubiquitin-dependent protein catabolism during transplant rejection while seeming to harness proliferative and immunosuppressive function during tolerance. We also demonstrate that this form of acquired transplant tolerance is associated with increased proliferation and PD-1 expression by Treg. Blocking PD-1 signaling with a neutralizing anti-PD-1 antibody leads to reduced Treg proliferation and graft rejection. Our results suggest that short-term coreceptor and costimulation blockade mediates immune tolerance to hESC-derived pancreatic islets by promoting Treg proliferation through engagement of PD-1. Our findings could give new insights into clinical development of hESC-derived pancreatic tissues, combined with immunotherapies that expand intragraft Treg, as a potentially sustainable alternative treatment for T1D.

Resolving the fibrotic niche of human liver cirrhosis at single-cell level

Nature (IF: 46.49), 2019-10, S A Teichmann et al. DOI: 10.1038/s41586-019-1631-3 PubMed: 31597160

Single Cell Gene Expression, Human, Mouse, Cell Atlas, Immunology.

Abstract

Liver cirrhosis is a major cause of death worldwide and is characterized by extensive fibrosis. There are currently no effective antifibrotic therapies available. To obtain a better understanding of the cellular and molecular mechanisms involved in disease pathogenesis and enable the discovery of therapeutic targets, here we profile the transcriptomes of more than 100,000 single human cells, yielding molecular definitions for non-parenchymal cell types that are found in healthy and cirrhotic human liver. We identify a scar-associated TREM2CD9 subpopulation of macrophages, which expands in liver fibrosis, differentiates from circulating monocytes and is pro-fibrogenic. We also define ACKR1 and PLVAP endothelial cells that expand in cirrhosis, are topographically restricted to the fibrotic niche and enhance the transmigration of leucocytes. Multi-lineage modelling of ligand and receptor interactions between the scar-associated macrophages, endothelial cells and PDGFR α collagen-producing mesenchymal cells reveals intra-scar activity of several pro-fibrogenic pathways including TNFRSF12A, PDGFR and NOTCH signalling. Our work dissects unanticipated aspects of the cellular and molecular basis of human organ fibrosis at a single-cell level, and provides a conceptual framework for the discovery of rational therapeutic targets in liver cirrhosis.

Decoding human fetal liver haematopoiesis

Nature (IF: 46.49), 2019-10, Sarah A Teichmann et al. DOI: 10.1038/s41586-019-1652 PubMed: 31597962

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Developmental Biology.

Abstract

Definitive haematopoiesis in the fetal liver supports self-renewal and differentiation of haematopoietic stem cells and multipotent progenitors (HSC/MPPs) but remains poorly defined in humans. Here, using single-cell transcriptome profiling of approximately 140,000 liver and 74,000 skin, kidney and yolk sac cells, we identify the repertoire of human blood and immune cells during development. We infer differentiation trajectories from HSC/MPPs and evaluate the influence of the tissue microenvironment on blood and immune cell development. We reveal physiological erythropoiesis in fetal skin and the presence of mast cells, natural killer and innate lymphoid cell precursors in the yolk sac. We demonstrate a shift in the haematopoietic composition of fetal liver during gestation away from being predominantly erythroid, accompanied by a parallel change in differentiation potential of HSC/MPPs, which we functionally validate. Our integrated map of fetal liver haematopoiesis provides a blueprint for the study of paediatric blood and immune disorders, and a reference for harnessing the therapeutic potential of HSC/MPPs.

Onco-fetal Reprogramming of Endothelial Cells Drives Immunosuppressive Macrophages in Hepatocellular Carcinoma

Cell (IF: 38.62), 2020-10, Florent Ginhoux et al. DOI: 10.1016/j.cell.2020.08.040 PubMed: 32976798

Single Cell Gene Expression, Human & Mouse, Cancer Research, Cell Atlas.

Abstract

We employed scRNA sequencing to extensively characterize the cellular landscape of human liver from development to disease. Analysis of ~212,000 cells representing human fetal, hepatocellular carcinoma (HCC), and mouse liver revealed remarkable fetal-like reprogramming of the tumor microenvironment. Specifically, the HCC ecosystem displayed features reminiscent of fetal development, including re-emergence of fetal-associated endothelial cells (PLVAP/VEGFR2) and fetal-like (FOLR2) tumor-associated macrophages. In a cross-species comparative analysis, we discovered remarkable similarity between mouse embryonic, fetal-liver, and tumor macrophages. Spatial transcriptomics further revealed a shared onco-fetal ecosystem between fetal liver and HCC. Furthermore, gene regulatory analysis, spatial transcriptomics, and in vitro functional assays implicated VEGF and NOTCH signaling in maintaining onco-fetal ecosystem. Taken together, we report a shared immunosuppressive onco-fetal ecosystem in fetal liver and HCC. Our results unravel a previously unexplored onco-fetal reprogramming of the tumor ecosystem, provide novel targets for therapeutic interventions in HCC, and open avenues for identifying similar paradigms in other cancers and disease.

Liver Immune Profiling Reveals Pathogenesis and Therapeutics for Biliary Atresia

Cell (IF: 38.62), 2020-11, Zhe Wen et al. DOI: 10.1016/j.cell.2020.10.048 PubMed: 33248023

Single Cell Immune Profiling, Human, Genetic Health, Immunology.

Abstract

Biliary atresia (BA) is a severe cholangiopathy that leads to liver failure in infants, but its pathogenesis remains to be fully characterized. By single-cell RNA profiling, we observed macrophage hypo-inflammation, Kupffer cell scavenger function defects, cytotoxic T cell expansion, and deficiency of CX3CR1 effector T and natural killer (NK) cells in infants with BA. More importantly, we discovered that hepatic B cell lymphopoiesis did not cease after birth and that tolerance defects contributed to immunoglobulin G (IgG)-autoantibody accumulation in BA. In a rhesus-rotavirus induced BA model, depleting B cells or blocking antigen presentation ameliorated liver damage. In a pilot clinical study, we demonstrated that rituximab was effective in depleting hepatic B cells and restoring the functions of macrophages, Kupffer cells, and T cells to levels comparable to those of control subjects. In summary, our comprehensive immune profiling in infants with BA had elucidated that B-cell-modifying therapies may alleviate liver pathology.

Host-Viral Infection Maps Reveal Signatures of Severe COVID-19 Patients

Cell (IF: 38.62), 2020-6, Zheng Zhang et al. DOI: 10.1016/j.cell.2020.05.006 PubMed: 32479746

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Computational Method, Infectious Disease.

Abstract

Viruses are a constant threat to global health as highlighted by the current COVID-19 pandemic. Currently, lack of data underlying how the human host interacts with viruses, including the SARS-CoV-2 virus, limits effective therapeutic intervention. We introduce Viral-Track, a computational method that globally scans unmapped single-cell RNA sequencing (scRNA-seq) data for the presence of viral RNA, enabling transcriptional cell sorting of infected versus bystander cells. We demonstrate the sensitivity and specificity of Viral-Track to systematically detect viruses from multiple models of infection, including hepatitis B virus, in an unsupervised manner. Applying Viral-Track to bronchoalveolar-lavage samples from severe and mild COVID-19 patients reveals a dramatic impact of the virus on the immune system of severe patients compared to mild cases. Viral-Track detects an unexpected co-infection of the human metapneumovirus, present mainly in monocytes perturbed in type-I interferon (IFN)-signaling. Viral-Track provides a robust technology for dissecting the mechanisms of viral-infection and pathology.

Single-Cell RNA Sequencing Resolves Spatiotemporal Development of Pre-thymic Lymphoid Progenitors and Thymus Organogenesis in Human Embryos

Immunity (IF: 25.73), 2019-11, Bing Liu et al. DOI: 10.1016/j.immuni.2019.09.008 PubMed: 31604687

Single Cell Gene Expression, Human, Developmental Biology, Immunology.

Abstract

Generation of the first T lymphocytes in the human embryo involves the emergence, migration, and thymus seeding of lymphoid progenitors together with concomitant thymus organogenesis, which is the initial step to establish the entire adaptive immune system. However, the cellular and molecular programs regulating this process remain unclear. We constructed a single-cell transcriptional landscape of human early T lymphopoiesis by using cells from multiple hemogenic and hematopoietic sites spanning embryonic and fetal stages. Among heterogeneous early thymic progenitors, one subtype shared common features with a subset of lymphoid progenitors in fetal liver that are known as thymus-seeding progenitors. Unbiased bioinformatics analysis identified a distinct type of pre-thymic lymphoid progenitors in the aorta-gonad-mesonephros (AGM) region. In parallel, we investigated thymic epithelial cell development and potential cell-cell interactions during thymus organogenesis. Together, our data provide insights into human early T lymphopoiesis that prospectively direct T lymphocyte regeneration, which might lead to development of clinical applications.

Tumor Cell Biodiversity Drives Microenvironmental Reprogramming in Liver Cancer

Cancer Cell (IF: 30.24), 2019-10, Tim F Greten et al. DOI: 10.1016/j.ccell.2019.08.007 PubMed: 31588021

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Cellular diversity in tumors is a key factor for therapeutic failures and lethal outcomes of solid malignancies. Here, we determined the single-cell transcriptomic landscape of liver cancer biospecimens from 19 patients. We found varying degrees of heterogeneity in malignant cells within and between tumors and diverse landscapes of tumor microenvironment (TME). Strikingly, tumors with higher transcriptomic diversity were associated with patient's worse overall survival. We found a link between hypoxia-dependent vascular endothelial growth factor expression in tumor diversity and TME polarization. Moreover, T cells from higher heterogeneous tumors showed lower cytolytic activities. Consistent results were found using bulk genomic and transcriptomic profiles of 765 liver tumors. Our results offer insight into the diverse ecosystem of liver cancer and its impact on patient prognosis.

Identification of FABP5 as an immunometabolic marker in human hepatocellular carcinoma

Journal for Immunotherapy of Cancer (IF: 9.91), 2020-7, Yinghong Shi et al. DOI: 10.1136/jitc-2019-000501 PubMed: 32611686

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Regulating T-cell metabolism is crucial for their anticancer activity. Therefore, understanding the function and metabolism of human tumor-infiltrating T cells is of broad interest and clinical importance. CD3CD45T cells were sorted from adjacent area or tumor core of human hepatocellular carcinoma (HCC), then the clusters and heterogeneity of T cells were further interrogated by single-cell transcriptomic profiling. 118 surgical samples from patients with HCC were histologically examined for infiltration of CD8T cells in tumor and adjacent tissue. Single-cell transcriptomic profiling indicated that several exhausted T-cell (Tex) populations differentially coexisted in the tumor and adjacent tissue. CD137 identifies and enriches Tex with superior effector functions and proliferation capacity. Furthermore, enhanced fatty acid-binding protein 5 (FABP5) expression along with increased mitochondrial oxidative metabolism were evident in these CD137-enriched Tex. Inhibiting FABP5 expression and mitochondrial fatty acid oxidation impaired the anti-apoptosis and proliferation of CD137-enriched Tex. These observations have been verified by generating CD137 CART. Immunohistochemistry staining on the tissue microarray of 118 patients with HCC showed intra-tumoral FABP5CD8T-cell infiltration was linked to overall and recurrence-free survival. The tumor microenvironment can impose metabolic restrictions on T-cell function. CD137, a costimulatory molecule highly expressed on some Tex, uses exogenous fatty acids and oxidative metabolism to mediate antitumor immunity. The immunometabolic marker FABP5 should be investigated in larger, longitudinal studies to determine their potential as prognostic biomarkers for HCC.

Single-cell analysis reveals new evolutionary complexity in uveal melanoma

Nature Communications (IF: 13.61), 2020-1, Alan S Livingstone et al. DOI: 10.1038/s41467-019-14256-1

PubMed: 31980621

Single Cell Immune Profiling & Single Cell CNV, Human, Cancer Research.

Abstract

Uveal melanoma (UM) is a highly metastatic cancer that, in contrast to cutaneous melanoma, is largely unresponsive to checkpoint immunotherapy. Here, we interrogate the tumor microenvironment at single-cell resolution using scRNA-seq of 59,915 tumor and non-neoplastic cells from 8 primary and 3 metastatic samples. Tumor cells reveal novel subclonal genomic complexity and transcriptional states. Tumor-infiltrating immune cells comprise a previously unrecognized diversity of cell types, including CD8T cells predominantly expressing the checkpoint marker LAG3, rather than PD1 or CTLA4. V(D)J analysis shows clonally expanded T cells, indicating that they are capable of mounting an immune response. An indolent liver metastasis from a class 1B UM is infiltrated with clonally expanded plasma cells, indicative of antibody-mediated immunity. This complex ecosystem of tumor and immune cells provides new insights into UM biology, and LAG3 is identified as a potential candidate for immune checkpoint blockade in patients with high risk UM.

Tumor Cell Biodiversity Drives Microenvironmental Reprogramming in Liver Cancer

Cancer Cell (IF: 30.24), 2019-10, Tim F Greten et al. DOI: 10.1016/j.ccell.2019.08.007 PubMed: 31588021

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Cellular diversity in tumors is a key factor for therapeutic failures and lethal outcomes of solid malignancies. Here, we determined the single-cell transcriptomic landscape of liver cancer biospecimens from 19 patients. We found varying degrees of heterogeneity in malignant cells within and between tumors and diverse landscapes of tumor microenvironment (TME). Strikingly, tumors with higher transcriptomic diversity were associated with patient's worse overall survival. We found a link between hypoxia-dependent vascular endothelial growth factor expression in tumor diversity and TME polarization. Moreover, T cells from higher heterogeneous tumors showed lower cytolytic activities. Consistent results were found using bulk genomic and transcriptomic profiles of 765 liver tumors. Our results offer insight into the diverse ecosystem of liver cancer and its impact on patient prognosis.

Acute liver failure is regulated by MYC- and microbiome-dependent programs

Nature Medicine (IF: 36.23), 2020-12, Ido Amit et al. DOI: 10.1038/s41591-020-1102-2 PubMed: 33106666

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

Acute liver failure (ALF) is a fulminant complication of multiple etiologies, characterized by rapid hepatic destruction, multi-organ failure and mortality. ALF treatment is mainly limited to supportive care and liver transplantation. Here we utilize the acetaminophen (APAP) and thioacetamide (TAA) ALF models in characterizing 56,527 single-cell transcriptomes to define the mouse ALF cellular atlas. We demonstrate that unique, previously uncharacterized stellate cell, endothelial cell, Kupffer cell, monocyte and neutrophil subsets, and their intricate intercellular crosstalk, drive ALF. We unravel a common MYC-dependent transcriptional program orchestrating stellate, endothelial and Kupffer cell activation during ALF, which is regulated by the gut microbiome through Toll-like receptor (TLR) signaling. Pharmacological inhibition of MYC, upstream TLR signaling checkpoints or microbiome depletion suppress this cell-specific, MYC-dependent program, thereby attenuating ALF. In humans, we demonstrate upregulated hepatic MYC expression in ALF transplant recipients compared to healthy donors. Collectively we demonstrate that detailed cellular/genetic decoding may enable pathway-specific ALF therapeutic intervention.

Niche-Specific Reprogramming of Epigenetic Landscapes Drives Myeloid Cell Diversity in Nonalcoholic Steatohepatitis

Immunity (IF: 25.73), 2020-6, Ronald N Germain et al. DOI: 10.1016/j.immuni.2020.04.001 PubMed: 32362324

Single Cell Gene Expression, Mouse, Genome Analysis, Immunology.

Abstract

Tissue-resident and recruited macrophages contribute to both host defense and pathology. Multiple macrophage phenotypes are represented in diseased tissues, but we lack deep understanding of mechanisms controlling diversification. Here, we investigate origins and epigenetic trajectories of hepatic macrophages during diet-induced non-alcoholic steatohepatitis (NASH). The NASH diet induced significant changes in Kupffer cell enhancers and gene expression, resulting in partial loss of Kupffer cell identity, induction of Trem2 and Cd9 expression, and cell death. Kupffer cell loss was compensated by gain of adjacent monocyte-derived macrophages that exhibited convergent epigenomes, transcriptomes, and functions. NASH-induced changes in Kupffer cell enhancers were driven by AP-1 and EGR that reprogrammed LXR functions required for Kupffer cell identity and survival to instead drive a scar-associated macrophage phenotype. These findings reveal mechanisms by which disease-associated environmental signals instruct resident and recruited macrophages to acquire distinct gene expression programs and corresponding functions.

Type I Interferon Signaling Disrupts the Hepatic Urea Cycle and Alters Systemic Metabolism to Suppress T Cell Function

Immunity (IF: 25.73), 2019-12, Kristaps Klavins et al. DOI: 10.1016/j.immuni.2019.10.014 PubMed: 31784108

Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

Infections induce complex host responses linked to antiviral defense, inflammation, and tissue damage and repair. We hypothesized that the liver, as a central metabolic hub, may orchestrate systemic metabolic changes during infection. We infected mice with chronic lymphocytic choriomeningitis virus (LCMV), performed RNA sequencing and proteomics of liver tissue, and integrated these data with serum metabolomics at different infection phases. Widespread reprogramming of liver metabolism occurred early after infection, correlating with type I interferon (IFN-I) responses. Viral infection induced metabolic alterations of the liver that depended on the interferon alpha/beta receptor (IFNAR1). Hepatocyte-intrinsic IFNAR1 repressed the transcription of metabolic genes, including *Otc* and *Ass1*, which encode urea cycle enzymes. This led to decreased arginine and increased ornithine concentrations in the circulation, resulting in suppressed virus-specific CD8T cell responses and ameliorated liver pathology. These findings establish IFN-I-induced modulation of hepatic metabolism and the urea cycle as an endogenous mechanism of immunoregulation. VIDEO ABSTRACT.

Generation of a p16 Reporter Mouse and Its Use to Characterize and Target p16^{high} Cells In Vivo

Cell Metabolism (IF: 24.29), 2020-11, Atsushi Miyajima et al. DOI: 10.1016/j.cmet.2020.09.006 PubMed: 32949498

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Cell senescence plays a key role in age-associated organ dysfunction, but the in vivo pathogenesis is largely unclear. Here, we generated a p16-Cre-tdTomato mouse model to analyze the in vivo characteristics of p16^{high} cells at a single-cell level. We found tdTomato-positive p16^{high} cells detectable in all organs, which were enriched with age. We also found that these cells failed to proliferate and had half-lives ranging from 2.6 to 4.2 months, depending on the tissue examined. Single-cell transcriptomics in the liver and kidneys revealed that p16^{high} cells were present in various cell types, though most dominant in hepatic endothelium and in renal proximal and distal tubule epithelia, and that these cells exhibited heterogeneous senescence-associated phenotypes. Further, elimination of p16^{high} cells ameliorated nonalcoholic steatohepatitis-related hepatic lipidosis and immune cell infiltration. Our new mouse model and single-cell analysis provide a powerful resource to enable the discovery of previously unidentified senescence functions in vivo.

Single-Cell Analysis of Neonatal HSC Ontogeny Reveals Gradual and Uncoordinated Transcriptional Reprogramming that Begins before Birth

Cell Stem Cell (IF: 23.45), 2020-11, Samantha A Morris et al. DOI: 10.1016/j.stem.2020.08.001 PubMed: 32822583
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Fetal and adult hematopoietic stem cells (HSCs) have distinct proliferation rates, lineage biases, gene expression profiles, and gene dependencies. Although these differences are widely recognized, it is not clear how the transition from fetal to adult identity is coordinated. Here we show that murine HSCs and committed hematopoietic progenitor cells (HPCs) undergo a gradual, rather than precipitous, transition from fetal to adult transcriptional states. The transition begins prior to birth and is punctuated by a late prenatal spike in type I interferon signaling that promotes perinatal HPC expansion and sensitizes progenitors to the leukemogenic FLT3 mutation. Most other changes in gene expression and enhancer activation are imprecisely timed and poorly coordinated. Thus, heterochronic enhancer elements, and their associated transcripts, are activated independently of one another rather than as part of a robust network. This simplifies the regulatory programs that guide neonatal HSC/HPC ontogeny, but it creates heterogeneity within these populations.

Generation of Functional Liver Sinusoidal Endothelial Cells from Human Pluripotent Stem-Cell-Derived Venous Angioblasts

Cell Stem Cell (IF: 23.45), 2020-8, Gary D Bader et al. DOI: 10.1016/j.stem.2020.06.007 PubMed: 32640183
Single Cell Gene Expression, Mouse, Cell Atlas, Developmental Biology.

Abstract

Liver sinusoidal endothelial cells (LSECs) form a highly specialized microvasculature that plays a critical role in liver function and disease. To better understand this role, we developed a strategy to generate LSECs from human pluripotent stem cells (hPSCs) by first optimizing the specification of arterial and venous angioblasts and derivative endothelial populations. Induction of a LSEC-like fate by hypoxia, cyclic AMP (cAMP) agonism, and transforming growth factor β (TGF- β) inhibition revealed that venous endothelial cells responded more rapidly and robustly than the arterial cells to upregulate LSEC markers and functions in vitro. Upon intrahepatic transplantation in neonates, venous angioblasts engrafted the liver and generated mature, fenestrated LSECs with scavenger functions and molecular profiles of primary human LSECs. When transplanted into the liver of adult mice, angioblasts efficiently gave rise to mature LSECs with robust factor VIII (FVIII) production. Humanization of the murine liver with hPSC-derived LSECs provides a tractable system for studying the biology of this key liver cell type.

YAP, but Not RSPO-LGR4/5, Signaling in Biliary Epithelial Cells Promotes a Ductular Reaction in Response to Liver Injury

Cell Stem Cell (IF: 23.45), 2019-7, Guglielmo Roma et al. DOI: 10.1016/j.stem.2019.04.005 PubMed: 31080135
Single Cell Gene Expression, Mouse, Functional Genomics.

Abstract

Biliary epithelial cells (BECs) form bile ducts in the liver and are facultative liver stem cells that establish a ductular reaction (DR) to support liver regeneration following injury. Liver damage induces periportal LGR5+ putative liver stem cells that can form BEC-like organoids, suggesting that RSPO-LGR4/5-mediated WNT/ β -catenin activity is important for a DR. We addressed the roles of this and other signaling pathways in a DR by performing a focused CRISPR-based loss-of-function screen in BEC-like organoids, followed by in vivo validation and single-cell RNA sequencing. We found that BECs lack and do not require LGR4/5-mediated WNT/ β -catenin signaling during a DR, whereas YAP and mTORC1 signaling are required for this process. Upregulation of AXIN2 and LGR5 is required in hepatocytes to enable their regenerative capacity in response to injury. Together, these data highlight heterogeneity within the BEC pool, delineate signaling pathways involved in a DR, and clarify the identity and roles of injury-induced periportal LGR5+ cells.

Transcriptional Dynamics of Hepatic Sinusoid - Associated Cells After Liver Injury

Hepatology (IF: 13.66), 2020-3, Henrik Dimke et al. DOI: 10.1002/hep.31215 PubMed: 32145072
Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Hepatic sinusoidal cells are known actors in the fibrogenic response to injury. Activated hepatic stellate cells (HSCs), liver sinusoidal endothelial cells, and Kupffer cells are responsible for sinusoidal capillarization and perisinusoidal matrix deposition, impairing vascular exchange and heightening the risk of advanced fibrosis. While the overall pathogenesis is well understood, functional relations between cellular transitions during fibrogenesis are only beginning to be resolved. At single-cell resolution, we here explored the heterogeneity of individual cell types and dissected their transitions and crosstalk during fibrogenesis. We applied single-cell transcriptomics to map the heterogeneity of sinusoid-associated cells in healthy and injured livers and reconstructed the single-lineage HSC trajectory from pericyte to myofibroblast. Stratifying each sinusoidal cell population by activation state, we projected shifts in sinusoidal communication upon injury. Weighted gene correlation network analysis of the HSC trajectory led to the identification of core genes whose expression proved highly predictive of advanced fibrosis in patients with nonalcoholic steatohepatitis (NASH). Among the core members of the injury-repressed gene module, we identified plasmalemma vesicle-associated protein (PLVAP) as a protein amply expressed by mouse and human HSCs. PLVAP expression was suppressed in activated HSCs upon injury and may hence define hitherto unknown roles for HSCs in the regulation of microcirculatory exchange and its breakdown in chronic liver disease. Our study offers a single-cell resolved account of drug-induced injury of the mammalian liver and identifies key genes that may serve important roles in sinusoidal integrity and as markers of advanced fibrosis in human NASH.

Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis

Molecular Cell (IF: 16.13), 2019-8, Jun Z Li et al. DOI: 10.1016/j.molcel.2019.07.028 PubMed: 31398325

Single Cell Gene Expression, Mouse, Cell Atlas, Cell Biology.

Abstract

Cell-cell communication via ligand-receptor signaling is a fundamental feature of complex organs. Despite this, the global landscape of intercellular signaling in mammalian liver has not been elucidated. Here we perform single-cell RNA sequencing on non-parenchymal cells isolated from healthy and NASH mouse livers. Secretome gene analysis revealed a highly connected network of intrahepatic signaling and disruption of vascular signaling in NASH. We uncovered the emergence of NASH-associated macrophages (NAMs), which are marked by high expression of triggering receptors expressed on myeloid cells 2 (Trem2), as a feature of mouse and human NASH that is linked to disease severity and highly responsive to pharmacological and dietary interventions. Finally, hepatic stellate cells (HSCs) serve as a hub of intrahepatic signaling via HSC-derived stellakines and their responsiveness to vasoactive hormones. These results provide unprecedented insights into the landscape of intercellular crosstalk and reprogramming of liver cells in health and disease.

Myeloid cells in liver and bone marrow acquire a functionally distinct inflammatory phenotype during obesity-related steatohepatitis

Gut (IF: 17.75), 2020-3, Ivan G Costa et al. DOI: 10.1136/gutjnl-2019-318382 PubMed: 31076404

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Bone marrow-derived myeloid cells accumulate in the liver as monocytes and macrophages during the progression of obesity-related non-alcoholic fatty liver disease (NAFLD) to steatohepatitis (NASH). Myeloid cells comprise heterogeneous subsets, and dietary overnutrition may affect macrophages in the liver and bone marrow. We therefore aimed at characterising in depth the functional adaptations of myeloid cells in fatty liver. We employed single-cell RNA sequencing to comprehensively assess the heterogeneity of myeloid cells in the liver and bone marrow during NAFLD, by analysing C57BL/6 mice fed with a high-fat, high-sugar, high-cholesterol 'Western diet' for 16 weeks. We also characterised NAFLD-driven functional adaptations of macrophages *in vitro* and their functional relevance during steatohepatitis *in vivo*. Single-cell RNA sequencing identified distinct myeloid cell clusters in the liver and bone marrow. In both compartments, monocyte-derived populations were largely expanded in NASH-affected mice. Importantly, the liver myeloid compartment adapted a unique inflammatory phenotype during NAFLD progression, exemplarily characterised by downregulated inflammatory calprotectin (S100A8/A9) in macrophage and dendritic cell subsets. This distinctive gene signature was also found in their bone marrow precursors. The NASH myeloid phenotype was principally recapitulated by *in vitro* exposure of bone marrow-derived macrophages with fatty acids, depended on toll-like receptor 4 signalling and defined a characteristic response pattern to lipopolysaccharide stimulation. This imprinted and stable NASH myeloid immune phenotype functionally determined inflammatory responses following acute liver injury (acetaminophen poisoning) *in vivo*. Liver myeloid leucocytes and their bone marrow precursors adapt a common and functionally relevant inflammatory signature during NAFLD progression.

Tissue Repair in the Mouse Liver Following Acute Carbon Tetrachloride Depends on Injury - Induced Wnt/ β - Catenin Signaling

Hepatology (IF: 13.66), 2019-5, Bruce Wang et al. DOI: 10.1002/hep.30563 PubMed: 30762896

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

In the liver, Wnt/ β -catenin signaling is involved in regulating zonation and hepatocyte proliferation during homeostasis. We examined Wnt gene expression and signaling after injury, and we show by in situ hybridization that Wnts are activated by acute carbon tetrachloride (CCl₄) toxicity. Following injury, peri-injury hepatocytes become Wnt-responsive, expressing the Wnt target gene axis inhibition protein 2 (Axin2). Lineage tracing of peri-injury Axin2 hepatocytes shows that during recovery the injured parenchyma becomes repopulated and repaired by Axin2 descendants. Using single-cell RNA sequencing, we show that endothelial cells are the major source of Wnts following acute CCl₄ toxicity. Induced loss of β -catenin in peri-injury hepatocytes results in delayed repair and ultimately injury-induced lethality, while loss of Wnt production from endothelial cells leads to a delay in the proliferative response after injury. Conclusion: Our findings highlight the importance of the Wnt/ β -catenin signaling pathway in restoring tissue integrity following acute liver toxicity and establish a role of endothelial cells as an important Wnt-producing regulator of liver tissue repair following localized liver injury.

Dynamics of the 4D genome during in vivo lineage specification and differentiation

Nature Communications (IF: 13.61), 2020-6, Douglas R Higgs et al. DOI: 10.1038/s41467-020-16598-7

PubMed: 32483172

Single Cell Gene Expression, Mouse, Developmental Biology, Genome Analysis.

Abstract

Mammalian gene expression patterns are controlled by regulatory elements, which interact within topologically associating domains (TADs). The relationship between activation of regulatory elements, formation of structural chromatin interactions and gene expression during development is unclear. Here, we present Tiled-C, a low-input chromosome conformation capture (3C) technique. We use this approach to study chromatin architecture at high spatial and temporal resolution through in vivo mouse erythroid differentiation. Integrated analysis of chromatin accessibility and single-cell expression data shows that regulatory elements gradually become accessible within pre-existing TADs during early differentiation. This is followed by structural re-organization within the TAD and formation of specific contacts between enhancers and promoters. Our high-resolution data show that these enhancer-promoter interactions are not established prior to gene expression, but formed gradually during differentiation, concomitant with progressive upregulation of gene activity. Together, these results provide new insight into the close, interdependent relationship between chromatin architecture and gene regulation during development.

Hemolysis transforms liver macrophages into anti-inflammatory erythrophagocytes

Journal of clinical investigation (IF: 12.81), 2020-10, Dominik J Schaer et al. DOI: 10.1172/JCI137282

PubMed: 32663195

Single Cell Gene Expression & Single Cell ATAC, Mouse, Immunology.

Abstract

During hemolysis, macrophages in the liver phagocytose damaged erythrocytes to prevent the toxic effects of cell-free hemoglobin and heme. It remains unclear how this homeostatic process modulates phagocyte functions in inflammatory diseases. Using a genetic mouse model of spherocytosis and single-cell RNA sequencing, we found that erythrophagocytosis skewed liver macrophages into an antiinflammatory phenotype that we defined as MarcohiHmoxhiMHC class IIo erythrophagocytes. This phenotype transformation profoundly mitigated disease expression in a model of an anti-CD40-induced hyperinflammatory syndrome with necrotic hepatitis and in a nonalcoholic steatohepatitis model, representing 2 macrophage-driven sterile inflammatory diseases. We reproduced the antiinflammatory erythrophagocyte transformation in vitro by heme exposure of mouse and human macrophages, yielding a distinctive transcriptional signature that segregated heme-polarized from M1- and M2-polarized cells. Mapping transposase-accessible chromatin in single cells by sequencing defined the transcription factor NFE2L2/NRF2 as a critical driver of erythrophagocytes, and Nfe2l2/Nrf2 deficiency restored heme-suppressed inflammation. Our findings point to a pathway that regulates macrophage functions to link erythrocyte homeostasis with innate immunity.

Epithelial splicing regulatory protein 2-mediated alternative splicing reprograms hepatocytes in severe alcoholic hepatitis

Journal of clinical investigation (IF: 12.81), 2020-4, Auinash Kalsotra et al. DOI: 10.1172/JCI132691

PubMed: 31945016

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Severe alcoholic hepatitis (SAH) is a deadly liver disease without an effective medical therapy. Although SAH mortality is known to correlate with hepatic accumulation of immature liver cells, why this occurs and how it causes death are unclear. Here, we demonstrate that expression of epithelial splicing regulatory protein 2 (ESRP2), an RNA-splicing factor that maintains the nonproliferative, mature phenotype of adult hepatocytes, was suppressed in both human SAH and various mouse models of SAH in parallel with the severity of alcohol consumption and liver damage. Inflammatory cytokines released by excessive alcohol ingestion reprogrammed adult hepatocytes into proliferative, fetal-like cells by suppressing ESRP2. Sustained loss of ESRP2 permitted reemergence of a fetal RNA-splicing program that attenuates the Hippo signaling pathway and thus allows fetal transcriptional regulators to accumulate in adult liver. We further showed that depleting ESRP2 in mice exacerbated alcohol-induced steatohepatitis, enabling surviving hepatocytes to shed adult hepatocyte functions and become more regenerative, but threatening overall survival by populating the liver with functionally immature hepatocytes. Our findings revealed a mechanism that explains why liver failure develops in patients with the clinical syndrome of SAH, suggesting that recovery from SAH might be improved by limiting adult-to-fetal reprogramming in hepatocytes.

Constructing and deconstructing GATA2-regulated cell fate programs to establish developmental trajectories

Journal of experimental medicine (IF: 11.32), 2020-11, Sunduz Keles et al. DOI: 10.1084/jem.20191526

PubMed: 32736380

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Stem and progenitor cell fate transitions constitute key decision points in organismal development that enable access to a developmental path or actively preclude others. Using the hematopoietic system, we analyzed the relative importance of cell fate-promoting mechanisms versus negating fate-suppressing mechanisms to engineer progenitor cells with multilineage differentiation potential. Deletion of the murine Gata2-77 enhancer, with a human equivalent that causes leukemia, downregulates the transcription factor GATA2 and blocks progenitor differentiation into erythrocytes, megakaryocytes, basophils, and granulocytes, but not macrophages. Using multiomics and single-cell analyses, we demonstrated that the enhancer orchestrates a balance between pro- and anti-fate circuitry in single cells. By increasing GATA2 expression, the enhancer instigates a fate-promoting mechanism while abrogating an innate immunity-linked, fate-suppressing mechanism. During embryogenesis, the suppressing mechanism dominated in enhancer mutant progenitors, thus yielding progenitors with a predominant monocytic differentiation potential. Coordinating fate-promoting and -suppressing circuits therefore averts deconstruction of a multifate system into a monopotent system and maintains critical progenitor heterogeneity and functionality.

Membrane budding is a major mechanism of in vivo platelet biogenesis

Journal of experimental medicine (IF: 11.32), 2020-9, Edwin D Hawkins et al. DOI: 10.1084/jem.20191206

PubMed: 32706855

Single Cell Gene Expression, Mouse, Immunology.

Abstract

How platelets are produced by megakaryocytes in vivo remains controversial despite more than a century of investigation. Megakaryocytes readily produce proplatelet structures in vitro; however, visualization of platelet release from proplatelets in vivo has remained elusive. We show that within the native prenatal and adult environments, the frequency and rate of proplatelet formation is incompatible with the physiological demands of platelet replacement. We resolve this inconsistency by performing in-depth analysis of plasma membrane budding, a cellular process that has previously been dismissed as a source of platelet production. Our studies demonstrate that membrane budding results in the sustained release of platelets directly into the peripheral circulation during both fetal and adult life without induction of cell death or proplatelet formation. In support of this model, we demonstrate that in mice deficient for NF-E2 (the thrombopoietic master regulator), the absence of membrane budding correlates with failure of in vivo platelet production. Accordingly, we propose that membrane budding, rather than proplatelet formation, supplies the majority of the platelet biomass.

Hepatocyte-specific TAK1 deficiency drives RIPK1 kinase-dependent inflammation to promote liver fibrosis and hepatocellular carcinoma

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, Nan Liu et al. DOI: 10.1073/pnas.2005353117 PubMed: 32513687

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Transforming growth factor β -activated kinase1 (TAK1) encoded by the gene MAP3K7 regulates multiple important downstream effectors involved in immune response, cell death, and carcinogenesis. Hepatocyte-specific deletion of TAK1 in mice promotes liver fibrosis and hepatocellular carcinoma (HCC) formation. Here, we report that genetic inactivation of RIPK1 kinase using a kinase dead knock in D138N mutation in mice inhibits the expression of liver tumor biomarkers, liver fibrosis, and HCC formation. Inhibition of RIPK1, however, has no or minimum effect on hepatocyte loss and compensatory proliferation, which are the recognized factors important for liver fibrosis and HCC development. Using single-cell RNA sequencing, we discovered that inhibition of RIPK1 strongly suppresses inflammation induced by hepatocyte-specific loss of TAK1. Activation of RIPK1 promotes the transcription of key proinflammatory cytokines, such as CCL2, and CCR2 macrophage infiltration. Our study demonstrates the role and mechanism of RIPK1 kinase in promoting inflammation, both cell-autonomously and cell-nonautonomously, in the development of liver fibrosis and HCC, independent of cell death, and compensatory proliferation. We suggest the possibility of inhibiting RIPK1 kinase as a therapeutic strategy for reducing liver fibrosis and HCC development by inhibiting inflammation.

Peripheral PDGFR α +gp38+ mesenchymal cells support the differentiation of fetal liver-derived ILC2

Journal of experimental medicine (IF: 11.32), 2018-6, Shigeo Koyasu et al. DOI: 10.1084/jem.20172310

PubMed: 29728440

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Group 2 innate lymphoid cells (ILC2s) are derived from common lymphoid progenitors (CLPs) via several specific precursors, and the transcription factors essential for ILC2 differentiation have been extensively studied. However, the external factors regulating commitment to the ILC lineage as well as the sites and stromal cells that constitute the optimal microenvironment for ILC2-specific differentiation are not fully defined. In this study, we demonstrate that three key external factors, the concentration of interleukin 7 (IL-7) and strength and duration of Notch signaling, coordinately determine the fate of CLP toward the T, B, or ILC lineage. Additionally, we identified three stages of ILC2 in the fetal mesentery that require STAT5 signals for maturation: ILC progenitors, CCR9 ILC2 progenitors, and KLRG1 immature ILC2. We further demonstrate that ILC2 development is supported by mesenteric platelet-derived growth factor receptor α (PDGFR α) glycoprotein 38 (gp38) mesenchymal cells. Collectively, our results suggest that early differentiation of ILC2 occurs in the fetal liver via IL-7 and Notch signaling, whereas final differentiation occurs in the periphery with the aid of PDGFR α gp38 cells.

Enhancement of LIN28B-induced hematopoietic reprogramming by IGF2BP3

Genes & development (IF: 10.33), 2019-8, Markus Hafner et al. DOI: 10.1101/gad.325100.119 PubMed: 31221665
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Fetal hematopoietic stem and progenitor cells (HSPCs) hold promise to cure a wide array of hematological diseases, and we previously found a role for the RNA-binding protein (RBP) Lin28b in respecifying adult HSPCs to resemble their fetal counterparts. Here we show by single-cell RNA sequencing that Lin28b alone was insufficient for complete reprogramming of gene expression from the adult toward the fetal pattern. Using proteomics and in situ analyses, we found that Lin28b (and its closely related paralog, Lin28a) directly interacted with Igf2bp3, another RBP, and their enforced co-expression in adult HSPCs reactivated fetal-like B-cell development in vivo more efficiently than either factor alone. In B-cell progenitors, Lin28b and Igf2bp3 jointly stabilized thousands of mRNAs by binding at the same sites, including those of the B-cell regulators and as well as mRNA itself, forming an autoregulatory loop. Our results suggest that Lin28b and Igf2bp3 are at the center of a gene regulatory network that mediates the fetal-adult hematopoietic switch. A method to efficiently generate induced fetal-like hematopoietic stem cells (ifHSCs) will facilitate basic studies of their biology and possibly pave a path toward their clinical application.

Distinct Waves from the Hemogenic Endothelium Give Rise to Layered Lymphoid Tissue Inducer Cell Ontogeny

Cell Reports (IF: 8.82), 2020-8, Rachel Golub et al. DOI: 10.1016/j.celrep.2020.108004 PubMed: 32783932
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

During embryogenesis, lymphoid tissue inducer (LTi) cells are essential for lymph node organogenesis. These cells are part of the innate lymphoid cell (ILC) family. Although their earliest embryonic hematopoietic origin is unclear, other innate immune cells have been shown to be derived from early hemogenic endothelium in the yolk sac as well as the aorta-gonad-mesonephros. A proper model to discriminate between these locations was unavailable. In this study, using a Cxcr4-CreERT2 lineage tracing model, we identify a major contribution from embryonic hemogenic endothelium, but not the yolk sac, toward LTi progenitors. Conversely, embryonic LTi cells are replaced by hematopoietic stem cell-derived cells in adults. We further show that, in the fetal liver, common lymphoid progenitors differentiate into highly dynamic alpha-lymphoid precursor cells that, at this embryonic stage, preferentially mature into LTi precursors and establish their functional LTi cell identity only after reaching the periphery.

Mitigating Antagonism between Transcription and Proliferation Allows Near-Deterministic Cellular Reprogramming

Cell Stem Cell (IF: 23.45), 2019-10, Berislav V Zlokovic et al. DOI: 10.1016/j.stem.2019.08.005 PubMed: 31523028
Single Cell Gene Expression, Human & Mouse, Developmental Biology, Functional Genomics.

Abstract

Although cellular reprogramming enables the generation of new cell types for disease modeling and regenerative therapies, reprogramming remains a rare cellular event. By examining reprogramming of fibroblasts into motor neurons and multiple other somatic lineages, we find that epigenetic barriers to conversion can be overcome by endowing cells with the ability to mitigate an inherent antagonism between transcription and DNA replication. We show that transcription factor overexpression induces unusually high rates of transcription and that sustaining hypertranscription and transgene expression in hyperproliferative cells early in reprogramming is critical for successful lineage conversion. However, hypertranscription impedes DNA replication and cell proliferation, processes that facilitate reprogramming. We identify a chemical and genetic cocktail that dramatically increases the number of cells capable of simultaneous hypertranscription and hyperproliferation by activating topoisomerases. Further, we show that hypertranscribing, hyperproliferating cells reprogram at 100-fold higher, near-deterministic rates. Therefore, relaxing biophysical constraints overcomes molecular barriers to cellular reprogramming.

Human Pluripotency Is Initiated and Preserved by a Unique Subset of Founder Cells

Cell (IF: 38.62), 2019-5, Andras NagyMickie Bhatia DOI: 10.1016/j.cell.2019.03.013 PubMed: 30982595
Single Cell Gene Expression, Human, Developmental Biology.

Abstract

The assembly of organized colonies is the earliest manifestation in the derivation or induction of pluripotency in vitro. However, the necessity and origin of this assemblance is unknown. Here, we identify human pluripotent founder cells (hPFCs) that initiate, as well as preserve and establish, pluripotent stem cell (PSC) cultures. PFCs are marked by N-cadherin expression (NCAD) and reside exclusively at the colony boundary of primate PSCs. As demonstrated by functional analysis, hPFCs harbor the clonogenic capacity of PSC cultures and emerge prior to commitment events or phenotypes associated with pluripotent reprogramming. Comparative single-cell analysis with pre- and post-implantation primate embryos revealed hPFCs share hallmark properties with primitive endoderm (PrE) and can be regulated by non-canonical Wnt signaling. Uniquely informed by primate embryo organization in vivo, our study defines a subset of founder cells critical to the establishment pluripotent state.

Multi-layered Spatial Transcriptomics Identify Secretory Factors Promoting Human Hematopoietic Stem Cell Development

Cell Stem Cell (IF: 23.45), 2020-11, Richard A Anderson et al. DOI: 10.1016/j.stem.2020.08.004 PubMed: 32946788
Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Hematopoietic stem cells (HSCs) first emerge in the embryonic aorta-gonad-mesonephros (AGM) region. Studies of model organisms defined intersecting signaling pathways that converge to promote HSC emergence predominantly in the ventral domain of the dorsal aorta. Much less is known about mechanisms driving HSC development in humans. Here, to identify secreted signals underlying human HSC development, we combined spatial transcriptomics analysis of dorsoventral polarized signaling in the aorta with gene expression profiling of sorted cell populations and single cells. Our analysis revealed a subset of aortic endothelial cells with a downregulated arterial signature and a predicted lineage relationship with the emerging HSC/progenitor population. Analysis of the ventrally polarized molecular landscape identified endothelin 1 as an important secreted regulator of human HSC development. The obtained gene expression datasets will inform future studies on mechanisms of HSC development in vivo and on generation of clinically relevant HSCs in vitro.

Modeling Monogenic Diabetes using Human ESCs Reveals Developmental and Metabolic Deficiencies Caused by Mutations in HNF1A

Cell Stem Cell (IF: 23.45), 2019-8, Deborah L French et al. DOI: 10.1016/j.stem.2019.07.007 PubMed: 31374199
Single Cell Gene Expression, Human, Developmental Biology, Functional Genomics.

Abstract

Human monogenic diabetes, caused by mutations in genes involved in beta cell development and function, has been a challenge to study because multiple mouse models have not fully recapitulated the human disease. Here, we use genome edited human embryonic stem cells to understand the most common form of monogenic diabetes, MODY3, caused by mutations in the transcription factor HNF1A. We found that HNF1A is necessary to repress an alpha cell gene expression signature, maintain endocrine cell function, and regulate cellular metabolism. In addition, we identified the human-specific long non-coding RNA, LINKA, as an HNF1A target necessary for normal mitochondrial respiration. These findings provide a possible explanation for the species difference in disease phenotypes observed with HNF1A mutations and offer mechanistic insights into how the HNF1A gene may also influence type 2 diabetes.

Single-cell patterning and axis characterization in the murine and human definitive endoderm

Cell Research (IF: 20.4), 2020-10, Jun Gu et al. DOI: 10.1038/s41422-020-00426-0 PubMed: 33106598

Single Cell Gene Expression, Human & Mouse, Developmental Biology.

Abstract

Defining the precise regionalization of specified definitive endoderm progenitors is critical for understanding the mechanisms underlying the generation and regeneration of respiratory and digestive organs, yet the patterning of endoderm progenitors remains unresolved, particularly in humans. We performed single-cell RNA sequencing on endoderm cells during the early somitogenesis stages in mice and humans. We developed molecular criteria to define four major endoderm regions (foregut, lip of anterior intestinal portal, midgut, and hindgut) and their developmental pathways. We identified the cell subpopulations in each region and their spatial distributions and characterized key molecular features along the body axes. Dorsal and ventral pancreatic progenitors appear to originate from the midgut population and follow distinct pathways to develop into an identical cell type. Finally, we described the generally conserved endoderm patterning in humans and clear differences in dorsal cell distribution between species. Our study comprehensively defines single-cell endoderm patterning and provides novel insights into the spatiotemporal process that drives establishment of early endoderm domains.

Tracing the first hematopoietic stem cell generation in human embryo by single-cell RNA sequencing

Cell Research (IF: 20.4), 2019-9, Yu Lan et al. DOI: 10.1038/s41422-019-0228-6 PubMed: 31501518

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Tracing the emergence of the first hematopoietic stem cells (HSCs) in human embryos, particularly the scarce and transient precursors thereof, is so far challenging, largely due to the technical limitations and the material rarity. Here, using single-cell RNA sequencing, we constructed the first genome-scale gene expression landscape covering the entire course of endothelial-to-HSC transition during human embryogenesis. The transcriptomically defined HSC-primed hemogenic endothelial cells (HECs) were captured at Carnegie stage (CS) 12-14 in an unbiased way, showing an unambiguous feature of arterial endothelial cells (ECs) with the up-regulation of RUNX1, MYB and ANGPT1. Importantly, subcategorizing CD34CD45ECs into a CD44 population strikingly enriched HECs by over 10-fold. We further mapped the developmental path from arterial ECs via HSC-primed HECs to hematopoietic stem progenitor cells, and revealed a distinct expression pattern of genes that were transiently over-represented upon the hemogenic fate choice of arterial ECs, including EMCN, PROCR and RUNX1T1. We also uncovered another temporally and molecularly distinct intra-embryonic HEC population, which was detected mainly at earlier CS 10 and lacked the arterial feature. Finally, we revealed the cellular components of the putative aortic niche and potential cellular interactions acting on the HSC-primed HECs. The cellular and molecular programs that underlie the generation of the first HSCs from HECs in human embryos, together with the ability to distinguish the HSC-primed HECs from others, will shed light on the strategies for the production of clinically useful HSCs from pluripotent stem cells.

HMGB2 Loss upon Senescence Entry Disrupts Genomic Organization and Induces CTCF Clustering across Cell Types

Molecular Cell (IF: 16.13), 2018-5, Karsten RippeArgyris Papanonis et al. DOI: 10.1016/j.molcel.2018.03.030

PubMed: 29706538

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Processes like cellular senescence are characterized by complex events giving rise to heterogeneous cell populations. However, the early molecular events driving this cascade remain elusive. We hypothesized that senescence entry is triggered by an early disruption of the cells' three-dimensional (3D) genome organization. To test this, we combined Hi-C, single-cell and population transcriptomics, imaging, and in silico modeling of three distinct cell types entering senescence. Genes involved in DNA conformation maintenance are suppressed upon senescence entry across all cell types. We show that nuclear depletion of the abundant HMGB2 protein occurs early on the path to senescence and coincides with the dramatic spatial clustering of CTCF. Knocking down HMGB2 suffices for senescence-induced CTCF clustering and for loop reshuffling, while ectopically expressing HMGB2 rescues these effects. Our data suggest that HMGB2-mediated genomic reorganization constitutes a primer for the ensuing senescent program.

Human pre-valvular endocardial cells derived from pluripotent stem cells recapitulate cardiac pathophysiological valvulogenesis

Nature Communications (IF: 13.61), 2019-4, Roger Markwald Michel Puc  at et al. DOI: 10.1038/s41467-019-09459-5 PubMed: 31028265

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Genetically modified mice have advanced our understanding of valve development and disease. Yet, human pathophysiological valvulogenesis remains poorly understood. Here we report that, by combining single cell sequencing and in vivo approaches, a population of human pre-valvular endocardial cells (HPVCs) can be derived from pluripotent stem cells. HPVCs express gene patterns conforming to the E9.0 mouse atrio-ventricular canal (AVC) endocardium signature. HPVCs treated with BMP2, cultured on mouse AVC cushions, or transplanted into the AVC of embryonic mouse hearts, undergo endothelial-to-mesenchymal transition and express markers of valve interstitial cells of different valvular layers, demonstrating cell specificity. Extending this model to patient-specific induced pluripotent stem cells recapitulates features of mitral valve prolapse and identified dysregulation of the SHH pathway. Concurrently increased ECM secretion can be rescued by SHH inhibition, thus providing a putative therapeutic target. In summary, we report a human cell model of valvulogenesis that faithfully recapitulates valve disease in a dish.

An accurate and robust imputation method scImpute for single-cell RNA-seq data

Nature Communications (IF: 13.61), 2018-12, Wei Vivian Li et al. DOI: 10.1038/s41467-018-03405-7

PubMed: 29520097

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

The emerging single-cell RNA sequencing (scRNA-seq) technologies enable the investigation of transcriptomic landscapes at the single-cell resolution. ScRNA-seq data analysis is complicated by excess zero counts, the so-called dropouts due to low amounts of mRNA sequenced within individual cells. We introduce scImpute, a statistical method to accurately and robustly impute the dropouts in scRNA-seq data. scImpute automatically identifies likely dropouts, and only perform imputation on these values without introducing new biases to the rest data. scImpute also detects outlier cells and excludes them from imputation. Evaluation based on both simulated and real human and mouse scRNA-seq data suggests that scImpute is an effective tool to recover transcriptome dynamics masked by dropouts. scImpute is shown to identify likely dropouts, enhance the clustering of cell subpopulations, improve the accuracy of differential expression analysis, and aid the study of gene expression dynamics.

Generation of pulmonary neuroendocrine cells and SCLC-like tumors from human embryonic stem cells

Journal of experimental medicine (IF: 11.32), 2019-3, Hans-Willem Snoeck Harold Varmus et al.

DOI: 10.1084/jem.20181155 PubMed: 30737256

Single Cell Gene Expression, Human, Cancer Research, Developmental Biology.

Abstract

Cancer models based on cells derived from human embryonic stem cells (hESCs) may reveal why certain constellations of genetic changes drive carcinogenesis in specialized lineages. Here we demonstrate that inhibition of NOTCH signaling induces up to 10% of lung progenitor cells to form pulmonary neuroendocrine cells (PNECs), putative precursors to small cell lung cancers (SCLCs), and we can increase PNECs by reducing levels of retinoblastoma (RB) proteins with inhibitory RNA. Reducing levels of TP53 protein or expressing mutant genes did not induce or expand PNECs, but tumors resembling early-stage SCLC grew in immunodeficient mice after subcutaneous injection of PNEC-containing cultures in which expression of both and was blocked. Single-cell RNA profiles of PNECs are heterogeneous; when RB levels are reduced, the profiles resemble those from early-stage SCLC; and when both RB and TP53 levels are reduced, the transcriptome is enriched with cell cycle-specific RNAs. Our findings suggest that genetic manipulation of hESC-derived pulmonary cells will enable studies of this recalcitrant cancer.

Epigenetic regulator function through mouse gastrulation

Nature (IF: 46.49), 2020-8, Shankar Mukherji et al. DOI: 10.1038/s41586-020-2552 PubMed: 32728215

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

During ontogeny, proliferating cells become restricted in their fate through the combined action of cell-type-specific transcription factors and ubiquitous epigenetic machinery, which recognizes universally available histone residues or nucleotides in a context-dependent manner. The molecular functions of these regulators are generally well understood, but assigning direct developmental roles to them is hampered by complex mutant phenotypes that often emerge after gastrulation. Single-cell RNA sequencing and analytical approaches have explored this highly conserved, dynamic period across numerous model organisms, including mouse. Here we advance these strategies using a combined zygotic perturbation and single-cell RNA-sequencing platform in which many mutant mouse embryos can be assayed simultaneously, recovering robust morphological and transcriptional information across a panel of ten essential regulators. Deeper analysis of central Polycomb repressive complex (PRC) 1 and 2 components indicates substantial cooperativity, but distinguishes a dominant role for PRC2 in restricting the germline. Moreover, PRC mutant phenotypes emerge after gross epigenetic and transcriptional changes within the initial conceptus prior to gastrulation. Our experimental framework may eventually lead to a fully quantitative view of how cellular diversity emerges using an identical genetic template and from a single totipotent cell.

Molecular design of hypothalamus development

Nature (IF: 46.49), 2020-6, Erik Keimpema et al. DOI: 10.1038/s41586-020-2266-0 PubMed: 32499648

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

A wealth of specialized neuroendocrine command systems intercalated within the hypothalamus control the most fundamental physiological needs in vertebrates. Nevertheless, we lack a developmental blueprint that integrates the molecular determinants of neuronal and glial diversity along temporal and spatial scales of hypothalamus development. Here we combine single-cell RNA sequencing of 51,199 mouse cells of ectodermal origin, gene regulatory network (GRN) screens in conjunction with genome-wide association study-based disease phenotyping, and genetic lineage reconstruction to show that nine glial and thirty-three neuronal subtypes are generated by mid-gestation under the control of distinct GRNs. Combinatorial molecular codes that arise from neurotransmitters, neuropeptides and transcription factors are minimally required to decode the taxonomical hierarchy of hypothalamic neurons. The differentiation of γ -aminobutyric acid (GABA) and dopamine neurons, but not glutamate neurons, relies on quasi-stable intermediate states, with a pool of GABA progenitors giving rise to dopamine cells. We found an unexpected abundance of chemotropic proliferation and guidance cues that are commonly implicated in dorsal (cortical) patterning in the hypothalamus. In particular, loss of SLIT-ROBO signalling impaired both the production and positioning of periventricular dopamine neurons. Overall, we identify molecular principles that shape the developmental architecture of the hypothalamus and show how neuronal heterogeneity is transformed into a multimodal neural unit to provide virtually infinite adaptive potential throughout life.

Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids

Nature (IF: 46.49), 2020-6, Alfonso Martinez Arias et al. DOI: 10.1038/s41586-020-2024-3 PubMed: 32076263

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Gastruloids are three-dimensional aggregates of embryonic stem cells that display key features of mammalian development after implantation, including germ-layer specification and axial organization. To date, the expression pattern of only a small number of genes in gastruloids has been explored with microscopy, and the extent to which genome-wide expression patterns in gastruloids mimic those in embryos is unclear. Here we compare mouse gastruloids with mouse embryos using single-cell RNA sequencing and spatial transcriptomics. We identify various embryonic cell types that were not previously known to be present in gastruloids, and show that key regulators of somitogenesis are expressed similarly between embryos and gastruloids. Using live imaging, we show that the somitogenesis clock is active in gastruloids and has dynamics that resemble those in vivo. Because gastruloids can be grown in large quantities, we performed a small screen that revealed how reduced FGF signalling induces a short-tail phenotype in embryos. Finally, we demonstrate that embedding in Matrigel induces gastruloids to generate somites with the correct rostral-caudal patterning, which appear sequentially in an anterior-to-posterior direction over time. This study thus shows the power of gastruloids as a model system for exploring development and somitogenesis in vitro in a high-throughput manner.

Molecular recording of mammalian embryogenesis

Nature (IF: 46.49), 2019-5, Alexander Meissner et al. DOI: 10.1038/s41586-019-1184-5 PubMed: 31086336

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Ontogeny describes the emergence of complex multicellular organisms from single totipotent cells. This field is particularly challenging in mammals, owing to the indeterminate relationship between self-renewal and differentiation, variation in progenitor field sizes, and internal gestation in these animals. Here we present a flexible, high-information, multi-channel molecular recorder with a single-cell readout and apply it as an evolving lineage tracer to assemble mouse cell-fate maps from fertilization through gastrulation. By combining lineage information with single-cell RNA sequencing profiles, we recapitulate canonical developmental relationships between different tissue types and reveal the nearly complete transcriptional convergence of endodermal cells of extra-embryonic and embryonic origins. Finally, we apply our cell-fate maps to estimate the number of embryonic progenitor cells and their degree of asymmetric partitioning during specification. Our approach enables massively parallel, high-resolution recording of lineage and other information in mammalian systems, which will facilitate the construction of a quantitative framework for understanding developmental processes.

Single-cell analysis of cardiogenesis reveals basis for organ-level developmental defects

Nature (IF: 46.49), 2019-1, Casey A Gifford et al. DOI: 10.1038/s41586-019-1414 PubMed: 31341279

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Organogenesis involves integration of diverse cell types; dysregulation of cell-type-specific gene networks results in birth defects, which affect 5% of live births. Congenital heart defects are the most common malformations, and result from disruption of discrete subsets of cardiac progenitor cells, but the transcriptional changes in individual progenitors that lead to organ-level defects remain unknown. Here we used single-cell RNA sequencing to interrogate early cardiac progenitor cells as they become specified during normal and abnormal cardiogenesis, revealing how dysregulation of specific cellular subpopulations has catastrophic consequences. A network-based computational method for single-cell RNA-sequencing analysis that predicts lineage-specifying transcription factors identified Hand2 as a specifier of outflow tract cells but not right ventricular cells, despite the failure of right ventricular formation in Hand2-null mice. Temporal single-cell-transcriptome analysis of Hand2-null embryos revealed failure of outflow tract myocardium specification, whereas right ventricular myocardium was specified but failed to properly differentiate and migrate. Loss of Hand2 also led to dysregulation of retinoic acid signalling and disruption of anterior-posterior patterning of cardiac progenitors. This work reveals transcriptional determinants that specify fate and differentiation in individual cardiac progenitor cells, and exposes mechanisms of disrupted cardiac development at single-cell resolution, providing a framework for investigating congenital heart defects.

The emergent landscape of the mouse gut endoderm at single-cell resolution

Nature (IF: 46.49), 2019-4, Anna-Katerina Hadjantonakis et al. DOI: 10.1038/s41586-019-1127-1

PubMed: 30959515

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Here we delineate the ontogeny of the mammalian endoderm by generating 112,217 single-cell transcriptomes, which represent all endoderm populations within the mouse embryo until midgestation. We use graph-based approaches to model differentiating cells, which provides a spatio-temporal characterization of developmental trajectories and defines the transcriptional architecture that accompanies the emergence of the first (primitive or extra-embryonic) endodermal population and its sister pluripotent (embryonic) epiblast lineage. We uncover a relationship between descendants of these two lineages, in which epiblast cells differentiate into endoderm at two distinct time points-before and during gastrulation. Trajectories of endoderm cells were mapped as they acquired embryonic versus extra-embryonic fates and as they spatially converged within the nascent gut endoderm, which revealed these cells to be globally similar but retain aspects of their lineage history. We observed the regionalized identity of cells along the anterior-posterior axis of the emergent gut tube, which reflects their embryonic or extra-embryonic origin, and the coordinated patterning of these cells into organ-specific territories.

Unblending of Transcriptional Condensates in Human Repeat Expansion Disease

Cell (IF: 38.62), 2020-5, Daniel M Ibrahim et al. DOI: 10.1016/j.cell.2020.04.018 PubMed: 32386547

Single Cell Gene Expression, Mouse, Developmental Biology, Genetic Health.

Abstract

Expansions of amino acid repeats occur in >20 inherited human disorders, and many occur in intrinsically disordered regions (IDRs) of transcription factors (TFs). Such diseases are associated with protein aggregation, but the contribution of aggregates to pathology has been controversial. Here, we report that alanine repeat expansions in the HOXD13 TF, which cause hereditary synpolydactyly in humans, alter its phase separation capacity and its capacity to co-condense with transcriptional co-activators. HOXD13 repeat expansions perturb the composition of HOXD13-containing condensates in vitro and in vivo and alter the transcriptional program in a cell-specific manner in a mouse model of synpolydactyly. Disease-associated repeat expansions in other TFs (HOXA13, RUNX2, and TBP) were similarly found to alter their phase separation. These results suggest that unblending of transcriptional condensates may underlie human pathologies. We present a molecular classification of TF IDRs, which provides a framework to dissect TF function in diseases associated with transcriptional dysregulation.

Defining murine organogenesis at single-cell resolution reveals a role for the leukotriene pathway in regulating blood progenitor formation

Nature cell biology (IF: 20.96), 2018-2, Berthold Göttgens et al. DOI: 10.1038/s41556-017-0013

PubMed: 29311656

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

During gastrulation, cell types from all three germ layers are specified and the basic body plan is established. However, molecular analysis of this key developmental stage has been hampered by limited numbers and a paucity of markers. Single-cell RNA sequencing circumvents these problems, but has so far been limited to specific organ systems. Here, we report single-cell transcriptomic characterization of >20,000 cells immediately following gastrulation at E8.25 of mouse development. We identify 20 major cell types, which frequently contain substructure, including three distinct signatures in early foregut cells. Pseudo-space ordering of somitic progenitor cells identifies dynamic waves of transcription and candidate regulators, which are validated by molecular characterization of spatially resolved regions of the embryo. Within the endothelial population, cells that transition from haemogenic endothelial to erythro-myeloid progenitors specifically express Alox5 and its co-factor Alox5ap, which control leukotriene production. Functional assays using mouse embryonic stem cells demonstrate that leukotrienes promote haematopoietic progenitor cell generation. Thus, this comprehensive single-cell map can be exploited to reveal previously unrecognized pathways that contribute to tissue development.

The Transcription Factor ZEB2 Is Required to Maintain the Tissue-Specific Identities of Macrophages

Immunity (IF: 25.73), 2018-8, Yvan Saeys et al. DOI: 10.1016/j.immuni.2018.07.004 PubMed: 30076102

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Heterogeneity between different macrophage populations has become a defining feature of this lineage. However, the conserved factors defining macrophages remain largely unknown. The transcription factor ZEB2 is best described for its role in epithelial to mesenchymal transition; however, its role within the immune system is only now being elucidated. We show here that Zeb2 expression is a conserved feature of macrophages. Using Clec4f-cre, Itgax-cre, and Fcgr1-cre mice to target five different macrophage populations, we found that loss of ZEB2 resulted in macrophage disappearance from the tissues, coupled with their subsequent replenishment from bone-marrow precursors in open niches. Mechanistically, we found that ZEB2 functioned to maintain the tissue-specific identities of macrophages. In Kupffer cells, ZEB2 achieved this by regulating expression of the transcription factor LXR α , removal of which recapitulated the loss of Kupffer cell identity and disappearance. Thus, ZEB2 expression is required in macrophages to preserve their tissue-specific identities.

The cellular and molecular landscape of hypothalamic patterning and differentiation from embryonic to late postnatal development

Nature Communications (IF: 13.61), 2020-8, Lizhi Jiang et al. DOI: 10.1038/s41467-020-18231 PubMed: 32868762

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The hypothalamus is a central regulator of many innate behaviors essential for survival, but the molecular mechanisms controlling hypothalamic patterning and cell fate specification are poorly understood. To identify genes that control hypothalamic development, we have used single-cell RNA sequencing (scRNA-Seq) to profile mouse hypothalamic gene expression across 12 developmental time points between embryonic day 10 and postnatal day 45. This identified genes that delineated clear developmental trajectories for all major hypothalamic cell types, and readily distinguished major regional subdivisions of the developing hypothalamus. By using our developmental dataset, we were able to rapidly annotate previously unidentified clusters from existing scRNA-Seq datasets collected during development and to identify the developmental origins of major neuronal populations of the ventromedial hypothalamus. We further show that our approach can rapidly and comprehensively characterize mutants that have altered hypothalamic patterning, identifying Nkx2.1 as a negative regulator of prethalamic identity. These data serve as a resource for further studies of hypothalamic development, physiology, and dysfunction.

HOX13-dependent chromatin accessibility underlies the transition towards the digit development program

Nature Communications (IF: 13.61), 2020-5, Jacques Drouin et al. DOI: 10.1038/s41467-020-16317-2

PubMed: 32427842

Single Cell ATAC, Mouse, Developmental Biology.

Abstract

Hox genes encode transcription factors (TFs) that establish morphological diversity in the developing embryo. The similar DNA-binding motifs of the various HOX TFs contrast with the wide-range of HOX-dependent genetic programs. The influence of the chromatin context on HOX binding specificity remains elusive. Here, we used the developing limb as a model system to compare the binding specificity of HOXA13 and HOXD13 (HOX13 hereafter), which are required for digit formation, and HOXA11, involved in forearm/leg development. We find that upon ectopic expression in distal limb buds, HOXA11 binds sites normally HOX13-specific. Importantly, these sites are loci whose chromatin accessibility relies on HOX13. Moreover, we show that chromatin accessibility specific to the distal limb requires HOX13 function. Based on these results, we propose that HOX13 TFs pioneer the distal limb-specific chromatin accessibility landscape for the proper implementation of the distal limb developmental program.

Single cell transcriptomics identifies a signaling network coordinating endoderm and mesoderm diversification during foregut organogenesis

Nature Communications (IF: 13.61), 2020-8, Takanori Takebe et al. DOI: 10.1038/s41467-020-17968

PubMed: 32855417

Single Cell Immune Profiling, Mouse, Developmental Biology.

Abstract

Visceral organs, such as the lungs, stomach and liver, are derived from the fetal foregut through a series of inductive interactions between the definitive endoderm (DE) and the surrounding splanchnic mesoderm (SM). While DE patterning is fairly well studied, the paracrine signaling controlling SM regionalization and how this is coordinated with epithelial identity is obscure. Here, we use single cell transcriptomics to generate a high-resolution cell state map of the embryonic mouse foregut. This identifies a diversity of SM cell types that develop in close register with the organ-specific epithelium. We infer a spatiotemporal signaling network of endoderm-mesoderm interactions that orchestrate foregut organogenesis. We validate key predictions with mouse genetics, showing the importance of endoderm-derived signals in mesoderm patterning. Finally, leveraging these signaling interactions, we generate different SM subtypes from human pluripotent stem cells (hPSCs), which previously have been elusive. The single cell data can be explored at: <https://research.cchmc.org/ZornLab-singlecell>.

Ash2l interacts with Oct4-stemness circuitry to promote super-enhancer-driven pluripotency network

Nucleic Acids Research (IF: 11.8), 2019-11, Chung-Hsuan Chen et al. DOI: 10.1093/nar/gkz801 PubMed: 31555818
Single Cell Gene Expression, Mouse, Developmental Biology, Functional Genomics.

Abstract

Pluripotency and cell fates can be modulated through the regulation of super-enhancers; however, the underlying mechanisms are unclear. Here, we showed a novel mechanism in which Ash2l directly binds to super-enhancers of several stemness genes to regulate pluripotency and self-renewal in pluripotent stem cells. Ash2l recruits Oct4/Sox2/Nanog (OSN) to form Ash2l/OSN complex at the super-enhancers of *Jarid2*, *Nanog*, *Sox2* and *Oct4*, and further drives enhancer activation, upregulation of stemness genes, and maintains the pluripotent circuitry. Ash2l knockdown abrogates the OSN recruitment to all super-enhancers and further hinders the enhancer activation. In addition, CRISPRi/dCas9-mediated blocking of Ash2l-binding motifs at these super-enhancers also prevents OSN recruitment and enhancer activation, validating that Ash2l directly binds to super-enhancers and initiates the pluripotency network. Transfection of Ash2l with W118A mutation to disrupt Ash2l-Oct4 interaction fails to rescue Ash2l-driven enhancer activation and pluripotent gene upregulation in Ash2l-depleted pluripotent stem cells. Together, our data demonstrated Ash2l formed an enhancer-bound Ash2l/OSN complex that can drive enhancer activation, govern pluripotency network and stemness circuitry.

Single-Cell Transcriptomic Analyses of the Developing Meninges Reveal Meningeal Fibroblast Diversity and Function

Developmental Cell (IF: 10.13), 2020-7, Kenneth L Jones et al. DOI: 10.1016/j.devcel.2020.06.009
PubMed: 32634398

Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

The meninges are a multilayered structure composed of fibroblasts, blood and lymphatic vessels, and immune cells. Meningeal fibroblasts secrete a variety of factors that control CNS development, yet strikingly little is known about their heterogeneity or development. Using single-cell sequencing, we report distinct transcriptional signatures for fibroblasts in the embryonic dura, arachnoid, and pia. We define new markers for meningeal layers and show conservation in human meninges. We find that embryonic meningeal fibroblasts are transcriptionally distinct between brain regions and identify a regionally localized pial subpopulation marked by the expression of μ -crystallin. Developmental analysis reveals a progressive, ventral-to-dorsal maturation of telencephalic meninges. Our studies have generated an unparalleled view of meningeal fibroblasts, providing molecular profiles of embryonic meningeal fibroblasts by layer and yielding insights into the mechanisms of meninges development and function.

Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion

Nature Biotechnology (IF: 42.3), 2019-8, William J Greenleaf et al. PubMed: 31375813

Single Cell ATAC, Human, Cancer Research, Immunology.

Abstract

Understanding complex tissues requires single-cell deconstruction of gene regulation with precision and scale. Here, we assess the performance of a massively parallel droplet-based method for mapping transposase-accessible chromatin in single cells using sequencing (scATAC-seq). We apply scATAC-seq to obtain chromatin profiles of more than 200,000 single cells in human blood and basal cell carcinoma. In blood, application of scATAC-seq enables marker-free identification of cell type-specific cis- and trans-regulatory elements, mapping of disease-associated enhancer activity and reconstruction of trajectories of cellular differentiation. In basal cell carcinoma, application of scATAC-seq reveals regulatory networks in malignant, stromal and immune cells in the tumor microenvironment. Analysis of scATAC-seq profiles from serial tumor biopsies before and after programmed cell death protein 1 blockade identifies chromatin regulators of therapy-responsive T cell subsets and reveals a shared regulatory program that governs intratumoral CD8T cell exhaustion and CD4T follicular helper cell development. We anticipate that scATAC-seq will enable the unbiased discovery of gene regulatory factors across diverse biological systems.

Decoding human fetal liver haematopoiesis

Nature (IF: 46.49), 2019-10, Sarah A Teichmann et al. DOI: 10.1038/s41586-019-1652 PubMed: 31597962

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Developmental Biology.

Abstract

Definitive haematopoiesis in the fetal liver supports self-renewal and differentiation of haematopoietic stem cells and multipotent progenitors (HSC/MPPs) but remains poorly defined in humans. Here, using single-cell transcriptome profiling of approximately 140,000 liver and 74,000 skin, kidney and yolk sac cells, we identify the repertoire of human blood and immune cells during development. We infer differentiation trajectories from HSC/MPPs and evaluate the influence of the tissue microenvironment on blood and immune cell development. We reveal physiological erythropoiesis in fetal skin and the presence of mast cells, natural killer and innate lymphoid cell precursors in the yolk sac. We demonstrate a shift in the haemopoietic composition of fetal liver during gestation away from being predominantly erythroid, accompanied by a parallel change in differentiation potential of HSC/MPPs, which we functionally validate. Our integrated map of fetal liver haematopoiesis provides a blueprint for the study of paediatric blood and immune disorders, and a reference for harnessing the therapeutic potential of HSC/MPPs.

A Cancer Cell Program Promotes T Cell Exclusion and Resistance to Checkpoint Blockade

Cell (IF: 38.62), 2018-11, Benjamin Izar et al. DOI: 10.1016/j.cell.2018.09.006 PubMed: 30388455

Single Cell Gene Expression, Human, Mouse, Immuno-oncology.

Abstract

Immune checkpoint inhibitors (ICIs) produce durable responses in some melanoma patients, but many patients derive no clinical benefit, and the molecular underpinnings of such resistance remain elusive. Here, we leveraged single-cell RNA sequencing (scRNA-seq) from 33 melanoma tumors and computational analyses to interrogate malignant cell states that promote immune evasion. We identified a resistance program expressed by malignant cells that is associated with T cell exclusion and immune evasion. The program is expressed prior to immunotherapy, characterizes cold niches in situ, and predicts clinical responses to anti-PD-1 therapy in an independent cohort of 112 melanoma patients. CDK4/6-inhibition represses this program in individual malignant cells, induces senescence, and reduces melanoma tumor outgrowth in mouse models in vivo when given in combination with immunotherapy. Our study provides a high-resolution landscape of ICI-resistant cell states, identifies clinically predictive signatures, and suggests new therapeutic strategies to overcome immunotherapy resistance.

Transcriptional Basis of Mouse and Human Dendritic Cell Heterogeneity

Cell (IF: 38.62), 2019-10, Dana Pe'er et al. DOI: 10.1016/j.cell.2019.09.035 PubMed: 31668803

Single Cell Gene Expression, Human, Mouse, Immunology.

Abstract

Dendritic cells (DCs) play a critical role in orchestrating adaptive immune responses due to their unique ability to initiate T cell responses and direct their differentiation into effector lineages. Classical DCs have been divided into two subsets, cDC1 and cDC2, based on phenotypic markers and their distinct abilities to prime CD8 and CD4 T cells. While the transcriptional regulation of the cDC1 subset has been well characterized, cDC2 development and function remain poorly understood. By combining transcriptional and chromatin analyses with genetic reporter expression, we identified two principal cDC2 lineages defined by distinct developmental pathways and transcriptional regulators, including T-bet and ROR γ t, two key transcription factors known to define innate and adaptive lymphocyte subsets. These novel cDC2 lineages were characterized by distinct metabolic and functional programs. Extending our findings to humans revealed conserved DC heterogeneity and the presence of the newly defined cDC2 subsets in human cancer.

Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma

Cell (IF: 38.62), 2020-7, Joakim Lundeberg et al. DOI: 10.1016/j.cell.2020.05.039 PubMed: 32579974

Single Cell Gene Expression & Spatial Gene Expression, Human, Cancer Research.

Abstract

To define the cellular composition and architecture of cutaneous squamous cell carcinoma (cSCC), we combined single-cell RNA sequencing with spatial transcriptomics and multiplexed ion beam imaging from a series of human cSCCs and matched normal skin. cSCC exhibited four tumor subpopulations, three recapitulating normal epidermal states, and a tumor-specific keratinocyte (TSK) population unique to cancer, which localized to a fibrovascular niche. Integration of single-cell and spatial data mapped ligand-receptor networks to specific cell types, revealing TSK cells as a hub for intercellular communication. Multiple features of potential immunosuppression were observed, including T regulatory cell (Treg) co-localization with CD8 T cells in compartmentalized tumor stroma. Finally, single-cell characterization of human tumor xenografts and *in vivo* CRISPR screens identified essential roles for specific tumor subpopulation-enriched gene networks in tumorigenesis. These data define cSCC tumor and stromal cell subpopulations, the spatial niches where they interact, and the communicating gene networks that they engage in cancer.

Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome: a case report

Nature Medicine (IF: 36.23), 2020-1, Alexandra F Freeman et al. DOI: 10.1038/s41591-019-0733-7

PubMed: 31959990

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immunology.

Abstract

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) is a potentially fatal multiorgan inflammatory disease associated with herpesvirus reactivation and subsequent onset of autoimmune diseases. Pathophysiology remains elusive and therapeutic options are limited. Cases refractory to corticosteroid therapy pose a clinical challenge and approximately 30% of patients with DiHS/DRESS develop complications, including infections and inflammatory and autoimmune diseases. Progress in single-cell RNA sequencing (scRNA-seq) provides an opportunity to dissect human disease pathophysiology at unprecedented resolutions, particularly in diseases lacking animal models, such as DiHS/DRESS. We performed scRNA-seq on skin and blood from a patient with refractory DiHS/DRESS, identifying the JAK-STAT signaling pathway as a potential target. We further showed that central memory CD4T cells were enriched with DNA from human herpesvirus 6b. Intervention via tofacitinib enabled disease control and tapering of other immunosuppressive agents. Tofacitinib, as well as antiviral agents, suppressed culprit-induced T cell proliferation *in vitro*, further supporting the roles of the JAK-STAT pathway and herpesviruses in mediating the adverse drug reaction. Thus, scRNA-seq analyses guided successful therapeutic intervention in the patient with refractory DiHS/DRESS. scRNA-seq may improve our understanding of complicated human disease pathophysiology and provide an alternative approach in personalized medicine.

Clonal replacement of tumor-specific T cells following PD-1 blockade

Nature Medicine (IF: 36.23), 2019-7, Anne Lynn S Chang et al. DOI: 10.1038/s41591-019-0522-3

PubMed: 31359002

Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Immunotherapies that block inhibitory checkpoint receptors on T cells have transformed the clinical care of patients with cancer. However, whether the T cell response to checkpoint blockade relies on reinvigoration of pre-existing tumor-infiltrating lymphocytes or on recruitment of novel T cells remains unclear. Here we performed paired single-cell RNA and T cell receptor sequencing on 79,046 cells from site-matched tumors from patients with basal or squamous cell carcinoma before and after anti-PD-1 therapy. Tracking T cell receptor clones and transcriptional phenotypes revealed coupling of tumor recognition, clonal expansion and T cell dysfunction marked by clonal expansion of CD8CD39T cells, which co-expressed markers of chronic T cell activation and exhaustion. However, the expansion of T cell clones did not derive from pre-existing tumor-infiltrating T lymphocytes; instead, the expanded clones consisted of novel clonotypes that had not previously been observed in the same tumor. Clonal replacement of T cells was preferentially observed in exhausted CD8T cells and evident in patients with basal or squamous cell carcinoma. These results demonstrate that pre-existing tumor-specific T cells may have limited reinvigoration capacity, and that the T cell response to checkpoint blockade derives from a distinct repertoire of T cell clones that may have just recently entered the tumor.

Epigenomics and Single-Cell Sequencing Define a Developmental Hierarchy in Langerhans Cell Histiocytosis

Cancer Discovery (IF: 28.3), 2019-10, Christoph Bock et al. DOI: 10.1158/2159-8290.CD-19-0138

PubMed: 31345789

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

Langerhans cell histiocytosis (LCH) is a rare neoplasm predominantly affecting children. It occupies a hybrid position between cancers and inflammatory diseases, which makes it an attractive model for studying cancer development. To explore the molecular mechanisms underlying the pathophysiology of LCH and its characteristic clinical heterogeneity, we investigated the transcriptomic and epigenomic diversity in primary LCH lesions. Using single-cell RNA sequencing, we identified multiple recurrent types of LCH cells within these biopsies, including putative LCH progenitor cells and several subsets of differentiated LCH cells. We confirmed the presence of proliferative LCH cells in all analyzed biopsies using IHC, and we defined an epigenomic and gene-regulatory basis of the different LCH-cell subsets by chromatin-accessibility profiling. In summary, our single-cell analysis of LCH uncovered an unexpected degree of cellular, transcriptomic, and epigenomic heterogeneity among LCH cells, indicative of complex developmental hierarchies in LCH lesions. SIGNIFICANCE: This study sketches a molecular portrait of LCH lesions by combining single-cell transcriptomics with epigenome profiling. We uncovered extensive cellular heterogeneity, explained in part by an intrinsic developmental hierarchy of LCH cells. Our findings provide new insights and hypotheses for advancing LCH research and a starting point for personalizing therapy...

Excessive Polyamine Generation in Keratinocytes Promotes Self-RNA Sensing by Dendritic Cells in Psoriasis

Immunity (IF: 25.73), 2020-7, Florent Ginhoux et al. DOI: 10.1016/j.immuni.2020.06.004 PubMed: 32553276

Single Cell Gene Expression, Human & Mouse, Immunology.

Abstract

Psoriasis is a chronic inflammatory disease whose etiology is multifactorial. The contributions of cellular metabolism to psoriasis are unclear. Here, we report that interleukin-17 (IL-17) downregulated Protein Phosphatase 6 (PP6) in psoriatic keratinocytes, causing phosphorylation and activation of the transcription factor C/EBP- β and subsequent generation of arginase-1. Mice lacking Pp6 in keratinocytes were predisposed to psoriasis-like skin inflammation. Accumulation of arginase-1 in Pp6-deficient keratinocytes drove polyamine production from the urea cycle. Polyamines protected self-RNA released by psoriatic keratinocytes from degradation and facilitated the endocytosis of self-RNA by myeloid dendritic cells to promote toll-like receptor-7 (TLR7)-dependent RNA sensing and IL-6 production. An arginase inhibitor improved skin inflammation in murine and non-human primate models of psoriasis. Our findings suggest that urea cycle hyperreactivity and excessive polyamine generation in psoriatic keratinocytes promote self-RNA sensation and PP6 deregulation in keratinocytes is a pivotal event that amplifies the inflammatory circuits in psoriasis.

Genome-wide transcriptome analysis of the STAT6-regulated genes in advanced-stage cutaneous T-cell lymphoma

Blood (IF: 14.82), 2020-10, Larisa J Geskin et al. DOI: 10.1182/blood.2019004725 PubMed: 32438399

Single Cell Gene Expression, Human, Cancer Research, Immunology.

Abstract

The signal transducer and activator of transcription 6 (STAT6) is a critical up-stream mediator of interleukin-13 (IL-13) and IL-4 signaling and is constitutively activated in malignant lymphocytes from Sezary syndrome (SS) and mycosis fungoides (MF), the most common subtypes of cutaneous T-cell lymphomas. By combining genome-wide expression profiling with pharmacological STAT6 inhibition, we have identified the genes regulated by STAT6 in MF/SS tumors. We found that STAT6 regulates several common pathways in MF/SS malignant lymphocytes that are associated with control of cell-cycle progression and genomic stability as well as production of Th2 cytokines. Using ex vivo skin explants from cutaneous MF tumors as well as Sezary cells derived from the blood of SS patients, we demonstrated that inhibition of STAT6 activation downregulates cytokine production and induces cell-cycle arrest in MF/SS malignant lymphocytes, inhibiting their proliferation but not their survival. Furthermore, we show that STAT6 promotes the protumoral M2-like phenotype of tumor-associated macrophages in the tumor microenvironment of advanced stage MF by upregulating the expression of genes associated with immunosuppression, chemotaxis, and tumor matrix remodeling. Thus, we show STAT6 to be a major factor in the pathogenesis and progression of MF/SS, promoting proliferation and invasion of the malignant lymphocytes while inducing a progressive depression of the antitumor immune response. Together, our results provide new insights into disease pathogenesis and offer new prospective targets for therapeutic intervention.

Single-Cell Transcriptomics of Regulatory T Cells Reveals Trajectories of Tissue Adaptation

Immunity (IF: 25.73), 2019-2, Fiona Powrie et al. DOI: 10.1016/j.immuni.2019.01.001 PubMed: 30737144

Single Cell Gene Expression, Human & Mouse, Immunology.

Abstract

Non-lymphoid tissues (NLTs) harbor a pool of adaptive immune cells with largely unexplored phenotype and development. We used single-cell RNA-seq to characterize 35,000 CD4regulatory (Treg) and memory (Tmem) T cells in mouse skin and colon, their respective draining lymph nodes (LNs) and spleen. In these tissues, we identified Treg cell subpopulations with distinct degrees of NLT phenotype. Subpopulation pseudotime ordering and gene kinetics were consistent in recruitment to skin and colon, yet the initial NLT-priming in LNs and the final stages of NLT functional adaptation reflected tissue-specific differences. Predicted kinetics were recapitulated using an in vivo melanoma-induction model, validating key regulators and receptors. Finally, we profiled human blood and NLT Treg and Tmem cells, and identified cross-mammalian conserved tissue signatures. In summary, we describe the relationship between Treg cell heterogeneity and recruitment to NLTs through the combined use of computational prediction and in vivo validation.

Phagocytosis of Wnt inhibitor SFRP4 by late wound macrophages drives chronic Wnt activity for fibrotic skin healing

Science Advances (IF: 14.09), 2020-3, Maksim V Plikus et al. DOI: 10.1126/sciadv.aay3704 PubMed: 32219160

Single Cell Gene Expression, Human & Mouse, Developmental Biology.

Abstract

Human and murine skin wounding commonly results in fibrotic scarring, but the murine wounding model wound-induced hair neogenesis (WIHN) can frequently result in a regenerative repair response. Here, we show in single-cell RNA sequencing comparisons of semi-regenerative and fibrotic WIHN wounds, increased expression of phagocytic/lysosomal genes in macrophages associated with predominance of fibrotic myofibroblasts in fibrotic wounds. Investigation revealed that macrophages in the late wound drive fibrosis by phagocytizing dermal Wnt inhibitor SFRP4 to establish persistent Wnt activity. In accordance, phagocytosis abrogation resulted in transient Wnt activity and a more regenerative healing. Phagocytosis of SFRP4 was integrin-mediated and dependent on the interaction of SFRP4 with the EDA splice variant of fibronectin. In the human skin condition hidradenitis suppurativa, phagocytosis of SFRP4 by macrophages correlated with fibrotic wound repair. These results reveal that macrophages can modulate a key signaling pathway via phagocytosis to alter the skin wound healing fate.

A micropeptide encoded by lncRNA MIR155HG suppresses autoimmune inflammation via modulating antigen presentation

Science Advances (IF: 14.09), 2020-5, Li Fan et al. DOI: 10.1126/sciadv.aaz2059 PubMed: 32937302

Single Cell Gene Expression, Human, Immunology.

Abstract

Many annotated long noncoding RNAs (lncRNAs) harbor predicted short open reading frames (sORFs), but the coding capacities of these sORFs and the functions of the resulting micropeptides remain elusive. Here, we report that human lncRNA MIR155HG encodes a 17-amino acid micropeptide, which we termed miPEP155 (P155). MIR155HG is highly expressed by inflamed antigen-presenting cells, leading to the discovery that P155 interacts with the adenosine 5'-triphosphate binding domain of heat shock cognate protein 70 (HSC70), a chaperone required for antigen trafficking and presentation in dendritic cells (DCs). P155 modulates major histocompatibility complex class II-mediated antigen presentation and T cell priming by disrupting the HSC70-HSP90 machinery. Exogenously injected P155 improves two classical mouse models of DC-driven auto inflammation. Collectively, we demonstrate the endogenous existence of a micropeptide encoded by a transcript annotated as non-protein coding and characterize a micropeptide as a regulator of antigen presentation and a suppressor of inflammatory diseases.

Elucidating the fundamental fibrotic processes driving abdominal adhesion formation

Nature Communications (IF: 13.61), 2020-8, Gerlinde Wernig et al. DOI: 10.1038/s41467-020-17883-1

PubMed: 32792541

Single Cell Gene Expression, Human & Mouse, Cell Biology.

Abstract

Adhesions are fibrotic scars that form between abdominal organs following surgery or infection, and may cause bowel obstruction, chronic pain, or infertility. Our understanding of adhesion biology is limited, which explains the paucity of anti-adhesion treatments. Here we present a systematic analysis of mouse and human adhesion tissues. First, we show that adhesions derive primarily from the visceral peritoneum, consistent with our clinical experience that adhesions form primarily following laparotomy rather than laparoscopy. Second, adhesions are formed by poly-clonal proliferating tissue-resident fibroblasts. Third, using single cell RNA-sequencing, we identify heterogeneity among adhesion fibroblasts, which is more pronounced at early timepoints. Fourth, JUN promotes adhesion formation and results in upregulation of PDGFRA expression. With JUN suppression, adhesion formation is diminished. Our findings support JUN as a therapeutic target to prevent adhesions. An anti-JUN therapy that could be applied intra-operatively to prevent adhesion formation could dramatically improve the lives of surgical patients.

A Bayesian mixture model for clustering droplet-based single-cell transcriptomic data from population studies

Nature Communications (IF: 13.61), 2019-4, Ming Hu et al. DOI: 10.1038/s41467-019-09639-3

PubMed: 30967541

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

The recently developed droplet-based single-cell transcriptome sequencing (scRNA-seq) technology makes it feasible to perform a population-scale scRNA-seq study, in which the transcriptome is measured for tens of thousands of single cells from multiple individuals. Despite the advances of many clustering methods, there are few tailored methods for population-scale scRNA-seq studies. Here, we develop a Bayesian mixture model for single-cell sequencing (BAMM-SC) method to cluster scRNA-seq data from multiple individuals simultaneously. BAMM-SC takes raw count data as input and accounts for data heterogeneity and batch effect among multiple individuals in a unified Bayesian hierarchical model framework. Results from extensive simulation studies and applications of BAMM-SC to in-house experimental scRNA-seq datasets using blood, lung and skin cells from humans or mice demonstrate that BAMM-SC outperformed existing clustering methods with considerable improved clustering accuracy, particularly in the presence of heterogeneity among individuals.

AP-1 and TGF β cooperativity drives non-canonical Hedgehog signaling in resistant basal cell carcinoma

Nature Communications (IF: 13.61), 2020-10, Kerri Rieger et al. DOI: 10.1038/s41467-020-18762-5

PubMed: 33033234

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Tumor heterogeneity and lack of knowledge about resistant cell states remain a barrier to targeted cancer therapies. Basal cell carcinomas (BCCs) depend on Hedgehog (Hh)/Gli signaling, but can develop mechanisms of Smoothened (SMO) inhibitor resistance. We previously identified a nuclear myocardin-related transcription factor (nMRTF) resistance pathway that amplifies noncanonical Gli1 activity, but characteristics and drivers of the nMRTF cell state remain unknown. Here, we use single cell RNA-sequencing of patient tumors to identify three prognostic surface markers (LYPD3, TACSTD2, and LY6D) which correlate with nMRTF and resistance to SMO inhibitors. The nMRTF cell state resembles transit-amplifying cells of the hair follicle matrix, with AP-1 and TGF β cooperativity driving nMRTF activation. JNK/AP-1 signaling commissions chromatin accessibility and Smad3 DNA binding leading to a transcriptional program of RhoGEFs that facilitate nMRTF activity. Importantly, small molecule AP-1 inhibitors selectively target LYPD3+/TACSTD2+/LY6D+ nMRTF human BCCs ex vivo, opening an avenue for improving combinatorial therapies.

Single cell transcriptome in aneuploidies reveals mechanisms of gene dosage imbalance

Nature Communications (IF: 13.61), 2019-10, Federico Santoni et al. DOI: 10.1038/s41467-019-12273-8

PubMed: 31582743

Single Cell Gene Expression, Human, Genome Analysis.

Abstract

Aneuploidy is a major source of gene dosage imbalance due to copy number alterations (CNA), and viable human trisomies are model disorders of altered gene expression. We study gene and allele-specific expression (ASE) of 9668 single-cell fibroblasts from trisomy 21 (T21) discordant twins and from mosaic T21, T18, T13 and T8. We examine 928 single cells with deep scRNAseq. Expected and observed overexpression of trisomic genes in trisomic vs. diploid bulk RNAseq is not detectable in trisomic vs. diploid single cells. Instead, for trisomic genes with low-to-average expression, their altered gene dosage is mainly due to the higher fraction of trisomic cells simultaneously expressing these genes, in agreement with a stochastic 2-state burst-like model of transcription. These results, confirmed in a further analysis of 8740 single fibroblasts with shallow scRNAseq, suggest that the specific transcriptional profile of each gene contributes to the phenotypic variability of trisomies. We propose an improved model to understand the effects of CNA and, generally, of gene regulation on gene dosage imbalance.

Single-cell transcriptomics combined with interstitial fluid proteomics defines cell type-specific immune regulation in atopic dermatitis

Journal of Allergy and Clinical Immunology (IF: 12.04), 2020-11, Michael Mildner et al.

DOI: 10.1016/j.jaci.2020.03.041 PubMed: 32344053

Single Cell Gene Expression, Human, Immunology.

Abstract

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, but its complex pathogenesis is only insufficiently understood, resulting in still limited treatment options. We sought to characterize AD on both transcriptomic and proteomic levels in humans. We used skin suction blistering, a painless and nonscarring procedure that can simultaneously sample skin cells and interstitial fluid. We then compared results with conventional biopsies. Suction blistering captured epidermal and most immune cells equally well as biopsies, except for mast cells and nonmigratory CD163⁺ macrophages that were only present in biopsy isolates. Using single-cell RNA sequencing, we found comparable transcriptional profiles of key inflammatory pathways between blister and biopsy AD, but suction blistering was superior in cell-specific resolution for high-abundance transcripts (KRT1/KRT10, KRT16/KRT6A, S100A8/S100A9), which showed some background signals in biopsy isolates. Compared with healthy controls, we found characteristic upregulation of AD-typical cytokines such as IL13 and IL22 in T2 and T22 cells, respectively, but we also discovered these mediators in proliferating T cells and natural killer T cells, that also expressed the antimicrobial cytokine IL26. Overall, not T cells, but myeloid cells were most strongly enriched in AD, and we found dendritic cell (CLEC7A, amphiregulin/AREG, EREG) and macrophage products (CCL13) among the top upregulated proteins in AD blister fluid proteomic analyses. These data show that by using cutting-edge technology, suction blistering offers several advantages over conventional biopsies, including better transcriptomic resolution of skin cells, combined with proteomic information from interstitial fluid, unraveling novel inflammatory players that shape the cellular and proteomic microenvironment of AD.

Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA

Nature Communications (IF: 13.61), 2018-9, P Nghiem A G Chapuis DOI: 10.1038/s41467-018-06300-3

PubMed: 30250229

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Understanding mechanisms of late/acquired cancer immunotherapy resistance is critical to improve outcomes; cellular immunotherapy trials offer a means to probe complex tumor-immune interfaces through defined T cell/antigen interactions. We treated two patients with metastatic Merkel cell carcinoma with autologous Merkel cell polyomavirus specific CD8⁺ T cells and immune-checkpoint inhibitors. In both cases, dramatic remissions were associated with dense infiltration of activated CD8⁺s into the regressing tumors. However, late relapses developed at 22 and 18 months, respectively. Here we report single cell RNA sequencing identified dynamic transcriptional suppression of the specific HLA genes presenting the targeted viral epitope in the resistant tumor as a consequence of intense CD8-mediated immunologic pressure; this is distinguished from genetic HLA-loss by its reversibility with drugs. Transcriptional suppression of Class I loci may underlie resistance to other immunotherapies, including checkpoint inhibitors, and have implications for the design of improved immunotherapy treatments.

Single-cell transcriptome analysis of human skin identifies novel fibroblast subpopulation and enrichment of immune subsets in atopic dermatitis

Journal of Allergy and Clinical Immunology (IF: 12.04), 2020-6, Thomas Tuschl et al.

DOI: 10.1016/j.jaci.2020.01.042 PubMed: 32035984

Single Cell Gene Expression, Human, Immunology.

Abstract

Atopic dermatitis (AD) is a prevalent inflammatory skin disease with a complex pathogenesis involving immune cell and epidermal abnormalities. Despite whole tissue biopsy studies that have advanced the mechanistic understanding of AD, single cell-based molecular alterations are largely unknown. Our aims were to construct a detailed, high-resolution atlas of cell populations and assess variability in cell composition and cell-specific gene expression in the skin of patients with AD versus in controls. We performed single-cell RNA sequencing on skin biopsy specimens from 5 patients with AD (4 lesional samples and 5 nonlesional samples) and 7 healthy control subjects, using 10× Genomics. We created transcriptomic profiles for 39,042 AD (lesional and nonlesional) and healthy skin cells. Fibroblasts demonstrated a novel COL6A5COL18A1 subpopulation that was unique to lesional AD and expressed CCL2 and CCL19 cytokines. A corresponding LAMP3 dendritic cell (DC) population that expressed the CCL19 receptor CCR7 was also unique to AD lesions, illustrating a potential role for fibroblast signaling to immune cells. The lesional AD samples were characterized by expansion of inflammatory DCs (CD1AFCER1A) and tissue-resident memory T cells (CD69CD103). The frequencies of type 2 (IL13)/type 22 (IL22) T cells were higher than those of type 1 (IFNG) in lesional AD, whereas this ratio was slightly diminished in nonlesional AD and further diminished in controls. AD lesions were characterized by expanded type 2/type 22 T cells and inflammatory DCs, and by a unique inflammatory fibroblast that may interact with immune cells to regulate lymphoid cell organization and type 2 inflammation.

Single-cell lymphocyte heterogeneity in advanced Cutaneous T-Cell Lymphoma skin tumors

Clinical cancer research (IF: 10.12), 2019-7, Robert LafyatisPatrizia Fuschiotti DOI: 10.1158/1078-0432.CCR-19-0148 PubMed: 31010835

Single Cell Gene Expression, Human, Cancer Research.

Abstract

The heterogeneity of tumor cells presents a major challenge to cancer diagnosis and therapy. Cutaneous T-cell lymphomas (CTCL) are a group of T lymphocyte malignancies that primarily affect skin. Lack of highly specific markers for malignant lymphocytes prevents early diagnosis, while only limited treatment options are available for patients with advanced stage CTCL. Droplet-based single-cell transcriptome analysis of CTCL skin biopsies opens avenues for dissecting patient-specific T lymphocyte heterogeneity, providing a basis for identifying specific markers for diagnosis and cure of CTCL. Single-cell RNA-sequencing was performed by Droplet-based sequencing (10X Genomics), focusing on 14,056 CD3 lymphocytes (448 cells from normal and 13,608 cells from CTCL skin samples) from skin biopsies of 5 patients with advanced-stage CTCL and 4 healthy donors. Protein expression of identified genes was validated in advanced stage CTCL skin tumors by immunohistochemistry and confocal immunofluorescence microscopy. Our analysis revealed a large inter- and intratumor gene expression heterogeneity in the T lymphocyte subset, as well as a common gene expression signature in highly proliferating lymphocytes that was validated in multiple advanced-stage skin tumors. In addition, we established the immunologic state of reactive lymphocytes and found heterogeneity in effector and exhaustion programs across patient samples. Single-cell analysis of CTCL skin tumor samples reveals patient-specific landscapes of malignant and reactive lymphocytes within the local microenvironment of each tumor, giving an unprecedented view of lymphocyte heterogeneity and identifying tumor-specific molecular signatures, with important implications for diagnosis and personalized disease treatment.

Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming

Communications Biology (IF: 11.2), 2020-4, Manuel Rodriguez-Paredes et al. DOI: 10.1038/s42003-020-0922-4 PubMed: 32327715

Single Cell Gene Expression, Human, Cell Atlas, Functional Genomics.

Abstract

Fibroblasts are an essential cell population for human skin architecture and function. While fibroblast heterogeneity is well established, this phenomenon has not been analyzed systematically yet. We have used single-cell RNA sequencing to analyze the transcriptomes of more than 5,000 fibroblasts from a sun-protected area in healthy human donors. Our results define four main subpopulations that can be spatially localized and show differential secretory, mesenchymal and pro-inflammatory functional annotations. Importantly, we found that this fibroblast 'priming' becomes reduced with age. We also show that aging causes a substantial reduction in the predicted interactions between dermal fibroblasts and other skin cells, including undifferentiated keratinocytes at the dermal-epidermal junction. Our work thus provides evidence for a functional specialization of human dermal fibroblasts and identifies the partial loss of cellular identity as an important age-related change in the human dermis. These findings have important implications for understanding human skin aging and its associated phenotypes.

Gene expression variability across cells and species shapes innate immunity

Nature (IF: 46.49), 2018-10, Michael Lässig Sarah A Teichmann DOI: 10.1038/s41586-018-0657-2

PubMed: 30356220

Single Cell Gene Expression, Rat, Cell Atlas.

Abstract

As the first line of defence against pathogens, cells mount an innate immune response, which varies widely from cell to cell. The response must be potent but carefully controlled to avoid self-damage. How these constraints have shaped the evolution of innate immunity remains poorly understood. Here we characterize the innate immune response's transcriptional divergence between species and variability in expression among cells. Using bulk and single-cell transcriptomics in fibroblasts and mononuclear phagocytes from different species, challenged with immune stimuli, we map the architecture of the innate immune response. Transcriptionally diverging genes, including those that encode cytokines and chemokines, vary across cells and have distinct promoter structures. Conversely, genes that are involved in the regulation of this response, such as those that encode transcription factors and kinases, are conserved between species and display low cell-to-cell variability in expression. We suggest that this expression pattern, which is observed across species and conditions, has evolved as a mechanism for fine-tuned regulation to achieve an effective but balanced response.

MAIT cells are imprinted by the microbiota in early life and promote tissue repair

Science (IF: 44.37), 2019-10, Michael A Fischbach et al. DOI: 10.1126/science.aax6624 PubMed: 31649166

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

How early-life colonization and subsequent exposure to the microbiota affect long-term tissue immunity remains poorly understood. Here, we show that the development of mucosal-associated invariant T (MAIT) cells relies on a specific temporal window, after which MAIT cell development is permanently impaired. This imprinting depends on early-life exposure to defined microbes that synthesize riboflavin-derived antigens. In adults, cutaneous MAIT cells are a dominant population of interleukin-17A (IL-17A)-producing lymphocytes, which display a distinct transcriptional signature and can subsequently respond to skin commensals in an IL-1-, IL-18-, and antigen-dependent manner. Consequently, local activation of cutaneous MAIT cells promotes wound healing. Together, our work uncovers a privileged interaction between defined members of the microbiota and MAIT cells, which sequentially controls both tissue-imprinting and subsequent responses to injury.

Defining the Design Principles of Skin Epidermis Postnatal Growth

Cell (IF: 38.62), 2020-4, Benjamin D Simons et al. DOI: 10.1016/j.cell.2020.03.015 PubMed: 32259486

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

During embryonic and postnatal development, organs and tissues grow steadily to achieve their final size at the end of puberty. However, little is known about the cellular dynamics that mediate postnatal growth. By combining in vivo clonal lineage tracing, proliferation kinetics, single-cell transcriptomics, and in vitro micro-pattern experiments, we resolved the cellular dynamics taking place during postnatal skin epidermis expansion. Our data revealed that harmonious growth is engineered by a single population of developmental progenitors presenting a fixed fate imbalance of self-renewing divisions with an ever-decreasing proliferation rate. Single-cell RNA sequencing revealed that epidermal developmental progenitors form a more uniform population compared with adult stem and progenitor cells. Finally, we found that the spatial pattern of cell division orientation is dictated locally by the underlying collagen fiber orientation. Our results uncover a simple design principle of organ growth where progenitors and differentiated cells expand in harmony with their surrounding tissues.

Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury

Science (IF: 44.37), 2019-1, John J O'Shea et al. DOI: 10.1126/science.aat6280 PubMed: 30523076

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Barrier tissues are primary targets of environmental stressors and are home to the largest number of antigen-experienced lymphocytes in the body, including commensal-specific T cells. We found that skin-resident commensal-specific T cells harbor a paradoxical program characterized by a type 17 program associated with a poised type 2 state. Thus, in the context of injury and exposure to inflammatory mediators such as interleukin-18, these cells rapidly release type 2 cytokines, thereby acquiring contextual functions. Such acquisition of a type 2 effector program promotes tissue repair. Aberrant type 2 responses can also be unleashed in the context of local defects in immunoregulation. Thus, commensal-specific T cells co-opt tissue residency and cell-intrinsic flexibility as a means to promote both local immunity and tissue adaptation to injury.

Homeostatic Control of Sebaceous Glands by Innate Lymphoid Cells Regulates Commensal Bacteria Equilibrium

Cell (IF: 38.62), 2019-2, Heidi H Kong et al. DOI: 10.1016/j.cell.2018.12.031 PubMed: 30712873

Single Cell Gene Expression, Mouse, Cell Biology, Microbiology.

Abstract

Immune cells and epithelium form sophisticated barrier systems in symbiotic relationships with microbiota. Evidence suggests that immune cells can sense microbes through intact barriers, but regulation of microbial commensalism remain largely unexplored. Here, we uncovered spatial compartmentalization of skin-resident innate lymphoid cells (ILCs) and modulation of sebaceous glands by a subset of ROR γ ILCs residing within hair follicles in close proximity to sebaceous glands. Their persistence in skin required IL-7 and thymic stromal lymphopoietin, and localization was dependent on the chemokine receptor CCR6. ILC subsets expressed TNF receptor ligands, which limited sebocyte growth by repressing Notch signaling pathway. Consequently, loss of ILCs resulted in sebaceous hyperplasia with increased production of antimicrobial lipids and restricted commensalism of Gram-positive bacterial communities. Thus, epithelia-derived signals maintain skin-resident ILCs that regulate microbial commensalism through sebaceous gland-mediated tuning of the barrier surface, highlighting an immune-epithelia circuitry that facilitates host-microbe symbiosis.

Caloric Restriction Reprograms the Single Cell Transcriptional Landscape of Rattus Norvegicus Aging

Cell (IF: 38.62), 2020-3, Jing QuGuang-Hui Liu DOI: 10.1016/j.cell.2020.02.008 PubMed: 32109414

Single Cell Gene Expression, Rat, Developmental Biology, Immunology.

Abstract

Aging causes a functional decline in tissues throughout the body that may be delayed by caloric restriction (CR). However, the cellular profiles and signatures of aging, as well as those ameliorated by CR, remain unclear. Here, we built comprehensive single-cell and single-nucleus transcriptomic atlases across various rat tissues undergoing aging and CR. CR attenuated aging-related changes in cell type composition, gene expression, and core transcriptional regulatory networks. Immune cells were increased during aging, and CR favorably reversed the aging-disturbed immune ecosystem. Computational prediction revealed that the abnormal cell-cell communication patterns observed during aging, including the excessive proinflammatory ligand-receptor interplay, were reversed by CR. Our work provides multi-tissue single-cell transcriptional landscapes associated with aging and CR in a mammal, enhances our understanding of the robustness of CR as a geroprotective intervention, and uncovers how metabolic intervention can act upon the immune system to modify the process of aging.

Oncolytic Viruses Engineered to Enforce Leptin Expression Reprogram Tumor-Infiltrating T Cell Metabolism and Promote Tumor Clearance

Immunity (IF: 25.73), 2019-9, Stephen H Thorne et al. DOI: 10.1016/j.immuni.2019.07.003 PubMed: 31471106
Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

Immunotherapy can reinvigorate dormant responses to cancer, but response rates remain low. Oncolytic viruses, which replicate in cancer cells, induce tumor lysis and immune priming, but their immune consequences are unclear. We profiled the infiltrate of aggressive melanomas induced by oncolytic Vaccinia virus using RNA sequencing and found substantial remodeling of the tumor microenvironment, dominated by effector T cell influx. However, responses to oncolytic viruses were incomplete due to metabolic insufficiencies induced by the tumor microenvironment. We identified the adipokine leptin as a potent metabolic reprogramming agent that supported antitumor responses. Leptin metabolically reprogrammed T cells in vitro, and melanoma cells expressing leptin were immunologically controlled in mice. Engineering oncolytic viruses to express leptin in tumor cells induced complete responses in tumor-bearing mice and supported memory development in the tumor infiltrate. Thus, leptin can provide metabolic support to tumor immunity, and oncolytic viruses represent a platform to deliver metabolic therapy.

Dermal Adipocyte Lipolysis and Myofibroblast Conversion Are Required for Efficient Skin Repair

Cell Stem Cell (IF: 23.45), 2020-6, Mark B Gerstein et al. DOI: 10.1016/j.stem.2020.03.013 PubMed: 32302523
Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Mature adipocytes store fatty acids and are a common component of tissue stroma. Adipocyte function in regulating bone marrow, skin, muscle, and mammary gland biology is emerging, but the role of adipocyte-derived lipids in tissue homeostasis and repair is poorly understood. Here, we identify an essential role for adipocyte lipolysis in regulating inflammation and repair after injury in skin. Genetic mouse studies revealed that dermal adipocytes are necessary to initiate inflammation after injury and promote subsequent repair. We find through histological, ultrastructural, lipidomic, and genetic experiments in mice that adipocytes adjacent to skin injury initiate lipid release necessary for macrophage inflammation. Tamoxifen-inducible genetic lineage tracing of mature adipocytes and single-cell RNA sequencing revealed that dermal adipocytes alter their fate and generate ECM-producing myofibroblasts within wounds. Thus, adipocytes regulate multiple aspects of repair and may be therapeutic for inflammatory diseases and defective wound healing associated with aging and diabetes.

Distinct Regulatory Programs Control the Latent Regenerative Potential of Dermal Fibroblasts during Wound Healing

Cell Stem Cell (IF: 23.45), 2020-9, T Michael Underhill et al. DOI: 10.1016/j.stem.2020.07.008 PubMed: 32755548
Single Cell Gene Expression & Single Cell ATAC, Mouse, Cell Biology.

Abstract

Dermal fibroblasts exhibit considerable heterogeneity during homeostasis and in response to injury. Defining lineage origins of reparative fibroblasts and regulatory programs that drive fibrosis or, conversely, promote regeneration will be essential for improving healing outcomes. Using complementary fate-mapping approaches, we show that hair follicle mesenchymal progenitors make limited contributions to wound repair. In contrast, extrafollicular progenitors marked by the quiescence-associated factor *Hic1* generated the bulk of reparative fibroblasts and exhibited functional divergence, mediating regeneration in the center of the wound neodermis and scar formation in the periphery. Single-cell RNA-seq revealed unique transcriptional, regulatory, and epithelial-mesenchymal crosstalk signatures that enabled mesenchymal competence for regeneration. Integration with scATAC-seq highlighted changes in chromatin accessibility within regeneration-associated loci. Finally, pharmacological modulation of *RUNX1* and retinoic acid signaling or genetic deletion of *Hic1* within wound-activated fibroblasts was sufficient to modulate healing outcomes, suggesting that reparative fibroblasts have latent but modifiable regenerative capacity.

The Molecular Anatomy of Mouse Skin during Hair Growth and Rest

Cell Stem Cell (IF: 23.45), 2020-3, Rickard Sandberg et al. DOI: 10.1016/j.stem.2020.01.012 PubMed: 32109378
Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Skin homeostasis is orchestrated by dozens of cell types that together direct stem cell renewal, lineage commitment, and differentiation. Here, we use single-cell RNA sequencing and single-molecule RNA FISH to provide a systematic molecular atlas of full-thickness skin, determining gene expression profiles and spatial locations that define 56 cell types and states during hair growth and rest. These findings reveal how the outer root sheath (ORS) and inner hair follicle layers coordinate hair production. We found that the ORS is composed of two intermingling but transcriptionally distinct cell types with differing capacities for interactions with stromal cell types. Inner layer cells branch from transcriptionally uncommitted progenitors, and each lineage differentiation passes through an intermediate state. We also provide an online tool to explore this comprehensive skin cell atlas, including epithelial and stromal cells such as fibroblasts, vascular, and immune cells, to spur further discoveries in skin biology.

A Subset of TREM2⁺ Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth

Cell Stem Cell (IF: 23.45), 2019-4, Charles G Drake et al. DOI: 10.1016/j.stem.2019.01.011 PubMed: 30930146
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Hair growth can be induced from resting mouse hair follicles by topical application of JAK inhibitors, suggesting that JAK-STAT signaling is required for maintaining hair follicle stem cells (HFSCs) in a quiescent state. Here, we show that Oncostatin M (OSM), an IL-6 family cytokine, negatively regulates hair growth by signaling through JAK-STAT5 to maintain HFSC quiescence. Genetic deletion of the OSM receptor or STAT5 can induce premature HFSC activation, suggesting that the resting telogen stage is actively maintained by the hair follicle niche. Single-cell RNA sequencing revealed that the OSM source is not intrinsic to the hair follicle itself and is instead a subset of TREM2 macrophages that is enriched within the resting follicle and decreases immediately prior to HFSC activation. In vivo inhibition of macrophage function was sufficient to induce HFSC proliferation and hair cycle induction. Together these results clarify how JAK-STAT signaling actively inhibits hair growth.

A novel mouse model demonstrates that oncogenic melanocyte stem cells engender melanoma resembling human disease

Nature Communications (IF: 13.61), 2019-11, Emi K Nishimura et al. DOI: 10.1038/s41467-019-12733-1
PubMed: 31685822
Single Cell Gene Expression, Mouse, Assay Method, Cancer Research.

Abstract

Melanoma, the deadliest skin cancer, remains largely incurable at advanced stages. Currently, there is a lack of animal models that resemble human melanoma initiation and progression. Recent studies using a Tyr-CreER driven mouse model have drawn contradictory conclusions about the potential of melanocyte stem cells (McSCs) to form melanoma. Here, we employ a c-Kit-CreER-driven model that specifically targets McSCs to show that oncogenic McSCs are a bona fide source of melanoma that expand in the niche, and then establish epidermal melanomas that invade into the underlying dermis. Further, normal Wnt and Endothelin niche signals during hair anagen onset are hijacked to promote McSC malignant transformation during melanoma induction. Finally, molecular profiling reveals strong resemblance of murine McSC-derived melanoma to human melanoma in heterogeneity and gene signatures. These findings provide experimental validation of the human melanoma progression model and key insights into the transformation and heterogeneity of McSC-derived melanoma.

Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds

Nature Communications (IF: 13.61), 2019-2, George Cotsarelis et al. DOI: 10.1038/s41467-018-08247

PubMed: 30737373

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

During wound healing in adult mouse skin, hair follicles and then adipocytes regenerate. Adipocytes regenerate from myofibroblasts, a specialized contractile wound fibroblast. Here we study wound fibroblast diversity using single-cell RNA-sequencing. On analysis, wound fibroblasts group into twelve clusters. Pseudotime and RNA velocity analyses reveal that some clusters likely represent consecutive differentiation states toward a contractile phenotype, while others appear to represent distinct fibroblast lineages. One subset of fibroblasts expresses hematopoietic markers, suggesting their myeloid origin. We validate this finding using single-cell western blot and single-cell RNA-sequencing on genetically labeled myofibroblasts. Using bone marrow transplantation and Cre recombinase-based lineage tracing experiments, we rule out cell fusion events and confirm that hematopoietic lineage cells give rise to a subset of myofibroblasts and rare regenerated adipocytes. In conclusion, our study reveals that wounding induces a high degree of heterogeneity among fibroblasts and recruits highly plastic myeloid cells that contribute to adipocyte regeneration.

Hedgehog stimulates hair follicle neogenesis by creating inductive dermis during murine skin wound healing

Nature Communications (IF: 13.61), 2018-11, George Cotsarelis et al. DOI: 10.1038/s41467-018-07142-9

PubMed: 30464171

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Mammalian wounds typically heal by fibrotic repair without hair follicle (HF) regeneration. Fibrosis and regeneration are currently considered the opposite end of wound healing. This study sought to determine if scar could be remodeled to promote healing with HF regeneration. Here, we identify that activation of the Sonic hedgehog (Shh) pathway reinstalls a regenerative dermal niche, called dermal papilla, which is required and sufficient for HF neogenesis (HFN). Epidermal Shh overexpression or constitutive Smoothed dermal activation results in extensive HFN in wounds that otherwise end in scarring. While long-term Wnt activation is associated with fibrosis, Shh signal activation in Wnt active cells promotes the dermal papilla fate in scarring wounds. These studies demonstrate that mechanisms of scarring and regeneration are not distant from one another and that wound repair can be redirected to promote regeneration following injury by modifying a key dermal signal.

Dysfunction of Hair Follicle Mesenchymal Progenitors Contributes to Age-Associated Hair Loss

Developmental Cell (IF: 10.13), 2020-4, Jo Anne Stratton et al. DOI: 10.1016/j.devcel.2020.03.019 PubMed: 32315612

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Skin aging is accompanied by hair loss due to impairments in hair follicle (HF) epithelial progenitor cells and their mesenchymal niche. This inductive mesenchyme, called dermal papilla (DP), undergoes progressive cell loss and eventual miniaturization that contributes to HF pathogenesis. Using laser ablation and fate mapping, we show that HF dermal stem cells (hfDSCs) reconstitute the damaged DP and maintain hair growth, suggesting that hfDSC dysfunction may trigger degeneration of the inductive niche. Fate mapping over 24 months revealed progressive hfDSC depletion, and in vivo clonal analysis of aged hfDSCs showed impaired self-renewal and biased differentiation. Single-cell RNA-seq confirmed hfDSCs as a central precursor, giving rise to divergent mesenchymal trajectories. In aged skin, hfDSCs exhibited senescent-like characteristics, and senescence-associated secretory phenotypes were identified in the aging HF mesenchyme. These results clarify fibroblast dynamics within the HF and suggest that progressive dysfunction within the mesenchymal progenitor pool contributes to age-related hair loss.

GCN2 drives macrophage and MDSC function and immunosuppression in the tumor microenvironment

Science Immunology (IF: 13.41), 2019-12, David G Brooks et al. DOI: 10.1126/sciimmunol.aax8189

PubMed: 31836669

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

General control nonderepressible 2 (GCN2) is an environmental sensor controlling transcription and translation in response to nutrient availability. Although GCN2 is a putative therapeutic target for immuno-oncology, its role in shaping the immune response to tumors is poorly understood. Here, we used mass cytometry, transcriptomics, and transcription factor-binding analysis to determine the functional impact of GCN2 on the myeloid phenotype and immune responses in melanoma. We found that myeloid-lineage deletion of GCN2 drives a shift in the phenotype of tumor-associated macrophages and myeloid-derived suppressor cells (MDSCs) that promotes antitumor immunity. Time-of-flight mass cytometry (CyTOF) and single-cell RNA sequencing showed that this was due to changes in the immune microenvironment with increased proinflammatory activation of macrophages and MDSCs and interferon- γ expression in intratumoral CD8T cells. Mechanistically, GCN2 altered myeloid function by promoting increased translation of the transcription factor CREB-2/ATF4, which was required for maturation and polarization of macrophages and MDSCs in both mice and humans, whereas targeting by small interfering RNA knockdown reduced tumor growth. Last, analysis of patients with cutaneous melanoma showed that GCN2-dependent transcriptional signatures correlated with macrophage polarization, T cell infiltrates, and overall survival. Thus, these data reveal a previously unknown dependence of tumors on myeloid GCN2 signals for protection from immune attack.

Regulatory T cells in skin are uniquely poised to suppress profibrotic immune responses

Science Immunology (IF: 13.41), 2019-9, Francesco Boin et al. DOI: 10.1126/sciimmunol.aaw2910

PubMed: 31492709

Single Cell Gene Expression, Mouse, Immunology.

Abstract

At the center of fibrosing diseases is the aberrant activation of tissue fibroblasts. The cellular and molecular mechanisms of how the immune system augments fibroblast activation have been described; however, little is known about how the immune system controls fibroblast function in tissues. Here, we identify regulatory T cells (T) as important regulators of fibroblast activation in skin. Bulk cell and single-cell analysis of T_{in} murine skin and lungs revealed that T_{in} skin are transcriptionally distinct and skewed toward T helper 2 (T₂) differentiation. When compared with T_{in} lung, skin T_{in} preferentially expressed high levels of GATA3, the master T₂ transcription factor. Genes regulated by GATA3 were highly enriched in skin “T₂ T” subsets. In functional experiments, T_{in} depletion resulted in a preferential increase in T₂ cytokine production in skin. Both acute depletion and chronic reduction of T_{in} resulted in spontaneous skin fibroblast activation, profibrotic gene expression, and dermal fibrosis, all of which were exacerbated in a bleomycin-induced murine model of skin sclerosis. Lineage-specific deletion of T_{in} resulted in an exacerbation of T₂ cytokine secretion that was preferential to skin, resulting in enhanced fibroblast activation and dermal fibrosis. Together, we demonstrate that T_{in} play a critical role in regulating fibroblast activation in skin and do so by expressing a unique tissue-restricted transcriptional program that is mediated, at least in part, by GATA3.

Single-Cell RNA Sequencing Reveals a Dynamic Stromal Niche That Supports Tumor Growth

Cell Reports (IF: 8.82), 2020-5, Jacqueline D Shields et al. DOI: 10.1016/j.celrep.2020.107628 PubMed: 32433953

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Here, using single-cell RNA sequencing, we examine the stromal compartment in murine melanoma and draining lymph nodes (LNs) at points across tumor development, providing data at <http://www.teichlab.org/data/>. Naive lymphocytes from LNs undergo activation and clonal expansion within the tumor, before PD1 and Lag3 expression, while tumor-associated myeloid cells promote the formation of a suppressive niche. We identify three temporally distinct stromal populations displaying unique functional signatures, conserved across mouse and human tumors. Whereas immune stromal cells are observed in early tumors, contractile cells become more prevalent at later time points. Complement component C3 is specifically expressed in the immune population. Its cleavage product C3a supports the recruitment of C3aR macrophages, and perturbation of C3a and C3aR disrupts immune infiltration, slowing tumor growth. Our results highlight the power of scRNA-seq to identify complex interplays and increase stromal diversity as a tumor develops, revealing that stromal cells acquire the capacity to modulate immune landscapes from early disease.

The aging skin microenvironment dictates stem cell behavior

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-3, James G Krueger et al. DOI: 10.1073/pnas.1901720117 PubMed: 32094197

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Aging manifests with architectural alteration and functional decline of multiple organs throughout an organism. In mammals, aged skin is accompanied by a marked reduction in hair cycling and appearance of bald patches, leading researchers to propose that hair follicle stem cells (HFSCs) are either lost, differentiate, or change to an epidermal fate during aging. Here, we employed single-cell RNA-sequencing to interrogate aging-related changes in the HFSCs. Surprisingly, although numbers declined, aging HFSCs were present, maintained their identity, and showed no overt signs of shifting to an epidermal fate. However, they did exhibit prevalent transcriptional changes particularly in extracellular matrix genes, and this was accompanied by profound structural perturbations in the aging SC niche. Moreover, marked age-related changes occurred in many nonepithelial cell types, including resident immune cells, sensory neurons, and arrector pili muscles. Each of these SC niche components has been shown to influence HF regeneration. When we performed skin injuries that are known to mobilize young HFSCs to exit their niche and regenerate HFs, we discovered that aged skin is defective at doing so. Interestingly, however, in transplantation assays *in vivo*, aged HFSCs regenerated HFs when supported with young dermis, while young HFSCs failed to regenerate HFs when combined with aged dermis. Together, our findings highlight the importance of SC:niche interactions and favor a model where youthfulness of the niche microenvironment plays a dominant role in dictating the properties of its SCs and tissue health and fitness.

Single-Cell RNA Sequencing Reveals a Dynamic Stromal Niche That Supports Tumor Growth

Cell Reports (IF: 8.82), 2020-5, Jacqueline D Shields et al. DOI: 10.1016/j.celrep.2020.107628 PubMed: 32433953
Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Here, using single-cell RNA sequencing, we examine the stromal compartment in murine melanoma and draining lymph nodes (LNs) at points across tumor development, providing data at <http://www.teichlab.org/data/>. Naive lymphocytes from LNs undergo activation and clonal expansion within the tumor, before PD1 and Lag3 expression, while tumor-associated myeloid cells promote the formation of a suppressive niche. We identify three temporally distinct stromal populations displaying unique functional signatures, conserved across mouse and human tumors. Whereas “immune” stromal cells are observed in early tumors, “contractile” cells become more prevalent at later time points. Complement component C3 is specifically expressed in the immune population. Its cleavage product C3a supports the recruitment of C3aR macrophages, and perturbation of C3a and C3aR disrupts immune infiltration, slowing tumor growth. Our results highlight the power of scRNA-seq to identify complex interplays and increase stromal diversity as a tumor develops, revealing that stromal cells acquire the capacity to modulate immune landscapes from early disease.

A Spatiotemporal Organ-Wide Gene Expression and Cell Atlas of the Developing Human Heart

Cell (IF: 38.62), 2019-12, Christer Sylven et al. DOI: 10.1016/j.cell.2019.11.025 PubMed: 31835037

Single Cell Gene Expression & Spatial Gene Expression, Human, Developmental Biology.

Abstract

The process of cardiac morphogenesis in humans is incompletely understood. Its full characterization requires a deep exploration of the organ-wide orchestration of gene expression with a single-cell spatial resolution. Here, we present a molecular approach that reveals the comprehensive transcriptional landscape of cell types populating the embryonic heart at three developmental stages and that maps cell-type-specific gene expression to specific anatomical domains. Spatial transcriptomics identified unique gene profiles that correspond to distinct anatomical regions in each developmental stage. Human embryonic cardiac cell types identified by single-cell RNA sequencing confirmed and enriched the spatial annotation of embryonic cardiac gene expression. In situ sequencing was then used to refine these results and create a spatial subcellular map for the three developmental phases. Finally, we generated a publicly available web resource of the human developing heart to facilitate future studies on human cardiogenesis.

Transcriptional and Cellular Diversity of the Human Heart

Circulation (IF: 21.05), 2020-8, Christian M Stegmann et al. DOI: 10.1161/CIRCULATIONAHA.119.045401

PubMed: 32403949

Single Cell Gene Expression, Human, Cell Atlas, Genetic Health.

Abstract

The human heart requires a complex ensemble of specialized cell types to perform its essential function. A greater knowledge of the intricate cellular milieu of the heart is critical to increase our understanding of cardiac homeostasis and pathology. As recent advances in low-input RNA sequencing have allowed definitions of cellular transcriptomes at single-cell resolution at scale, we have applied these approaches to assess the cellular and transcriptional diversity of the nonfailing human heart. Microfluidic encapsulation and barcoding was used to perform single nuclear RNA sequencing with samples from 7 human donors, selected for their absence of overt cardiac disease. Individual nuclear transcriptomes were then clustered based on transcriptional profiles of highly variable genes. These clusters were used as the basis for between-chamber and between-sex differential gene expression analyses and intersection with genetic and pharmacologic data. We sequenced the transcriptomes of 287 269 single cardiac nuclei, revealing 9 major cell types and 20 subclusters of cell types within the human heart. Cellular subclasses include 2 distinct groups of resident macrophages, 4 endothelial subtypes, and 2 fibroblast subsets. Comparisons of cellular transcriptomes by cardiac chamber or sex reveal diversity not only in cardiomyocyte transcriptional programs but also in subtypes involved in extracellular matrix remodeling and vascularization. Using genetic association data, we identified strong enrichment for the role of cell subtypes in cardiac traits and diseases. Intersection of our data set with genes on cardiac clinical testing panels and the druggable genome reveals striking patterns of cellular specificity. Using large-scale single nuclei RNA sequencing, we defined the transcriptional and cellular diversity in the normal human heart. Our identification of discrete cell subtypes and differentially expressed genes within the heart will ultimately facilitate the development of new therapeutics for cardiovascular diseases.

BMP10 Signaling Promotes the Development of Endocardial Cells from Human Pluripotent Stem Cell-Derived Cardiovascular Progenitors

Cell Stem Cell (IF: 23.45), 2020-10, Mingxia Gu et al. DOI: 10.1016/j.stem.2020.10.003 PubMed: 33142114

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

The embryonic endocardium is essential for early heart development as it functions to induce trabecular myocardium, the first heart tissue to form, and is the source of the cells that make up the valves and a portion of the coronary vasculature. With this potential, human endocardial cells could provide unique therapeutic opportunities that include engineering biological valves and cell-based therapy strategies to replace coronary vasculature in damaged hearts. To access human endocardial cells, we generated a human pluripotent stem cell (hPSC)-derived endothelial population that displays many characteristics of endocardium, including expression of the cohort of genes that identifies this lineage in vivo, the capacity to induce a trabecular fate in immature cardiomyocytes in vitro, and the ability to undergo an endothelial-to-mesenchymal transition. Analyses of the signaling pathways required for development of the hPSC-derived endocardial cells identified a novel role for BMP10 in the specification of this lineage from cardiovascular mesoderm.

Intrinsic Endocardial Defects Contribute to Hypoplastic Left Heart Syndrome

Cell Stem Cell (IF: 23.45), 2020-10, Sean M Wu et al. DOI: 10.1016/j.stem.2020.07.015 PubMed: 32810435

Single Cell Gene Expression, Human, Developmental Biology, Genetic Health.

Abstract

Hypoplastic left heart syndrome (HLHS) is a complex congenital heart disease characterized by abnormalities in the left ventricle, associated valves, and ascending aorta. Studies have shown intrinsic myocardial defects but do not sufficiently explain developmental defects in the endocardial-derived cardiac valve, septum, and vasculature. Here, we identify a developmentally impaired endocardial population in HLHS through single-cell RNA profiling of hiPSC-derived endocardium and human fetal heart tissue with an underdeveloped left ventricle. Intrinsic endocardial defects contribute to abnormal endothelial-to-mesenchymal transition, NOTCH signaling, and extracellular matrix organization, key factors in valve formation. Endocardial abnormalities cause reduced cardiomyocyte proliferation and maturation by disrupting fibronectin-integrin signaling, consistent with recently described de novo HLHS mutations associated with abnormal endocardial gene and fibronectin regulation. Together, these results reveal a critical role for endocardium in HLHS etiology and provide a rationale for considering endocardial function in regenerative strategies.

Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease

Cell Stem Cell (IF: 23.45), 2020-6, Milena Bellin et al. DOI: 10.1016/j.stem.2020.05.004 PubMed: 32459996

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) are functionally immature, but this is improved by incorporation into engineered tissues or forced contraction. Here, we showed that tri-cellular combinations of hiPSC-derived CMs, cardiac fibroblasts (CFs), and cardiac endothelial cells also enhance maturation in easily constructed, scaffold-free, three-dimensional microtissues (MTs). hiPSC-CMs in MTs with CFs showed improved sarcomeric structures with T-tubules, enhanced contractility, and mitochondrial respiration and were electrophysiologically more mature than MTs without CFs. Interactions mediating maturation included coupling between hiPSC-CMs and CFs through connexin 43 (CX43) gap junctions and increased intracellular cyclic AMP (cAMP). Scaled production of thousands of hiPSC-MTs was highly reproducible across lines and differentiated cell batches. MTs containing healthy-control hiPSC-CMs but hiPSC-CFs from patients with arrhythmogenic cardiomyopathy strikingly recapitulated features of the disease. Our MT model is thus a simple and versatile platform for modeling multicellular cardiac diseases that will facilitate industry and academic engagement in high-throughput molecular screening.

TFPa/HADHA is required for fatty acid beta-oxidation and cardiolipin re-modeling in human cardiomyocytes

Nature Communications (IF: 13.61), 2019-10, Yuliang Wang et al. DOI: 10.1038/s41467-019-12482-1

PubMed: 31604922

Single Cell Gene Expression, Human, Cell Biology, Functional Genomics.

Abstract

Mitochondrial trifunctional protein deficiency, due to mutations in hydratase subunit A (HADHA), results in sudden infant death syndrome with no cure. To reveal the disease etiology, we generated stem cell-derived cardiomyocytes from HADHA-deficient hiPSCs and accelerated their maturation via an engineered microRNA maturation cocktail that upregulated the epigenetic regulator, HOPX. Here we report, matured HADHA mutant cardiomyocytes treated with an endogenous mixture of fatty acids manifest the disease phenotype: defective calcium dynamics and repolarization kinetics which results in a pro-arrhythmic state. Single cell RNA-seq reveals a cardiomyocyte developmental intermediate, based on metabolic gene expression. This intermediate gives rise to mature-like cardiomyocytes in control cells but, mutant cells transition to a pathological state with reduced fatty acid beta-oxidation, reduced mitochondrial proton gradient, disrupted cristae structure and defective cardiolipin remodeling. This study reveals that HADHA (tri-functional protein alpha), a monolysocardiolipin acyltransferase-like enzyme, is required for fatty acid beta-oxidation and cardiolipin remodeling, essential for functional mitochondria in human cardiomyocytes.

Defining human cardiac transcription factor hierarchies using integrated single-cell heterogeneity analysis

Nature Communications (IF: 13.61), 2018-11, Nathan Salomonis Joseph C Wu DOI: 10.1038/s41467-018-07333-4 PubMed: 30464173

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have become a powerful tool for human disease modeling and therapeutic testing. However, their use remains limited by their immaturity and heterogeneity. To characterize the source of this heterogeneity, we applied complementary single-cell RNA-seq and bulk RNA-seq technologies over time during hiPSC cardiac differentiation and in the adult heart. Using integrated transcriptomic and splicing analysis, more than half a dozen distinct single-cell populations were observed, several of which were coincident at a single time-point, day 30 of differentiation. To dissect the role of distinct cardiac transcriptional regulators associated with each cell population, we systematically tested the effect of a gain or loss of three transcription factors (NR2F2, TBX5, and HEY2), using CRISPR genome editing and ChIP-seq, in conjunction with patch clamp, calcium imaging, and CyTOF analysis. These targets, data, and integrative genomics analysis methods provide a powerful platform for understanding in vitro cellular heterogeneity.

The histone demethylase JMJD2B regulates endothelial-to-mesenchymal transition

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-2, Jes-Niels Boeckel et al. DOI: 10.1073/pnas.1913481117 PubMed: 32034099

Single Cell Gene Expression, Human & Mouse, Cell Biology.

Abstract

Endothelial cells play an important role in maintenance of the vascular system and the repair after injury. Under proinflammatory conditions, endothelial cells can acquire a mesenchymal phenotype by a process named endothelial-to-mesenchymal transition (EndMT), which affects the functional properties of endothelial cells. Here, we investigated the epigenetic control of EndMT. We show that the histone demethylase JMJD2B is induced by EndMT-promoting, proinflammatory, and hypoxic conditions. Silencing of JMJD2B reduced TGF- β 2-induced expression of mesenchymal genes, prevented the alterations in endothelial morphology and impaired endothelial barrier function. Endothelial-specific deletion of JMJD2B in vivo confirmed a reduction of EndMT after myocardial infarction. EndMT did not affect global H3K9me3 levels but induced a site-specific reduction of repressive H3K9me3 marks at promoters of mesenchymal genes, such as Calponin (CNN1), and genes involved in TGF- β signaling, such as AKT Serine/Threonine Kinase 3 (AKT3) and Sulfatase 1 (SULF1). Silencing of JMJD2B prevented the EndMT-induced reduction of H3K9me3 marks at these promoters and further repressed these EndMT-related genes. Our study reveals that endothelial identity and function is critically controlled by the histone demethylase JMJD2B, which is induced by EndMT-promoting, proinflammatory, and hypoxic conditions, and supports the acquirement of a mesenchymal phenotype.

Single-cell analysis of cardiogenesis reveals basis for organ-level developmental defects

Nature (IF: 46.49), 2019-1, Casey A Gifford et al. DOI: 10.1038/s41586-019-1414 PubMed: 31341279

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Organogenesis involves integration of diverse cell types; dysregulation of cell-type-specific gene networks results in birth defects, which affect 5% of live births. Congenital heart defects are the most common malformations, and result from disruption of discrete subsets of cardiac progenitor cells, but the transcriptional changes in individual progenitors that lead to organ-level defects remain unknown. Here we used single-cell RNA sequencing to interrogate early cardiac progenitor cells as they become specified during normal and abnormal cardiogenesis, revealing how dysregulation of specific cellular subpopulations has catastrophic consequences. A network-based computational method for single-cell RNA-sequencing analysis that predicts lineage-specifying transcription factors identified Hand2 as a specifier of outflow tract cells but not right ventricular cells, despite the failure of right ventricular formation in Hand2-null mice. Temporal single-cell-transcriptome analysis of Hand2-null embryos revealed failure of outflow tract myocardium specification, whereas right ventricular myocardium was specified but failed to properly differentiate and migrate. Loss of Hand2 also led to dysregulation of retinoic acid signalling and disruption of anterior-posterior patterning of cardiac progenitors. This work reveals transcriptional determinants that specify fate and differentiation in individual cardiac progenitor cells, and exposes mechanisms of disrupted cardiac development at single-cell resolution, providing a framework for investigating congenital heart defects.

Type V Collagen in Scar Tissue Regulates the Size of Scar after Heart Injury

Cell (IF: 38.62), 2020-8, Aldons J Lusis et al. DOI: 10.1016/j.cell.2020.06.030 PubMed: 32621799

Single Cell Gene Expression, Mouse, Cell Biology, Genetic Health.

Abstract

Scar tissue size following myocardial infarction is an independent predictor of cardiovascular outcomes, yet little is known about factors regulating scar size. We demonstrate that collagen V, a minor constituent of heart scars, regulates the size of heart scars after ischemic injury. Depletion of collagen V led to a paradoxical increase in post-infarction scar size with worsening of heart function. A systems genetics approach across 100 in-bred strains of mice demonstrated that collagen V is a critical driver of postinjury heart function. We show that collagen V deficiency alters the mechanical properties of scar tissue, and altered reciprocal feedback between matrix and cells induces expression of mechanosensitive integrins that drive fibroblast activation and increase scar size. Cilengitide, an inhibitor of specific integrins, rescues the phenotype of increased post-injury scarring in collagen-V-deficient mice. These observations demonstrate that collagen V regulates scar size in an integrin-dependent manner.

Pro-efferocytic nanoparticles are specifically taken up by lesional macrophages and prevent atherosclerosis

Nature Nanotechnology (IF: 40.3), 2020-1, Bryan Ronain Smith et al. DOI: 10.1038/s41565-019-0619-3

PubMed: 31988506

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Atherosclerosis is the process that underlies heart attack and stroke. A characteristic feature of the atherosclerotic plaque is the accumulation of apoptotic cells in the necrotic core. Prophagocytic antibody-based therapies are currently being explored to stimulate the phagocytic clearance of apoptotic cells; however, these therapies can cause off-target clearance of healthy tissues, which leads to toxicities such as anaemia. Here we developed a macrophage-specific nanotherapy based on single-walled carbon nanotubes loaded with a chemical inhibitor of the antiphagocytic CD47-SIRP α signalling axis. We demonstrate that these single-walled carbon nanotubes accumulate within the atherosclerotic plaque, reactivate lesional phagocytosis and reduce the plaque burden in atheroprone apolipoprotein-E-deficient mice without compromising safety, and thereby overcome a key translational barrier for this class of drugs. Single-cell RNA sequencing analysis reveals that prophagocytic single-walled carbon nanotubes decrease the expression of inflammatory genes linked to cytokine and chemokine pathways in lesional macrophages, which demonstrates the potential of 'Trojan horse' nanoparticles to prevent atherosclerotic cardiovascular disease.

Tissue-Specific Macrophage Responses to Remote Injury Impact the Outcome of Subsequent Local Immune Challenge

Immunity (IF: 25.73), 2019-11, Filip K Swirski et al. DOI: 10.1016/j.immuni.2019.10.010 PubMed: 31732166

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Myocardial infarction, stroke, and sepsis trigger systemic inflammation and organism-wide complications that are difficult to manage. Here, we examined the contribution of macrophages residing in vital organs to the systemic response after these injuries. We generated a comprehensive catalog of changes in macrophage number, origin, and gene expression in the heart, brain, liver, kidney, and lung of mice with myocardial infarction, stroke, or sepsis. Predominantly fueled by heightened local proliferation, tissue macrophage numbers increased systemically. Macrophages in the same organ responded similarly to different injuries by altering expression of tissue-specific gene sets. Preceding myocardial infarction improved survival of subsequent pneumonia due to enhanced bacterial clearance, which was caused by IFN γ priming of alveolar macrophages. Conversely, EGF receptor signaling in macrophages exacerbated inflammatory lung injury. Our data suggest that local injury activates macrophages in remote organs and that targeting macrophages could improve resilience against systemic complications following myocardial infarction, stroke, and sepsis.

Context-Specific Transcription Factor Functions Regulate Epigenomic and Transcriptional Dynamics during Cardiac Reprogramming

Cell Stem Cell (IF: 23.45), 2019-7, Katherine S Pollard et al. DOI: 10.1016/j.stem.2019.06.012 PubMed: 31271750
Single Cell Gene Expression & Single Cell Immune Profiling, Mouse, Developmental Biology, Functional Genomics.

Abstract

Ectopic expression of combinations of transcription factors (TFs) can drive direct lineage conversion, thereby reprogramming a somatic cell's identity. To determine the molecular mechanisms by which Gata4, Mef2c, and Tbx5 (GMT) induce conversion from a cardiac fibroblast toward an induced cardiomyocyte, we performed comprehensive transcriptomic, DNA-occupancy, and epigenomic interrogation throughout the reprogramming process. Integration of these datasets identified new TFs involved in cardiac reprogramming and revealed context-specific roles for GMT, including the ability of Mef2c and Tbx5 to independently promote chromatin remodeling at previously inaccessible sites. We also find evidence for cooperative facilitation and refinement of each TF's binding profile in a combinatorial setting. A reporter assay employing newly defined regulatory elements confirmed that binding of a single TF can be sufficient for gene activation, suggesting that co-binding events do not necessarily reflect synergy. These results shed light on fundamental mechanisms by which combinations of TFs direct lineage conversion.

Pathogenic Potential of Hic1-Expressing Cardiac Stromal Progenitors

Cell Stem Cell (IF: 23.45), 2020-2, T Michael Underhill et al. DOI: 10.1016/j.stem.2019.12.008 PubMed: 31978365
Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

The cardiac stroma contains multipotent mesenchymal progenitors. However, lineage relationships within cardiac stromal cells are poorly defined. Here, we identified heart-resident PDGFRaSCA-1 cells as cardiac fibro/adipogenic progenitors (cFAPs) and show that they respond to ischemic damage by generating fibrogenic cells. Pharmacological blockade of this differentiation step with an anti-fibrotic tyrosine kinase inhibitor decreases post-myocardial infarction (post-MI) remodeling and leads to improvement in cardiac function. In the undamaged heart, activation of cFAPs through lineage-specific deletion of the gene encoding the quiescence-associated factor HIC1 reveals additional pathogenic potential, causing fibrofatty infiltration within the myocardium and driving major pathological features pathognomonic in arrhythmogenic cardiomyopathy (AC). In this regard, cFAPs contribute to multiple pathogenic cell types within cardiac tissue and therapeutic strategies aimed at modifying their activity are expected to have tremendous benefit for the treatment of diverse cardiac diseases.

Endothelial Regeneration of Large Vessels Is a Biphasic Process Driven by Local Cells with Distinct Proliferative Capacities

Cell Stem Cell (IF: 23.45), 2018-8, Reza Ardehali et al. DOI: 10.1016/j.stem.2018.07.011 PubMed: 30075129
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The cellular and mechanistic bases underlying endothelial regeneration of adult large vessels have proven challenging to study. Using a reproducible in vivo aortic endothelial injury model, we characterized cellular dynamics underlying the regenerative process through a combination of multi-color lineage tracing, parabiosis, and single-cell transcriptomics. We found that regeneration is a biphasic process driven by distinct populations arising from differentiated endothelial cells. The majority of cells immediately adjacent to the injury site re-enter the cell cycle during the initial damage response, with a second phase driven by a highly proliferative subpopulation. Endothelial regeneration requires activation of stress response genes including Atf3, and aged aortas compromised in their reparative capacity express less Atf3. Deletion of Atf3 reduced endothelial proliferation and compromised the regeneration. These findings provide important insights into cellular dynamics and mechanisms that drive responses to large vessel injury.

Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction

Nature Immunology (IF: 22.3), 2018-12, Clinton S Robbins et al. DOI: 10.1038/s41590-018-0272-2

PubMed: 30538339

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Macrophages promote both injury and repair after myocardial infarction, but discriminating functions within mixed populations remains challenging. Here we used fate mapping, parabiosis and single-cell transcriptomics to demonstrate that at steady state, TIMD4LYVE1MHC-IICCR2resident cardiac macrophages self-renew with negligible blood monocyte input. Monocytes partially replaced resident TIMD4LYVE1MHC-IICCR2macrophages and fully replaced TIMD4LYVE1MHC-IICCR2macrophages, revealing a hierarchy of monocyte contribution to functionally distinct macrophage subsets. Ischemic injury reduced TIMD4and TIMD4resident macrophage abundance, whereas CCR2monocyte-derived macrophages adopted multiple cell fates within infarcted tissue, including those nearly indistinguishable from resident macrophages. Recruited macrophages did not express TIMD4, highlighting the ability of TIMD4 to track a subset of resident macrophages in the absence of fate mapping. Despite this similarity, inducible depletion of resident macrophages using a Cx3cr1-based system led to impaired cardiac function and promoted adverse remodeling primarily within the peri-infarct zone, revealing a nonredundant, cardioprotective role of resident cardiac macrophages.

Endothelial TGF- β signalling drives vascular inflammation and atherosclerosis

Nature Metabolism (IF: 18.57), 2019-8, George Tellides et al. DOI: 10.1038/s42255-019-0102-3

PubMed: 31572976

Single Cell Gene Expression, Mouse, Functional Genomics.

Abstract

Atherosclerosis is a progressive vascular disease triggered by interplay between abnormal shear stress and endothelial lipid retention. A combination of these and, potentially, other factors leads to a chronic inflammatory response in the vessel wall, which is thought to be responsible for disease progression characterized by a buildup of atherosclerotic plaques. Yet molecular events responsible for maintenance of plaque inflammation and plaque growth have not been fully defined. Here we show that endothelial TGF β signaling is one of the primary drivers of atherosclerosis-associated vascular inflammation. Inhibition of endothelial TGF β signaling in hyperlipidemic mice reduces vessel wall inflammation and vascular permeability and leads to arrest of disease progression and regression of established lesions. These pro-inflammatory effects of endothelial TGF β signaling are in stark contrast with its effects in other cell types and identify it as an important driver of atherosclerotic plaque growth and show the potential of cell-type specific therapeutic intervention aimed at control of this disease.

Platelet regulation of myeloid suppressor of cytokine signaling 3 accelerates atherosclerosis

Science Translational Medicine (IF: 18.56), 2019-11, Edward A Fisher et al. DOI: 10.1126/scitranslmed.aax0481

PubMed: 31694925

Single Cell Gene Expression, Mouse, Cell Biology, Immunology.

Abstract

Platelets are best known as mediators of hemostasis and thrombosis; however, their inflammatory effector properties are increasingly recognized. Atherosclerosis, a chronic vascular inflammatory disease, represents the interplay between lipid deposition in the artery wall and unresolved inflammation. Here, we reveal that platelets induce monocyte migration and recruitment into atherosclerotic plaques, resulting in plaque platelet-macrophage aggregates. In mice fed a Western diet, platelet depletion decreased plaque size and necrotic area and attenuated macrophage accumulation. Platelets drive atherogenesis by skewing plaque macrophages to an inflammatory phenotype, increasing myeloid suppressor of cytokine signaling 3 (SOCS3) expression and reducing theratio. Platelet-induced expression regulates plaque macrophage reprogramming by promoting inflammatory cytokine production (,, and) and impairing phagocytic capacity, dysfunctions that contribute to unresolved inflammation and sustained plaque growth. Translating our data to humans with cardiovascular disease, we found that women with, versus without, myocardial infarction have up-regulation of, lower, and increased monocyte-platelet aggregate. A second cohort of patients with lower extremity atherosclerosis demonstrated that and theratio correlated with platelet activity and inflammation. Collectively, these data provide a causative link between platelet-mediated myeloid inflammation and dysfunction,, and cardiovascular disease. Our findings define an atherogenic role of platelets and highlight how, in the absence of thrombosis, platelets contribute to inflammation.

Single-Cell RNA Sequencing to Dissect the Immunological Network of Autoimmune Myocarditis

Circulation (IF: 21.05), 2020-7, Mingyao Li et al. DOI: 10.1161/CIRCULATIONAHA.119.043545 PubMed: 32431172
Single Cell Immune Profiling, Mouse, Immunology.

Abstract

Myocarditis can develop into dilated cardiomyopathy, which may require heart transplantation. The immunological network of myocarditis phases remains unknown. This study aimed to investigate the immunological network during the transition from myocarditis to cardiomyopathy and to identify the genes contributing to the inflammatory response to myocarditis. Mice were treated with myosin heavy chain- α peptides to generate an experimental autoimmune myocarditis (EAM) model. We performed single-cell RNA sequencing analysis of cells extracted from mouse hearts during different EAM phases, including normal control, acute inflammatory, subacute inflammatory, and myopathy phases. Human heart tissues were collected from the surgically removed hearts of patients who had undergone heart transplantation. We identified 26 cell subtypes among 34 665 cells. Macrophages constituted the main immune cell population at all disease phases (>60%), and an inflammation-associated macrophage cluster was identified in which the expression of regulated genes was upregulated. The neutrophil population was increased after the induction of EAM, and neutrophils then released to participate in the EAM process. T cells were observed at the highest percentage at the subacute inflammatory phase. T-helper 17 cells, in which the expression of regulated genes was upregulated, constituted the main T-cell population detected at the acute inflammatory phase, whereas regulatory T cells were the main T-cell population detected at the subacute inflammatory phase, and $\gamma\delta$ T cells releasing were the main T-cell population observed at the myopathy phase. Moreover, the expression level correlated with the extent of inflammation. In addition, PX-478 could alleviate the inflammatory responses of the different EAM phases. Last, was expressed at higher levels in patients with acute autoimmune myocarditis than in patients with dilated cardiomyopathy and healthy control subjects. We present here a comprehensive single-cell landscape of the cardiac immune cells in different EAM phases. In addition, we elucidate the contribution of to the inflammatory response through the regulation of immune cell activity, particularly of macrophage cluster 2 and T-helper 17 cells. Moreover, an inhibitor alleviated inflammatory cell infiltration of the EAM model and may serve as a potential therapeutic target in the clinic.

Transcriptomic Profiling of the Developing Cardiac Conduction System at Single-Cell Resolution

Circulation Research (IF: 15.63), 2019-8, Joseph C Wu et al. DOI: 10.1161/CIRCRESAHA.118.314578

PubMed: 31284824

Single Cell Gene Expression, Mouse, Cell Atlas, Developmental Biology.

Abstract

The cardiac conduction system (CCS) consists of distinct components including the sinoatrial node, atrioventricular node, His bundle, bundle branches, and Purkinje fibers. Despite an essential role for the CCS in heart development and function, the CCS has remained challenging to interrogate because of inherent obstacles including small cell numbers, large cell-type heterogeneity, complex anatomy, and difficulty in isolation. Single-cell RNA-sequencing allows for genome-wide analysis of gene expression at single-cell resolution. Assess the transcriptional landscape of the entire CCS at single-cell resolution by single-cell RNA-sequencing within the developing mouse heart. Wild-type, embryonic day 16.5 mouse hearts (n=6 per zone) were harvested and 3 zones of microdissection were isolated, including: Zone I-sinoatrial node region; Zone II-atrioventricular node/His region; and Zone III-bundle branch/Purkinje fiber region. Tissue was digested into single-cell suspensions, cells isolated, mRNA reverse transcribed, and barcoded before high-throughput sequencing and bioinformatics analyses. Single-cell RNA-sequencing was performed on over 22 000 cells, and all major cell types of the murine heart were successfully captured including bona fide clusters of cells consistent with each major component of the CCS. Unsupervised weighted gene coexpression network analysis led to the discovery of a host of novel CCS genes, a subset of which were validated using fluorescent in situ hybridization as well as whole-mount immunolabeling with volume imaging (iDISCO+) in 3 dimensions on intact mouse hearts. Further, subcluster analysis unveiled isolation of distinct CCS cell subtypes, including the clinically relevant but poorly characterized transitional cells that bridge the CCS and surrounding myocardium. Our study represents the first comprehensive assessment of the transcriptional profiles from the entire CCS at single-cell resolution and provides a characterization in the context of development and disease.

Identification of a multipotent Twist2-expressing cell population in the adult heart

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2018-9, Eric N Olson et al. DOI: 10.1073/pnas.1800526115 PubMed: 30127033

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Twist transcription factors function as ancestral regulators of mesodermal cell fates in organisms ranging from to mammals. Through lineage tracing of Twist2 (Tw2)-expressing cells with tamoxifen-inducible Tw2-CreERT2 and tdTomato (tdTO) reporter mice, we discovered a unique cell population that progressively contributes to cardiomyocytes (CMs), endothelial cells, and fibroblasts in the adult heart. Clonal analysis confirmed the ability of Tw2-derived tdTO(Tw2-tdTO) cells to form CMs in vitro. Within the adult heart, Tw2-tdTOCMs accounted for ~13% of total CMs, the majority of which resulted from fusion of Tw2-tdTO cells with existing CMs. Tw2-tdTO cells also contribute to cardiac remodeling after injury. We conclude that Tw2-tdTO cells participate in lifelong maintenance of cardiac function, at least in part through de novo formation of CMs and fusion with preexisting CMs, as well as in the genesis of other cellular components of the adult heart.

Atlas of the Immune Cell Repertoire in Mouse Atherosclerosis Defined by Single-Cell RNA-Sequencing and Mass Cytometry

Circulation Research (IF: 15.63), 2018-6, Klaus Ley et al. DOI: 10.1161/CIRCRESAHA.117.312513

PubMed: 29545366

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

Atherosclerosis is a chronic inflammatory disease that is driven by the interplay of pro- and anti-inflammatory leukocytes in the aorta. Yet, the phenotypic and transcriptional diversity of aortic leukocytes is poorly understood. We characterized leukocytes from healthy and atherosclerotic mouse aortas in-depth by single-cell RNA-sequencing and mass cytometry (cytometry by time of flight) to define an atlas of the immune cell landscape in atherosclerosis. Using single-cell RNA-sequencing of aortic leukocytes from chow diet- and Western diet-fed and mice, we detected 11 principal leukocyte clusters with distinct phenotypic and spatial characteristics while the cellular repertoire in healthy aortas was less diverse. Gene set enrichment analysis on the single-cell level established that multiple pathways, such as for lipid metabolism, proliferation, and cytokine secretion, were confined to particular leukocyte clusters. Leukocyte populations were differentially regulated in atherosclerotic and mice. We confirmed the phenotypic diversity of these clusters with a novel mass cytometry 35-marker panel with metal-labeled antibodies and conventional flow cytometry. Cell populations retrieved by these protein-based approaches were highly correlated to transcriptionally defined clusters. In an integrated screening strategy of single-cell RNA-sequencing, mass cytometry, and fluorescence-activated cell sorting, we detected 3 principal B-cell subsets with alterations in surface markers, functional pathways, and in vitro cytokine secretion. Leukocyte cluster gene signatures revealed leukocyte frequencies in 126 human plaques by a genetic deconvolution strategy. This approach revealed that human carotid plaques and microdissected mouse plaques were mostly populated by macrophages, T-cells, and monocytes. In addition, the frequency of genetically defined leukocyte populations in carotid plaques predicted cardiovascular events in patients. The definition of leukocyte diversity by high-dimensional analyses enables a fine-grained analysis of aortic leukocyte subsets, reveals new immunologic mechanisms and cell-type-specific pathways, and establishes a functional relevance for lesional leukocytes in human atherosclerosis.

BNIP3L/NIX and FUNDC1-mediated mitophagy is required for mitochondrial network remodeling during cardiac progenitor cell differentiation

Autophagy (IF: 11.97), 2019-7, Mark A Sussman et al. DOI: 10.1080/15548627.2019.1580095 PubMed: 30741592
Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Cell-based therapies represent a very promising strategy to repair and regenerate the injured heart to prevent progression to heart failure. To date, these therapies have had limited success due to a lack of survival and retention of the infused cells. Therefore, it is important to increase our understanding of the biology of these cells and utilize this information to enhance their survival and function in the injured heart. Mitochondria are critical for progenitor cell function and survival. Here, we demonstrate the importance of mitochondrial autophagy, or mitophagy, in the differentiation process in adult cardiac progenitor cells (CPCs). We found that mitophagy was rapidly induced upon initiation of differentiation in CPCs. We also found that mitophagy was mediated by mitophagy receptors, rather than the PINK1-PRKN/PARKIN pathway. Mitophagy mediated by BNIP3L/NIX and FUNDC1 was not involved in regulating progenitor cell fate determination, mitochondrial biogenesis, or reprogramming. Instead, mitophagy facilitated the CPCs to undergo proper mitochondrial network reorganization during differentiation. Abrogating BNIP3L- and FUNDC1-mediated mitophagy during differentiation led to sustained mitochondrial fission and formation of donut-shaped impaired mitochondria. It also resulted in increased susceptibility to cell death and failure to survive the infarcted heart. Finally, aging is associated with accumulation of mitochondrial DNA (mtDNA) damage in cells and we found that acquiring mtDNA mutations selectively disrupted the differentiation-activated mitophagy program in CPCs. These findings demonstrate the importance of BNIP3L- and FUNDC1-mediated mitophagy as a critical regulator of mitochondrial network formation during differentiation, as well as the consequences of accumulating mtDNA mutations. Baf: bafilomycin A; BCL2L13: BCL2 like 13; BNIP3: BCL2 interacting protein 3; BNIP3L: BCL2 interacting protein 3 like; CPCs: cardiac progenitor cells; DM: differentiation media; DNM1L: dynamin 1 like; EPCs: endothelial progenitor cells; FCCP: carbonyl cyanide--(trifluoromethoxy)phenylhydrazone; FUNDC1: FUN14 domain containing 1; HSCs: hematopoietic stem cells; MAP1LC3B/LC3: microtubule-associated protein 1 light chain 3 beta; MFN1/2: mitofusin 1/2; MSCs: mesenchymal stem cells; mtDNA: mitochondrial DNA; OXPHOS: oxidative phosphorylation; PPARGC1A: PPARG coactivator 1 alpha; PHB2: prohibitin 2; POLG: DNA polymerase gamma, catalytic subunit; SQSTM1: sequestosome 1; TEM: transmission electron microscopy; TMRM: tetramethylrhodamine methyl ester.

Quantitative proteomics and single-nucleus transcriptomics of the sinus node elucidates the foundation of cardiac pacemaking

Nature Communications (IF: 13.61), 2019-6, Mark Boyett et al. DOI: 10.1038/s41467-019-10709-9

PubMed: 31253831

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

The sinus node is a collection of highly specialised cells constituting the heart's pacemaker. The molecular underpinnings of its pacemaking abilities are debated. Using high-resolution mass spectrometry, we here quantify >7,000 proteins from sinus node and neighbouring atrial muscle. Abundances of 575 proteins differ between the two tissues. By performing single-nucleus RNA sequencing of sinus node biopsies, we attribute measured protein abundances to specific cell types. The data reveal significant differences in ion channels responsible for the membrane clock, but not in Caclock proteins, suggesting that the membrane clock underpins pacemaking. Consistently, incorporation of ion channel expression differences into a biophysically-detailed atrial action potential model result in pacemaking and a sinus node-like action potential. Combining our quantitative proteomics data with computational modeling, we estimate ion channel copy numbers for sinus node myocytes. Our findings provide detailed insights into the unique molecular make-up of the cardiac pacemaker.

Single-cell and spatial transcriptomics enables probabilistic inference of cell type topography

Communications Biology (IF: 11.2), 2020-10, José Fernández Navarro et al. DOI: 10.1038/s42003-020-01247

PubMed: 33037292

Single Cell Gene Expression & Spatial Gene Expression, Mouse, Computational Method.

Abstract

The field of spatial transcriptomics is rapidly expanding, and with it the repertoire of available technologies. However, several of the transcriptome-wide spatial assays do not operate on a single cell level, but rather produce data comprised of contributions from a - potentially heterogeneous - mixture of cells. Still, these techniques are attractive to use when examining complex tissue specimens with diverse cell populations, where complete expression profiles are required to properly capture their richness. Motivated by an interest to put gene expression into context and delineate the spatial arrangement of cell types within a tissue, we here present a model-based probabilistic method that uses single cell data to deconvolve the cell mixtures in spatial data. To illustrate the capacity of our method, we use data from different experimental platforms and spatially map cell types from the mouse brain and developmental heart, which arrange as expected.

Disease-relevant transcriptional signatures identified in individual smooth muscle cells from healthy mouse vessels

Nature Communications (IF: 13.61), 2018-11, Mikhail Spivakov et al. DOI: 10.1038/s41467-018-06891

PubMed: 30385745

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Vascular smooth muscle cells (VSMCs) show pronounced heterogeneity across and within vascular beds, with direct implications for their function in injury response and atherosclerosis. Here we combine single-cell transcriptomics with lineage tracing to examine VSMC heterogeneity in healthy mouse vessels. The transcriptional profiles of single VSMCs consistently reflect their region-specific developmental history and show heterogeneous expression of vascular disease-associated genes involved in inflammation, adhesion and migration. We detect a rare population of VSMC-lineage cells that express the multipotent progenitor marker Sca1, progressively downregulate contractile VSMC genes and upregulate genes associated with VSMC response to inflammation and growth factors. We find that Sca1 upregulation is a hallmark of VSMCs undergoing phenotypic switching in vitro and in vivo, and reveal an equivalent population of Sca1-positive VSMC-lineage cells in atherosclerotic plaques. Together, our analyses identify disease-relevant transcriptional signatures in VSMC-lineage cells in healthy blood vessels, with implications for disease susceptibility, diagnosis and prevention.

Cardiac interstitial tetraploid cells can escape replicative senescence in rodents but not large mammals

Communications Biology (IF: 11.2), 2019-6, Michael E Davis et al. DOI: 10.1038/s42003-019-0453

PubMed: 31231694

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Cardiomyocyte ploidy has been described but remains obscure in cardiac interstitial cells. Ploidy of c-kit⁺ cardiac interstitial cells was assessed using confocal, karyotypic, and flow cytometric technique. Notable differences were found between rodent (rat, mouse) c-kit⁺ cardiac interstitial cells possessing mononuclear tetraploid (4n) content, compared to large mammals (human, swine) with mononuclear diploid (2n) content analysis, confirmed with fresh isolates, revealed diploid content in human c-kit⁺ cardiac interstitial cells and a mixture of diploid and tetraploid content in mouse. Downregulation of the p53 signaling pathway provides evidence why rodent, but not human, c-kit⁺ cardiac interstitial cells escape replicative senescence. Single cell transcriptional profiling reveals distinctions between diploid versus tetraploid populations in mouse c-kit⁺ cardiac interstitial cells, alluding to functional divergences. Collectively, these data reveal notable species-specific biological differences in c-kit⁺ cardiac interstitial cells, which could account for challenges in extrapolation of myocardial from preclinical studies to clinical trials.

Dynamic Transcriptional Responses to Injury of Regenerative and Non-regenerative Cardiomyocytes Revealed by Single-Nucleus RNA Sequencing

Developmental Cell (IF: 10.13), 2020-4, Rhonda Bassel-Duby et al. DOI: 10.1016/j.devcel.2020.02.019

PubMed: 32220304

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The adult mammalian heart is incapable of regeneration following injury. In contrast, the neonatal mouse heart can efficiently regenerate during the first week of life. The molecular mechanisms that mediate the regenerative response and its blockade in later life are not understood. Here, by single-nucleus RNA sequencing, we map the dynamic transcriptional landscape of five distinct cardiomyocyte populations in healthy, injured, and regenerating mouse hearts. We identify immature cardiomyocytes that enter the cell cycle following injury and disappear as the heart loses the ability to regenerate. These proliferative neonatal cardiomyocytes display a unique transcriptional program dependent on nuclear transcription factor Y subunit alpha (NFYa) and nuclear factor erythroid 2-like 1 (NFE2L1) transcription factors, which exert proliferative and protective functions, respectively. Cardiac overexpression of these two factors conferred protection against ischemic injury in mature mouse hearts that were otherwise non-regenerative. These findings advance our understanding of the cellular basis of neonatal heart regeneration and reveal a transcriptional landscape for heart repair following injury.

Cell Types of the Human Retina and Its Organoids at Single-Cell Resolution

Cell (IF: 38.62), 2020-9, Florian Nigsch et al. DOI: 10.1016/j.cell.2020.08.013 PubMed: 32946783

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Human organoids recapitulating the cell-type diversity and function of their target organ are valuable for basic and translational research. We developed light-sensitive human retinal organoids with multiple nuclear and synaptic layers and functional synapses. We sequenced the RNA of 285,441 single cells from these organoids at seven developmental time points and from the periphery, fovea, pigment epithelium and choroid of light-responsive adult human retinas, and performed histochemistry. Cell types in organoids matured in vitro to a stable developed state at a rate similar to human retina development in vivo. Transcriptomes of organoid cell types converged toward the transcriptomes of adult peripheral retinal cell types. Expression of disease-associated genes was cell-type-specific in adult retina, and cell-type specificity was retained in organoids. We implicate unexpected cell types in diseases such as macular degeneration. This resource identifies cellular targets for studying disease mechanisms in organoids and for targeted repair in human retinas.

An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma

Cell (IF: 38.62), 2019-8, Itay Tirosh et al. DOI: 10.1016/j.cell.2019.06.024 PubMed: 31327527

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Diverse genetic, epigenetic, and developmental programs drive glioblastoma, an incurable and poorly understood tumor, but their precise characterization remains challenging. Here, we use an integrative approach spanning single-cell RNA-sequencing of 28 tumors, bulk genetic and expression analysis of 401 specimens from the The Cancer Genome Atlas (TCGA), functional approaches, and single-cell lineage tracing to derive a unified model of cellular states and genetic diversity in glioblastoma. We find that malignant cells in glioblastoma exist in four main cellular states that recapitulate distinct neural cell types, are influenced by the tumor microenvironment, and exhibit plasticity. The relative frequency of cells in each state varies between glioblastoma samples and is influenced by copy number amplifications of the CDK4, EGFR, and PDGFRA loci and by mutations in the NF1 locus, which each favor a defined state. Our work provides a blueprint for glioblastoma, integrating the malignant cell programs, their plasticity, and their modulation by genetic drivers.

Single-Cell RNA Sequencing Maps Endothelial Metabolic Plasticity in Pathological Angiogenesis

Cell Metabolism (IF: 24.29), 2020-4, Yonglun Luo et al. DOI: 10.1016/j.cmet.2020.03.009 PubMed: 32268117

Single Cell Gene Expression, Human & Mouse, Cancer Research, Cell Atlas.

Abstract

Endothelial cell (EC) metabolism is an emerging target for anti-angiogenic therapy in tumor angiogenesis and choroidal neovascularization (CNV), but little is known about individual EC metabolic transcriptomes. By single-cell RNA sequencing 28,337 murine choroidal ECs (CECs) and sprouting CNV-ECs, we constructed a taxonomy to characterize their heterogeneity. Comparison with murine lung tumor ECs (TECs) revealed congruent marker gene expression by distinct EC phenotypes across tissues and diseases, suggesting similar angiogenic mechanisms. Trajectory inference predicted that differentiation of venous to angiogenic ECs was accompanied by metabolic transcriptome plasticity. ECs displayed metabolic transcriptome heterogeneity during cell-cycle progression and in quiescence. Hypothesizing that conserved genes are important, we used an integrated analysis, based on congruent transcriptome analysis, CEC-tailored genome-scale metabolic modeling, and gene expression meta-analysis in cross-species datasets, followed by in vitro and in vivo validation, to identify SQLE and ALDH18A1 as previously unknown metabolic angiogenic targets.

Deep generative modeling for single-cell transcriptomics

Nature Methods (IF: 36.15), 2018-11, Michael I Jordan et al. DOI: 10.1038/s41592-018-0229-2 PubMed: 30504886
Single Cell Gene Expression, Human, Mouse, Computational Method.

Abstract

Single-cell transcriptome measurements can reveal unexplored biological diversity, but they suffer from technical noise and bias that must be modeled to account for the resulting uncertainty in downstream analyses. Here we introduce single-cell variational inference (scVI), a ready-to-use scalable framework for the probabilistic representation and analysis of gene expression in single cells (<https://github.com/YosefLab/scVI>). scVI uses stochastic optimization and deep neural networks to aggregate information across similar cells and genes and to approximate the distributions that underlie observed expression values, while accounting for batch effects and limited sensitivity. We used scVI for a range of fundamental analysis tasks including batch correction, visualization, clustering, and differential expression, and achieved high accuracy for each task.

Co-expression of SARS-CoV-2 entry genes in the superficial adult human conjunctival, limbal and corneal epithelium suggests an additional route of entry via the ocular surface

Ocular Surface (IF: 12.33), 2020-6, Lyle Armstrong et al. DOI: 10.1016/j.jtos.2020.05.013 PubMed: 32502616
Single Cell Gene Expression & Single Cell ATAC, Human, Cell Atlas, Infectious Disease.

Abstract

The high infection rate of SARS-CoV-2 necessitates the need for multiple studies identifying the molecular mechanisms that facilitate the viral entry and propagation. Currently the potential extra-respiratory transmission routes of SARS-CoV-2 remain unclear. Using single-cell RNA Seq and ATAC-Seq datasets and immunohistochemical analysis, we investigated SARS-CoV-2 tropism in the embryonic, fetal and adult human ocular surface. The co-expression of ACE2 receptor and entry protease TMPRSS2 was detected in the human adult conjunctival, limbal and corneal epithelium, but not in the embryonic and fetal ocular surface up to 21 post conception weeks. These expression patterns were corroborated by the single cell ATAC-Seq data, which revealed a permissive chromatin in ACE2 and TMPRSS2 loci in the adult conjunctival, limbal and corneal epithelium. Co-expression of ACE2 and TMPRSS2 was strongly detected in the superficial limbal, corneal and conjunctival epithelium, implicating these as target entry cells for SARS-CoV-2 in the ocular surface. Strikingly, we also identified the key pro-inflammatory signals TNF, NFK β and IFNG as upstream regulators of the transcriptional profile of ACE2TMPRSS2 cells in the superficial conjunctival epithelium, suggesting that SARS-CoV-2 may utilise inflammatory driven upregulation of ACE2 and TMPRSS2 expression to enhance infection in ocular surface. Together our data indicate that the human ocular surface epithelium provides an additional entry portal for SARS-CoV-2, which may exploit inflammatory driven upregulation of ACE2 and TMPRSS2 entry factors to enhance infection.

Single-cell transcriptomic atlas of the human retina identifies cell types associated with age-related macular degeneration

Nature Communications (IF: 13.61), 2019-10, Manolis Kellis et al. DOI: 10.1038/s41467-019-12780-8

PubMed: 31653841

Single Cell Gene Expression, Human, Cell Atlas, Neuroscience.

Abstract

Genome-wide association studies (GWAS) have identified genetic variants associated with age-related macular degeneration (AMD), one of the leading causes of blindness in the elderly. However, it has been challenging to identify the cell types associated with AMD given the genetic complexity of the disease. Here we perform massively parallel single-cell RNA sequencing (scRNA-seq) of human retinas using two independent platforms, and report the first single-cell transcriptomic atlas of the human retina. Using a multi-resolution network-based analysis, we identify all major retinal cell types, and their corresponding gene expression signatures. Heterogeneity is observed within macroglia, suggesting that human retinal glia are more diverse than previously thought. Finally, GWAS-based enrichment analysis identifies glia, vascular cells, and cone photoreceptors to be associated with the risk of AMD. These data provide a detailed analysis of the human retina, and show how scRNA-seq can provide insight into cell types involved in complex, inflammatory genetic diseases.

Molecular taxonomy of human ocular outflow tissues defined by single-cell transcriptomics

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, W Daniel Stamer et al. DOI: 10.1073/pnas.2001896117 PubMed: 32439707

Single Cell Gene Expression, Human, Cell Atlas.

Abstract

The conventional outflow pathway is a complex tissue responsible for maintaining intraocular pressure (IOP) homeostasis. The coordinated effort of multiple cells with differing responsibilities ensures healthy outflow function and IOP maintenance. Dysfunction of one or more resident cell types results in ocular hypertension and risk for glaucoma, a leading cause of blindness. In this study, single-cell RNA sequencing was performed to generate a comprehensive cell atlas of human conventional outflow tissues. We obtained expression profiles of 17,757 genes from 8,758 cells from eight eyes of human donors representing the outflow cell transcriptome. Upon clustering analysis, 12 distinct cell types were identified, and region-specific expression of candidate genes was mapped in human tissues. Significantly, we identified two distinct expression patterns (myofibroblast- and fibroblast-like) from cells located in the trabecular meshwork (TM), the primary structural component of the conventional outflow pathway. We also located Schwann cell and macrophage signatures in the TM. The second primary component structure, Schlemm's canal, displayed a unique combination of lymphatic/blood vascular gene expression. Other expression clusters corresponded to cells from neighboring tissues, predominantly in the ciliary muscle/scleral spur, which together correspond to the uveoscleral outflow pathway. Importantly, the utility of our atlas was demonstrated by mapping glaucoma-relevant genes to outflow cell clusters. Our study provides a comprehensive molecular and cellular classification of conventional and unconventional outflow pathway structures responsible for IOP homeostasis.

Single-cell transcriptomics of the human retinal pigment epithelium and choroid in health and macular degeneration

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-11, Todd E Scheetz et al. DOI: 10.1073/pnas.1914143116 PubMed: 31712411

Single Cell Gene Expression, Human, Cell Biology, Method Comparison.

Abstract

The human retinal pigment epithelium (RPE) and choroid are complex tissues that provide crucial support to the retina. Disease affecting either of these supportive tissues can lead to irreversible blindness in the setting of age-related macular degeneration. In this study, single-cell RNA sequencing was performed on macular and peripheral regions of RPE-choroid from 7 human donor eyes in 2 independent experiments. In the first experiment, total RPE/choroid preparations were evaluated and expression profiles specific to RPE and major choroidal cell populations were identified. As choroidal endothelial cells represent a minority of the total RPE/choroidal cell population but are strongly implicated in age-related macular degeneration (AMD) pathogenesis, a second single-cell RNA-sequencing experiment was performed using endothelial cells enriched by magnetic separation. In this second study, we identified gene expression signatures along the choroidal vascular tree, classifying the transcriptome of human choriocapillaris, arterial, and venous endothelial cells. We found that the choriocapillaris highly and specifically expresses the regulator of cell cycle gene (), a gene that responds to complement activation and induces apoptosis in endothelial cells. In addition, was the most up-regulated choriocapillaris gene in a donor diagnosed with AMD. These results provide a characterization of the human RPE and choriocapillaris transcriptome, offering potential insight into the mechanisms of choriocapillaris response to complement injury and choroidal vascular disease in age-related macular degeneration.

A single-cell transcriptome atlas of the adult human retina

EMBO Journal, The (IF: 10.16), 2019-9, Joseph E Powell et al. DOI: 10.15252/embj.2018100811 PubMed: 31436334

Single Cell Gene Expression, Human, Cell Atlas.

Abstract

The retina is a specialized neural tissue that senses light and initiates image processing. Although the functional organization of specific retina cells has been well studied, the molecular profile of many cell types remains unclear in humans. To comprehensively profile the human retina, we performed single-cell RNA sequencing on 20,009 cells from three donors and compiled a reference transcriptome atlas. Using unsupervised clustering analysis, we identified 18 transcriptionally distinct cell populations representing all known neural retinal cells: rod photoreceptors, cone photoreceptors, Müller glia, bipolar cells, amacrine cells, retinal ganglion cells, horizontal cells, astrocytes, and microglia. Our data captured molecular profiles for healthy and putative early degenerating rod photoreceptors, and revealed the loss of MALAT1 expression with longer post-mortem time, which potentially suggested a novel role of MALAT1 in rod photoreceptor degeneration. We have demonstrated the use of this retina transcriptome atlas to benchmark pluripotent stem cell-derived cone photoreceptors and an adult Müller glia cell line. This work provides an important reference with unprecedented insights into the transcriptional landscape of human retinal cells, which is fundamental to understanding retinal biology and disease.

Single-Cell Analysis of Human Retina Identifies Evolutionarily Conserved and Species-Specific Mechanisms Controlling Development

Developmental Cell (IF: 10.13), 2020-5, Xiaoqun Wang et al. DOI: 10.1016/j.devcel.2020.04.009

PubMed: 32386599

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

The development of single-cell RNA sequencing (scRNA-seq) has allowed high-resolution analysis of cell-type diversity and transcriptional networks controlling cell-fate specification. To identify the transcriptional networks governing human retinal development, we performed scRNA-seq analysis on 16 time points from developing retina as well as four early stages of retinal organoid differentiation. We identified evolutionarily conserved patterns of gene expression during retinal progenitor maturation and specification of all seven major retinal cell types. Furthermore, we identified gene-expression differences between developing macula and periphery and between distinct populations of horizontal cells. We also identified species-specific patterns of gene expression during human and mouse retinal development. Finally, we identified an unexpected role for ATOH7 expression in regulation of photoreceptor specification during late retinogenesis. These results provide a roadmap to future studies of human retinal development and may help guide the design of cell-based therapies for treating retinal dystrophies.

Gene regulatory networks controlling vertebrate retinal regeneration

Science (IF: 44.37), 2020-11, Jiang Qian et al. DOI: 10.1126/science.abb8598 PubMed: 33004674

Single Cell Gene Expression, Mouse, Zebrafish, Developmental Biology.

Abstract

Injury induces retinal Müller glia of certain cold-blooded vertebrates, but not those of mammals, to regenerate neurons. To identify gene regulatory networks that reprogram Müller glia into progenitor cells, we profiled changes in gene expression and chromatin accessibility in Müller glia from zebrafish, chick, and mice in response to different stimuli. We identified evolutionarily conserved and species-specific gene networks controlling glial quiescence, reactivity, and neurogenesis. In zebrafish and chick, the transition from quiescence to reactivity is essential for retinal regeneration, whereas in mice, a dedicated network suppresses neurogenic competence and restores quiescence. Disruption of nuclear factor I transcription factors, which maintain and restore quiescence, induces Müller glia to proliferate and generate neurons in adult mice after injury. These findings may aid in designing therapies to restore retinal neurons lost to degenerative diseases.

Microglial Function Is Distinct in Different Anatomical Locations during Retinal Homeostasis and Degeneration

Immunity (IF: 25.73), 2019-3, Miriam Merad et al. DOI: 10.1016/j.immuni.2019.02.007 PubMed: 30850344

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Microglia from different nervous system regions are molecularly and anatomically distinct, but whether they also have different functions is unknown. We combined lineage tracing, single-cell transcriptomics, and electrophysiology of the mouse retina and showed that adult retinal microglia shared a common developmental lineage and were long-lived but resided in two distinct niches. Microglia in these niches differed in their interleukin-34 dependency and functional contribution to visual-information processing. During certain retinal-degeneration models, microglia from both pools relocated to the subretinal space, an inducible disease-associated niche that was poorly accessible to monocyte-derived cells. This microglial transition involved transcriptional reprogramming of microglia, characterized by reduced expression of homeostatic checkpoint genes and upregulation of injury-responsive genes. This transition was associated with protection of the retinal pigmented epithelium from damage caused by disease. Together, our data demonstrate that microglial function varies by retinal niche, thereby shedding light on the significance of microglia heterogeneity.

A new neutrophil subset promotes CNS neuron survival and axon regeneration

Nature Immunology (IF: 22.3), 2020-12, Roman J Giger et al. DOI: 10.1038/s41590-020-00813-0

PubMed: 33106668

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Transected axons typically fail to regenerate in the central nervous system (CNS), resulting in chronic neurological disability in individuals with traumatic brain or spinal cord injury, glaucoma and ischemia-reperfusion injury of the eye. Although neuroinflammation is often depicted as detrimental, there is growing evidence that alternatively activated, reparative leukocyte subsets and their products can be deployed to improve neurological outcomes. In the current study, we identify a unique granulocyte subset, with characteristics of an immature neutrophil, that had neuroprotective properties and drove CNS axon regeneration in vivo, in part via secretion of a cocktail of growth factors. This pro-regenerative neutrophil promoted repair in the optic nerve and spinal cord, demonstrating its relevance across CNS compartments and neuronal populations. Our findings could ultimately lead to the development of new immunotherapies that reverse CNS damage and restore lost neurological function across a spectrum of diseases.

Binary Fate Choice between Closely Related Interneuronal Types Is Determined by a Fezf1-Dependent Postmitotic Transcriptional Switch

Neuron (IF: 16), 2020-2, Alex L Kolodkin et al. DOI: 10.1016/j.neuron.2019.11.002 PubMed: 31812516

Single Cell Gene Expression, Mouse, Functional Genomics, Neuroscience.

Abstract

Many neuronal types occur as pairs that are similar in most respects but differ in a key feature. In some pairs of retinal neurons, called paramorphic, one member responds to increases and the other to decreases in luminance (ON and OFF responses). Here, we focused on one such pair, starburst amacrine cells (SACs), to explore how closely related neuronal types diversify. We find that ON and OFF SACs are transcriptionally distinct prior to their segregation, dendritic outgrowth, and synapse formation. The transcriptional repressor Fezf1 is selectively expressed by postmitotic ON SACs and promotes the ON fate and gene expression program while repressing the OFF fate and program. The atypical Rho GTPase Rnd3 is selectively expressed by OFF SACs and regulates their migration but is repressed by Fezf1 in ON SACs, enabling differential positioning of the two types. These results define a transcriptional program that controls diversification of a paramorphic pair.

Single-Cell Profiles of Retinal Ganglion Cells Differing in Resilience to Injury Reveal Neuroprotective Genes

Neuron (IF: 16), 2019-12, Zhigang He et al. DOI: 10.1016/j.neuron.2019.11.006 PubMed: 31784286

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Neuronal types in the central nervous system differ dramatically in their resilience to injury or other insults. Here we studied the selective resilience of mouse retinal ganglion cells (RGCs) following optic nerve crush (ONC), which severs their axons and leads to death of ~80% of RGCs within 2 weeks. To identify expression programs associated with differential resilience, we first used single-cell RNA-seq (scRNA-seq) to generate a comprehensive molecular atlas of 46 RGC types in adult retina. We then tracked their survival after ONC; characterized transcriptomic, physiological, and morphological changes that preceded degeneration; and identified genes selectively expressed by each type. Finally, using loss- and gain-of-function assays in vivo, we showed that manipulating some of these genes improved neuronal survival and axon regeneration following ONC. This study provides a systematic framework for parsing type-specific responses to injury and demonstrates that differential gene expression can be used to reveal molecular targets for intervention.

Nucleome Dynamics during Retinal Development

Neuron (IF: 16), 2019-11, Xiang Chen et al. DOI: 10.1016/j.neuron.2019.08.002 PubMed: 31493975

Single Cell Gene Expression & Single Cell ATAC, Mouse, Developmental Biology, Neuroscience.

Abstract

More than 8,000 genes are turned on or off as progenitor cells produce the 7 classes of retinal cell types during development. Thousands of enhancers are also active in the developing retinae, many having features of cell- and developmental stage-specific activity. We studied dynamic changes in the 3D chromatin landscape important for precisely orchestrated changes in gene expression during retinal development by ultra-deep in situ Hi-C analysis on murine retinae. We identified developmental-stage-specific changes in chromatin compartments and enhancer-promoter interactions. We developed a machine learning-based algorithm to map euchromatin and heterochromatin domains genome-wide and overlaid it with chromatin compartments identified by Hi-C. Single-cell ATAC-seq and RNA-seq were integrated with our Hi-C and previous ChIP-seq data to identify cell- and developmental-stage-specific super-enhancers (SEs). We identified a bipolar neuron-specific core regulatory circuit SE upstream of *Vsx2*, whose deletion in mice led to the loss of bipolar neurons.

Forward genetic analysis using OCT screening identifies *Sfxn3* mutations leading to progressive outer retinal degeneration in mice

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-6, Bruce Beutler et al. DOI: 10.1073/pnas.1921224117 PubMed: 32457148

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Retinal disease and loss of vision can result from any disruption of the complex pathways controlling retinal development and homeostasis. Forward genetics provides an excellent tool to find, in an unbiased manner, genes that are essential to these processes. Using ethylnitrosourea mutagenesis in mice in combination with a screening protocol using optical coherence tomography (OCT) and automated meiotic mapping, we identified 11 mutations presumably causative of retinal phenotypes in genes previously known to be essential for retinal integrity. In addition, we found multiple statistically significant gene-phenotype associations that have not been reported previously and decided to target one of these genes, (encoding sideroflexin-3), using CRISPR/Cas9 technology. We demonstrate, using OCT, light microscopy, and electroretinography, that two mouse lines developed progressive and severe outer retinal degeneration. Electron microscopy showed thinning of the retinal pigment epithelium and disruption of the external limiting membrane. Using single-cell RNA sequencing of retinal cells isolated from C57BL/6J mice, we demonstrate that it is expressed in several bipolar cell subtypes, retinal ganglion cells, and some amacrine cell subtypes but not significantly in Müller cells or photoreceptors. In situ hybridization confirmed these findings. Furthermore, pathway analysis suggests that *Sfxn3* may be associated with synaptic homeostasis. Importantly, electron microscopy analysis showed disruption of synapses and synaptic ribbons in the outer plexiform layer of mice. Our work describes a previously unknown requirement for in retinal.

Single-cell profiling reveals an endothelium-mediated immunomodulatory pathway in the eye choroid

Journal of experimental medicine, The (IF: 11.32), 2020-6, Enrique Rodriguez-Boulan et al. DOI: 10.1084/jem.20190730 PubMed: 32196081

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

The activity and survival of retinal photoreceptors depend on support functions performed by the retinal pigment epithelium (RPE) and on oxygen and nutrients delivered by blood vessels in the underlying choroid. By combining single-cell and bulk RNA sequencing, we categorized mouse RPE/choroid cell types and characterized the tissue-specific transcriptomic features of choroidal endothelial cells. We found that choroidal endothelium adjacent to the RPE expresses high levels of Indian Hedgehog and identified its downstream target as stromal GLI1+ mesenchymal stem cell-like cells. In vivo genetic impairment of Hedgehog signaling induced significant loss of choroidal mast cells, as well as an altered inflammatory response and exacerbated visual function defects after retinal damage. Our studies reveal the cellular and molecular landscape of adult RPE/choroid and uncover a Hedgehog-regulated choroidal immunomodulatory signaling circuit. These results open new avenues for the study and treatment of retinal vascular diseases and choroid-related inflammatory blinding disorders.

Comprehensive identification of mRNA isoforms reveals the diversity of neural cell-surface molecules with roles in retinal development and disease

Nature Communications (IF: 13.61), 2020-7, Vadim Y Arshavsky et al. DOI: 10.1038/s41467-020-17009-7 PubMed: 32620864

Single Cell Gene Expression, Mouse, Cell Biology, Neuroscience.

Abstract

Genes encoding cell-surface proteins control nervous system development and are implicated in neurological disorders. These genes produce alternative mRNA isoforms which remain poorly characterized, impeding understanding of how disease-associated mutations cause pathology. Here we introduce a strategy to define complete portfolios of full-length isoforms encoded by individual genes. Applying this approach to neural cell-surface molecules, we identify thousands of unannotated isoforms expressed in retina and brain. By mass spectrometry we confirm expression of newly-discovered proteins on the cell surface in vivo. Remarkably, we discover that the major isoform of a retinal degeneration gene, CRB1, was previously overlooked. This CRB1 isoform is the only one expressed by photoreceptors, the affected cells in CRB1 disease. Using mouse mutants, we identify a function for this isoform at photoreceptor-glial junctions and demonstrate that loss of this isoform accelerates photoreceptor death. Therefore, our isoform identification strategy enables discovery of new gene functions relevant to disease.

Comprehensive analysis of a mouse model of spontaneous uveoretinitis using single-cell RNA sequencing

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-12, Loyal A Goff et al. DOI: 10.1073/pnas.1915571116 PubMed: 31843893

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

Autoimmune uveoretinitis is a significant cause of visual loss, and mouse models offer unique opportunities to study its disease mechanisms. Mice fail to express self-antigens in the thymus, exhibit reduced central tolerance, and develop a spontaneous, chronic, and progressive uveoretinitis. Using single-cell RNA sequencing (scRNA-seq), we characterized wild-type and retinas to define, in a comprehensive and unbiased manner, the cell populations and gene expression patterns associated with disease. Based on scRNA-seq, immunostaining, and in situ hybridization, we infer that 1) the dominant effector response in retinas is Th1-driven, 2) a subset of monocytes convert to either a macrophage microglia state or a dendritic cell state, 3) the development of tertiary lymphoid structures constitutes part of the retinal phenotype, 4) all major resident retinal cell types respond to interferon gamma (IFN γ) by changing their patterns of gene expression, and 5) Muller glia up-regulate specific genes in response to IFN γ and may act as antigen-presenting cells.

Retinal homeostasis and metformin-induced protection are not affected by retina-specific Ppar δ knockout

Redox Biology (IF: 9.79), 2020-8, Ekaterina Lobanova et al. DOI: 10.1016/j.redox.2020.101700 PubMed: 32863184

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Peroxisome proliferator-activated receptors (PPARs) are a family of three nuclear hormone receptors (PPAR α , PPAR δ , and PPAR γ) that are known to regulate expression of lipid metabolism and oxidative stress genes. Given their role in reducing oxidative stress in a variety of tissues, these genes are likely important for retinal homeostasis. This hypothesis has been further supported by recent studies suggesting that PPAR-activating drugs are protective against retinal degenerations. The objective of the present study was to determine the role of PPAR δ in the neuroretina. RNA-seq data show that Ppar α and Ppar δ are both expressed in the retina, but that Ppar δ is expressed at 4-fold higher levels. Single-cell RNAseq data show that Ppar δ is broadly expressed in all retinal cell types. To determine the importance of Ppar δ to the retina, we generated retina-specific Ppar δ knockout mice. We found that deletion of Ppar δ had a minimal effect on retinal function or morphology out to 12 months of age and did not increase retinal sensitivity to oxidative stress induced by exposure to bright light. While data show that PPAR δ levels were increased by the drug metformin, PPAR δ was not necessary for metformin-induced protection from light damage. These data suggest that Ppar δ either has a redundant function with Ppar α or is not essential for normal neuroretina function or resistance to oxidative stress.

Diabetes relief in mice by glucose-sensing insulin-secreting human α -cells

Nature (IF: 46.49), 2019-2, Fabrizio Thorel et al. DOI: 10.1038/s41586-019-0942-8 PubMed: 30760930

Single Cell Gene Expression, Human & Mouse, Assay Method, Cell Biology.

Abstract

Cell-identity switches, in which terminally differentiated cells are converted into different cell types when stressed, represent a widespread regenerative strategy in animals, yet they are poorly documented in mammals. In mice, some glucagon-producing pancreatic α -cells and somatostatin-producing δ -cells become insulin-expressing cells after the ablation of insulin-secreting β -cells, thus promoting diabetes recovery. Whether human islets also display this plasticity, especially in diabetic conditions, remains unknown. Here we show that islet non- β -cells, namely α -cells and pancreatic polypeptide (PPY)-producing γ -cells, obtained from deceased non-diabetic or diabetic human donors, can be lineage-traced and reprogrammed by the transcription factors PDX1 and MAFA to produce and secrete insulin in response to glucose. When transplanted into diabetic mice, converted human α -cells reverse diabetes and continue to produce insulin even after six months. Notably, insulin-producing α -cells maintain expression of α -cell markers, as seen by deep transcriptomic and proteomic characterization. These observations provide conceptual evidence and a molecular framework for a mechanistic understanding of in situ cell plasticity as a treatment for diabetes and other degenerative diseases.

Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors

Science (IF: 44.37), 2018-8, Menna ClatworthySam Behjati DOI: 10.1126/science.aat1699 PubMed: 30093597

Single Cell Gene Expression, Human, Cancer Research, Cell Atlas.

Abstract

Messenger RNA encodes cellular function and phenotype. In the context of human cancer, it defines the identities of malignant cells and the diversity of tumor tissue. We studied 72,501 single-cell transcriptomes of human renal tumors and normal tissue from fetal, pediatric, and adult kidneys. We matched childhood Wilms tumor with specific fetal cell types, thus providing evidence for the hypothesis that Wilms tumor cells are aberrant fetal cells. In adult renal cell carcinoma, we identified a canonical cancer transcriptome that matched a little-known subtype of proximal convoluted tubular cell. Analyses of the tumor composition defined cancer-associated normal cells and delineated a complex vascular endothelial growth factor (VEGF) signaling circuit. Our findings reveal the precise cellular identities and compositions of human kidney tumors.

Decoding myofibroblast origins in human kidney fibrosis

Nature (IF: 46.49), 2020-11, Neil C Henderson et al. DOI: 10.1038/s41586-020-2941-1 PubMed: 33176333

Single Cell Gene Expression, Human, Mouse, Cell Atlas.

Abstract

Kidney fibrosis is the hallmark of chronic kidney disease progression, however, currently no antifibrotic therapies exist. This is largely because the origin, functional heterogeneity and regulation of scar-forming cells during human kidney fibrosis remains poorly understood. Here, using single cell RNA-seq, we profiled the transcriptomes of proximal tubule and non-proximal tubule cells in healthy and fibrotic human kidneys to map the entire human kidney in an unbiased approach. This enabled mapping of all matrix-producing cells at high resolution, revealing distinct subpopulations of pericytes and fibroblasts as the major cellular sources of scar forming myofibroblasts during human kidney fibrosis. We used genetic fate-tracing, time-course single cell RNA-seq and ATAC-seq experiments in mice, and spatial transcriptomics in human kidney fibrosis to functionally interrogate these findings, shedding new light on the origin, heterogeneity and differentiation of human kidney myofibroblasts and their fibroblast and pericyte precursors at unprecedented resolution. Finally, we used this strategy to facilitate target discovery, identifying *Nkd2* as a myofibroblast-specific target in human kidney fibrosis.

High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade

Nature Medicine (IF: 36.23), 2020-5, Mahrukh A Huseni et al. DOI: 10.1038/s41591-020-0860-1

PubMed: 32405063

Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immuno-oncology.

Abstract

Although elevated plasma interleukin-8 (pIL-8) has been associated with poor outcome to immune checkpoint blockade, this has not been comprehensively evaluated in large randomized studies. Here we analyzed circulating pIL-8 and IL8 gene expression in peripheral blood mononuclear cells and tumors of patients treated with atezolizumab (anti-PD-L1 monoclonal antibody) from multiple randomized trials representing 1,445 patients with metastatic urothelial carcinoma (mUC) and metastatic renal cell carcinoma. High levels of IL-8 in plasma, peripheral blood mononuclear cells and tumors were associated with decreased efficacy of atezolizumab in patients with mUC and metastatic renal cell carcinoma, even in tumors that were classically CD8T cell inflamed. Low baseline pIL-8 in patients with mUC was associated with increased response to atezolizumab and chemotherapy. Patients with mUC who experienced on-treatment decreases in pIL-8 exhibited improved overall survival when treated with atezolizumab but not with chemotherapy. Single-cell RNA sequencing of the immune compartment showed that IL8 is primarily expressed in circulating and intratumoral myeloid cells and that high IL8 expression is associated with downregulation of the antigen-presentation machinery. Therapies that can reverse the impacts of IL-8-mediated myeloid inflammation will be essential for improving outcomes of patients treated with immune checkpoint inhibitors.

Spatiotemporal immune zonation of the human kidney

Science (IF: 44.37), 2019-9, Sam Behjati et al. DOI: 10.1126/science.aat5031 PubMed: 31604275

Single Cell Gene Expression, Human, Cell Atlas, Immunology.

Abstract

Tissue-resident immune cells are important for organ homeostasis and defense. The epithelium may contribute to these functions directly or by cross-talk with immune cells. We used single-cell RNA sequencing to resolve the spatiotemporal immune topology of the human kidney. We reveal anatomically defined expression patterns of immune genes within the epithelial compartment, with antimicrobial peptide transcripts evident in pelvic epithelium in the mature, but not fetal, kidney. A network of tissue-resident myeloid and lymphoid immune cells was evident in both fetal and mature kidney, with postnatal acquisition of transcriptional programs that promote infection-defense capabilities. Epithelial-immune cross-talk orchestrated localization of antibacterial macrophages and neutrophils to the regions of the kidney most susceptible to infection. Overall, our study provides a global overview of how the immune landscape of the human kidney is zoned to counter the dominant immunological challenge.

Generation of Human PSC-Derived Kidney Organoids with Patterned Nephron Segments and a De Novo Vascular Network

Cell Stem Cell (IF: 23.45), 2019-9, Juan Carlos Izpisua Belmonte et al. DOI: 10.1016/j.stem.2019.06.009

PubMed: 31303547

Single Cell Gene Expression, Human & Mouse, Developmental Biology.

Abstract

Human pluripotent stem cell-derived kidney organoids recapitulate developmental processes and tissue architecture, but intrinsic limitations, such as lack of vasculature and functionality, have greatly hampered their application. Here we establish a versatile protocol for generating vascularized three-dimensional (3D) kidney organoids. We employ dynamic modulation of WNT signaling to control the relative proportion of proximal versus distal nephron segments, producing a correlative level of vascular endothelial growth factor A (VEGFA) to define a resident vascular network. Single-cell RNA sequencing identifies a subset of nephron progenitor cells as a potential source of renal vasculature. These kidney organoids undergo further structural and functional maturation upon implantation. Using this kidney organoid platform, we establish an in vitro model of autosomal recessive polycystic kidney disease (ARPKD), the cystic phenotype of which can be effectively prevented by gene correction or drug treatment. Our studies provide new avenues for studying human kidney development, modeling disease pathogenesis, and performing patient-specific drug validation.

Evaluation of variability in human kidney organoids

Nature Methods (IF: 36.15), 2018-12, Alicia OshlackMelissa H Little DOI: 10.1038/s41592-018-0253-2

PubMed: 30573816

Single Cell Gene Expression, Human, Cell Biology.

Abstract

The utility of human pluripotent stem cell-derived kidney organoids relies implicitly on the robustness and transferability of the protocol. Here we analyze the sources of transcriptional variation in a specific kidney organoid protocol. Although individual organoids within a differentiation batch showed strong transcriptional correlation, we noted significant variation between experimental batches, particularly in genes associated with temporal maturation. Single-cell profiling revealed shifts in nephron patterning and proportions of component cells. Distinct induced pluripotent stem cell clones showed congruent transcriptional programs, with interexperimental and interclonal variation also strongly associated with nephron patterning. Epithelial cells isolated from organoids aligned with total organoids at the same day of differentiation, again implicating relative maturation as a confounder. This understanding of experimental variation facilitated an optimized analysis of organoid-based disease modeling, thereby increasing the utility of kidney organoids for personalized medicine and functional genomics.

Comparative Analysis and Refinement of Human PSC-Derived Kidney Organoid Differentiation with Single-Cell Transcriptomics

Cell Stem Cell (IF: 23.45), 2018-12, Samantha A MorrisBenjamin D Humphreys DOI: 10.1016/j.stem.2018.10.010

PubMed: 30449713

Single Cell Gene Expression, Human, Assay Method, Developmental Biology.

Abstract

Kidney organoids derived from human pluripotent stem cells have great utility for investigating organogenesis and disease mechanisms and, potentially, as a replacement tissue source, but how closely organoids derived from current protocols replicate adult human kidney is undefined. We compared two directed differentiation protocols by single-cell transcriptomics of 83,130 cells from 65 organoids with single-cell transcriptomes of fetal and adult kidney cells. Both protocols generate a diverse range of kidney cells with differing ratios, but organoid-derived cell types are immature, and 10%-20% of cells are non-renal. Reconstructing lineage relationships by pseudotemporal ordering identified ligands, receptors, and transcription factor networks associated with fate decisions. Brain-derived neurotrophic factor (BDNF) and its cognate receptor NTRK2 were expressed in the neuronal lineage during organoid differentiation. Inhibiting this pathway improved organoid formation by reducing neurons by 90% without affecting kidney differentiation, highlighting the power of single-cell technologies to characterize and improve organoid differentiation.

The immune cell landscape in kidneys of patients with lupus nephritis

Nature Immunology (IF: 22.3), 2019-6, Betty Diamond DOI: 10.1038/s41590-019-0398 PubMed: 31209404

Single Cell Gene Expression, Human, Immunology.

Abstract

Lupus nephritis is a potentially fatal autoimmune disease for which the current treatment is ineffective and often toxic. To develop mechanistic hypotheses of disease, we analyzed kidney samples from patients with lupus nephritis and from healthy control subjects using single-cell RNA sequencing. Our analysis revealed 21 subsets of leukocytes active in disease, including multiple populations of myeloid cells, T cells, natural killer cells and B cells that demonstrated both pro-inflammatory responses and inflammation-resolving responses. We found evidence of local activation of B cells correlated with an age-associated B-cell signature and evidence of progressive stages of monocyte differentiation within the kidney. A clear interferon response was observed in most cells. Two chemokine receptors, CXCR4 and CX3CR1, were broadly expressed, implying a potentially central role in cell trafficking. Gene expression of immune cells in urine and kidney was highly correlated, which would suggest that urine might serve as a surrogate for kidney biopsies.

Using single nucleotide variations in single-cell RNA-seq to identify subpopulations and genotype-phenotype linkage

Nature Communications (IF: 13.61), 2018-11, Travers Ching et al. DOI: 10.1038/s41467-018-07170-5

PubMed: 30459309

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

Despite its popularity, characterization of subpopulations with transcript abundance is subject to a significant amount of noise. We propose to use effective and expressed nucleotide variations (eeSNVs) from scRNA-seq as alternative features for tumor subpopulation identification. We develop a linear modeling framework, SSrGE, to link eeSNVs associated with gene expression. In all the datasets tested, eeSNVs achieve better accuracies than gene expression for identifying subpopulations. Previously validated cancer-relevant genes are also highly ranked, confirming the significance of the method. Moreover, SSrGE is capable of analyzing coupled DNA-seq and RNA-seq data from the same single cells, demonstrating its value in integrating multi-omics single cell techniques. In summary, SNV features from scRNA-seq data have merits for both subpopulation identification and linkage of genotype-phenotype relationship.

Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation

Nature Communications (IF: 13.61), 2019-11, Aviv Regev et al. DOI: 10.1038/s41467-019-13382-0

PubMed: 31784515

Single Cell Gene Expression, Human, Cell Atlas, Developmental Biology.

Abstract

Human iPSC-derived kidney organoids have the potential to revolutionize discovery, but assessing their consistency and reproducibility across iPSC lines, and reducing the generation of off-target cells remain an open challenge. Here, we profile four human iPSC lines for a total of 450,118 single cells to show how organoid composition and development are comparable to human fetal and adult kidneys. Although cell classes are largely reproducible across time points, protocols, and replicates, we detect variability in cell proportions between different iPSC lines, largely due to off-target cells. To address this, we analyze organoids transplanted under the mouse kidney capsule and find diminished off-target cells. Our work shows how single cell RNA-seq (scRNA-seq) can score organoids for reproducibility, faithfulness and quality, that kidney organoids derived from different iPSC lines are comparable surrogates for human kidney, and that transplantation enhances their formation by diminishing off-target cells.

An eQTL Landscape of Kidney Tissue in Human Nephrotic Syndrome

American Journal of Human Genetics (IF: 10.5), 2018-8, Xiaoquan WenMatthew G Sampson

DOI: 10.1016/j.ajhg.2018.07.004 PubMed: 30057032

Single Cell Gene Expression, Human, Genetic Health.

Abstract

Expression quantitative trait loci (eQTL) studies illuminate the genetics of gene expression and, in disease research, can be particularly illuminating when using the tissues directly impacted by the condition. In nephrology, there is a paucity of eQTL studies of human kidney. Here, we used whole-genome sequencing (WGS) and microdissected glomerular (GLOM) and tubulointerstitial (TI) transcriptomes from 187 individuals with nephrotic syndrome (NS) to describe the eQTL landscape in these functionally distinct kidney structures. Using MatrixEQTL, we performed cis-eQTL analysis on GLOM (n = 136) and TI (n = 166). We used the Bayesian Deterministic Approximation of Posteriors (DAP) to fine-map these signals, eQTLBMA to discover GLOM- or TI-specific eQTLs, and single-cell RNA-seq data of control kidney tissue to identify the cell type specificity of significant eQTLs. We integrated eQTL data with an IgA Nephropathy (IgAN) GWAS to perform a transcriptome-wide association study (TWAS). We discovered 894 GLOM eQTLs and 1,767 TI eQTLs at FDR < 0.05. 14% and 19% of GLOM and TI eQTLs, respectively, had >1 independent signal associated with its expression. 12% and 26% of eQTLs were GLOM specific and TI specific, respectively. GLOM eQTLs were most significantly enriched in podocyte transcripts and TI eQTLs in proximal tubules. The IgAN TWAS identified significant GLOM and TI genes, primarily at the HLA region. In this study, we discovered GLOM and TI eQTLs, identified those that were tissue specific, deconvoluted them into cell-specific signals, and used them to characterize known GWAS alleles. These data are available for browsing and download via our eQTL browser, nephQTL.

The single-cell transcriptomic landscape of early human diabetic nephropathy

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2019-9, Sushrut S Waikar et al. DOI: 10.1073/pnas.1908706116 PubMed: 31506348

Single Cell Gene Expression, Human, Cell Atlas, Immunology.

Abstract

Diabetic nephropathy is characterized by damage to both the glomerulus and tubulointerstitium, but relatively little is known about accompanying cell-specific changes in gene expression. We performed unbiased single-nucleus RNA sequencing (snRNA-seq) on cryopreserved human diabetic kidney samples to generate 23,980 single-nucleus transcriptomes from 3 control and 3 early diabetic nephropathy samples. All major cell types of the kidney were represented in the final dataset. Side-by-side comparison demonstrated cell-type-specific changes in gene expression that are important for ion transport, angiogenesis, and immune cell activation. In particular, we show that the diabetic thick ascending limb, late distal convoluted tubule, and principal cells all adopt a gene expression signature consistent with increased potassium secretion, including alterations in Na/K-ATPase, mineralocorticoid receptor, and expression, as well as decreased paracellular calcium and magnesium reabsorption. We also identify strong angiogenic signatures in glomerular cell types, proximal convoluted tubule, distal convoluted tubule, and principal cells. Taken together, these results suggest that increased potassium secretion and angiogenic signaling represent early kidney responses in human diabetic nephropathy.

In Vivo Developmental Trajectories of Human Podocyte Inform In Vitro Differentiation of Pluripotent Stem Cell-Derived Podocytes

Developmental Cell (IF: 10.13), 2019-7, Jill A McMahon et al. DOI: 10.1016/j.devcel.2019.06.001

PubMed: 31265809

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

The renal corpuscle of the kidney comprises a glomerular vasculature embraced by podocytes and supported by mesangial myofibroblasts, which ensure plasma filtration at the podocyte-generated slit diaphragm. With a spectrum of podocyte-expressed gene mutations causing chronic disease, an enhanced understanding of podocyte development and function to create relevant in vitro podocyte models is a clinical imperative. To characterize podocyte development, scRNA-seq was performed on human fetal kidneys, identifying distinct transcriptional signatures accompanying the differentiation of functional podocytes from progenitors. Interestingly, organoid-generated podocytes exhibited highly similar, progressive transcriptional profiles despite an absence of the vasculature, although abnormal gene expression was pinpointed in late podocytes. On transplantation into mice, organoid-derived podocytes recruited the host vasculature and partially corrected transcriptional profiles. Thus, human podocyte development is mostly intrinsically regulated and vascular interactions refine maturation. These studies support the application of organoid-derived podocytes to model disease and to restore or replace normal kidney functions.

Macrophage HIF-1 α Is an Independent Prognostic Indicator in Kidney Cancer

Clinical cancer research (IF: 10.12), 2020-9, Eric Jonasch et al. DOI: 10.1158/1078-0432.CCR-19-3890

PubMed: 32586940

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Clear cell renal cell carcinoma (ccRCC) is frequently associated with inactivation of the von Hippel-Lindau tumor suppressor, resulting in activation of HIF-1 α and HIF-2 α . The current paradigm, established using mechanistic cell-based studies, supports a tumor promoting role for HIF-2 α , and a tumor suppressor role for HIF-1 α . However, few studies have comprehensively examined the clinical relevance of this paradigm. Furthermore, the hypoxia-associated factor (HAF), which regulates the HIFs, has not been comprehensively evaluated in ccRCC. To assess the involvement of HAF/HIFs in ccRCC, we analyzed their relationship to tumor grade/stage/outcome using tissue from 380 patients, and validated these associations using tissue from 72 additional patients and a further 57 patients treated with antiangiogenic therapy for associations with response. Further characterization was performed using single-cell mRNA sequencing (scRNA-seq), RNA-hybridization (RNA-ISH), and IHC. HIF-1 α was primarily expressed in tumor-associated macrophages (TAMs), whereas HIF-2 α and HAF were expressed primarily in tumor cells. TAM-associated HIF-1 α was significantly associated with high tumor grade and increased metastasis and was independently associated with decreased overall survival. Furthermore, elevated TAM HIF-1 α was significantly associated with resistance to antiangiogenic therapy. In contrast, high HAF or HIF-2 α were associated with low grade, decreased metastasis, and increased overall survival. scRNA-seq, RNA-ISH, and Western blotting confirmed the expression of HIF-1 α in M2-polarized CD163-expressing TAMs. These findings highlight a potential role of TAM HIF-1 α in ccRCC progression and support the reevaluation of HIF-1 α as a therapeutic target and marker of disease progression.

Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease

Science (IF: 44.37), 2018-5, Jonathan Barasch et al. DOI: 10.1126/science.aar2131 PubMed: 29622724

Single Cell Gene Expression, Mouse, Cell Atlas, Cell Biology.

Abstract

Our understanding of kidney disease pathogenesis is limited by an incomplete molecular characterization of the cell types responsible for the organ's multiple homeostatic functions. To help fill this knowledge gap, we characterized 57,979 cells from healthy mouse kidneys by using unbiased single-cell RNA sequencing. On the basis of gene expression patterns, we infer that inherited kidney diseases that arise from distinct genetic mutations but share the same phenotypic manifestation originate from the same differentiated cell type. We also found that the collecting duct in kidneys of adult mice generates a spectrum of cell types through a newly identified transitional cell. Computational cell trajectory analysis and in vivo lineage tracing revealed that intercalated cells and principal cells undergo transitions mediated by the Notch signaling pathway. In mouse and human kidney disease, these transitions were shifted toward a principal cell fate and were associated with metabolic acidosis.

The Nuclear Receptor ESRRA Protects from Kidney Disease by Coupling Metabolism and Differentiation

Cell Metabolism (IF: 24.29), 2020-12, Nuria Montserrat et al. DOI: 10.1016/j.cmet.2020.11.011 PubMed: 33301705
Single Cell Gene Expression, Mouse, Cell Atlas, Genetic Health.

Abstract

Kidney disease is poorly understood because of the organ's cellular diversity. We used single-cell RNA sequencing not only in resolving differences in injured kidney tissue cellular composition but also in cell-type-specific gene expression in mouse models of kidney disease. This analysis highlighted major changes in cellular diversity in kidney disease, which markedly impacted whole-kidney transcriptomics outputs. Cell-type-specific differential expression analysis identified proximal tubule (PT) cells as the key vulnerable cell type. Through unbiased cell trajectory analyses, we show that PT cell differentiation is altered in kidney disease. Metabolism (fatty acid oxidation and oxidative phosphorylation) in PT cells showed the strongest and most reproducible association with PT cell differentiation and disease. Coupling of cell differentiation and the metabolism was established by nuclear receptors (estrogen-related receptor alpha [ESRRA] and peroxisomal proliferation-activated receptor alpha [PPARA]) that directly control metabolic and PT-cell-specific gene expression in mice and patient samples while protecting from kidney disease in the mouse model.

Generation of a p16 Reporter Mouse and Its Use to Characterize and Target p16^{high} Cells In Vivo

Cell Metabolism (IF: 24.29), 2020-11, Atsushi Miyajima et al. DOI: 10.1016/j.cmet.2020.09.006 PubMed: 32949498
Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Cell senescence plays a key role in age-associated organ dysfunction, but the in vivo pathogenesis is largely unclear. Here, we generated a p16-Cre-tdTomato mouse model to analyze the in vivo characteristics of p16^{high} cells at a single-cell level. We found tdTomato-positive p16^{high} cells detectable in all organs, which were enriched with age. We also found that these cells failed to proliferate and had half-lives ranging from 2.6 to 4.2 months, depending on the tissue examined. Single-cell transcriptomics in the liver and kidneys revealed that p16^{high} cells were present in various cell types, though most dominant in hepatic endothelium and in renal proximal and distal tubule epithelia, and that these cells exhibited heterogeneous senescence-associated phenotypes. Further, elimination of p16^{high} cells ameliorated nonalcoholic steatohepatitis-related hepatic lipidosis and immune cell infiltration. Our new mouse model and single-cell analysis provide a powerful resource to enable the discovery of previously unidentified senescence functions in vivo.

Solo: Doublet Identification in Single-Cell RNA-Seq via Semi-Supervised Deep Learning

Cell systems (IF: 11.45), 2020-7, David G Hendrickson et al. DOI: 10.1016/j.cels.2020.05.010 PubMed: 32592658
Single Cell Gene Expression, Mouse, Computational Method.

Abstract

Single-cell RNA sequencing (scRNA-seq) measurements of gene expression enable an unprecedented high-resolution view into cellular state. However, current methods often result in two or more cells that share the same cell-identifying barcode; these “doublets” violate the fundamental premise of single-cell technology and can lead to incorrect inferences. Here, we describe Solo, a semi-supervised deep learning approach that identifies doublets with greater accuracy than existing methods. Solo embeds cells unsupervised using a variational autoencoder and then appends a feed-forward neural network layer to the encoder to form a supervised classifier. We train this classifier to distinguish simulated doublets from the observed data. Solo can be applied in combination with experimental doublet detection methods to further purify scRNA-seq data to true single cells. It is freely available from <https://github.com/calico/solo>. A record of this paper's transparent peer review process is included in the Supplemental Information.

Altered proximal tubular cell glucose metabolism during acute kidney injury is associated with mortality

Nature Metabolism (IF: 18.57), 2020-8, Pietro E Cippà et al. DOI: 10.1038/s42255-020-0238-1 PubMed: 32694833
Single Cell Gene Expression, Mouse, Immunology.

Abstract

Acute kidney injury (AKI) is strongly associated with mortality, independently of its cause. The kidney contributes to up to 40% of systemic glucose production by gluconeogenesis during fasting and under stress conditions. Whether kidney gluconeogenesis is impaired during AKI and how this might influence systemic metabolism remain unknown. Here we show that glucose production and lactate clearance are impaired during human and experimental AKI by using renal arteriovenous catheterization in patients, lactate tolerance testing in mice and glucose isotope labelling in rats. Single-cell transcriptomics reveal that gluconeogenesis is impaired in proximal tubule cells during AKI. In a retrospective cohort of critically ill patients, we demonstrate that altered glucose metabolism during AKI is a major determinant of systemic glucose and lactate levels and is strongly associated with mortality. Thiamine supplementation increases lactate clearance without modifying renal function in mice with AKI, enhances glucose production by renal tubular cells ex vivo and is associated with reduced mortality and improvement of the metabolic pattern in a retrospective cohort of critically ill patients with AKI. This study highlights an unappreciated systemic role of renal glucose and lactate metabolism under stress conditions, delineates general mechanisms of AKI-associated mortality and introduces a potential intervention targeting metabolism for a highly prevalent clinical condition with limited therapeutic options.

Cell profiling of mouse acute kidney injury reveals conserved cellular responses to injury

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-7, Parker C Wilson et al. DOI: 10.1073/pnas.2005477117 PubMed: 32571916

Single Cell Gene Expression, Mouse, Immunology.

Abstract

After acute kidney injury (AKI), patients either recover or alternatively develop fibrosis and chronic kidney disease. Interactions between injured epithelia, stroma, and inflammatory cells determine whether kidneys repair or undergo fibrosis, but the molecular events that drive these processes are poorly understood. Here, we use single nucleus RNA sequencing of a mouse model of AKI to characterize cell states during repair from acute injury. We identify a distinct proinflammatory and profibrotic proximal tubule cell state that fails to repair. Deconvolution of bulk RNA-seq datasets indicates that this failed-repair proximal tubule cell (FR-PTC) state can be detected in other models of kidney injury, increasing during aging in rat kidney and over time in human kidney allografts. We also describe dynamic intercellular communication networks and discern transcriptional pathways driving successful vs. failed repair. Our study provides a detailed description of cellular responses after injury and suggests that the FR-PTC state may represent a therapeutic target to improve repair.

Single-Cell Transcriptome Profiling Reveals β Cell Maturation in Stem Cell-Derived Islets after Transplantation

Cell Reports (IF: 8.82), 2020-8, Leonardo Velazco-Cruz et al. DOI: 10.1016/j.celrep.2020.108067

PubMed: 32846125

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Human pluripotent stem cells differentiated to insulin-secreting β cells (SC- β cells) in islet organoids could provide an unlimited cell source for diabetes cell replacement therapy. However, current SC- β cells generated in vitro are transcriptionally and functionally immature compared to native adult β cells. Here, we use single-cell transcriptomic profiling to catalog changes that occur in transplanted SC- β cells. We find that transplanted SC- β cells exhibit drastic transcriptional changes and mature to more closely resemble adult β cells. Insulin and IAPP protein secretions increase upon transplantation, along with expression of maturation genes lacking with differentiation in vitro, including INS, MAFA, CHGB, and G6PC2. Other differentiated cell types, such as SC- α and SC-enterochromaffin (SC-EC) cells, also exhibit large transcriptional changes. This study provides a comprehensive resource for understanding human islet cell maturation and provides important insights into maturation of SC- β cells and other SC-islet cell types to enable future differentiation strategy improvements.

Single-Cell Profiling Reveals Sex, Lineage, and Regional Diversity in the Mouse Kidney

Developmental Cell (IF: 10.13), 2019-11, Junhyong Kim et al. DOI: 10.1016/j.devcel.2019.10.005

PubMed: 31689386

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Chronic kidney disease affects 10% of the population with notable differences in ethnic and sex-related susceptibility to kidney injury and disease. Kidney dysfunction leads to significant morbidity and mortality and chronic disease in other organ systems. A mouse-organ-centered understanding underlies rapid progress in human disease modeling and cellular approaches to repair damaged systems. To enhance an understanding of the mammalian kidney, we combined anatomy-guided single-cell RNA sequencing of the adult male and female mouse kidney with in situ expression studies and cell lineage tracing. These studies reveal cell diversity and marked sex differences, distinct organization and cell composition of nephrons dependent on the time of nephron specification, and lineage convergence, in which contiguous functionally related cell types are specified from nephron and collecting system progenitor populations. A searchable database, Kidney Cell Explorer (<https://cello.shinyapps.io/kidneycellexplorer/>), enables gene-cell relationships to be viewed in the anatomical framework of the kidney.

High-Resolution mRNA and Secretome Atlas of Human Enteroendocrine Cells

Cell (IF: 38.62), 2020-6, Wei Wu et al. DOI: 10.1016/j.cell.2020.04.036 PubMed: 32407674

Single Cell Gene Expression, Human, Cell Atlas, Developmental Biology.

Abstract

Enteroendocrine cells (EECs) sense intestinal content and release hormones to regulate gastrointestinal activity, systemic metabolism, and food intake. Little is known about the molecular make-up of human EEC subtypes and the regulated secretion of individual hormones. Here, we describe an organoid-based platform for functional studies of human EECs. EEC formation is induced in vitro by transient expression of NEUROG3. A set of gut organoids was engineered in which the major hormones are fluorescently tagged. A single-cell mRNA atlas was generated for the different EEC subtypes, and their secreted products were recorded by mass-spectrometry. We note key differences to murine EECs, including hormones, sensory receptors, and transcription factors. Notably, several hormone-like molecules were identified. Inter-EEC communication is exemplified by secretin-induced GLP-1 secretion. Indeed, individual EEC subtypes carry receptors for various EEC hormones. This study provides a rich resource to study human EEC development and function.

Single-Cell Analysis of Crohn's Disease Lesions Identifies a Pathogenic Cellular Module Associated with Resistance to Anti-TNF Therapy

Cell (IF: 38.62), 2019-9, Judy H Cho et al. DOI: 10.1016/j.cell.2019.08.008 PubMed: 31474370

Single Cell Gene Expression, Human, Cell Biology.

Abstract

Clinical benefits of cytokine blockade in ileal Crohn's disease (iCD) are limited to a subset of patients. Here, we applied single-cell technologies to iCD lesions to address whether cellular heterogeneity contributes to treatment resistance. We found that a subset of patients expressed a unique cellular module in inflamed tissues that consisted of IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells, which we named the GIMATS module. Analysis of ligand-receptor interaction pairs identified a distinct network connectivity that likely drives the GIMATS module. Strikingly, the GIMATS module was also present in a subset of patients in four independent iCD cohorts (n = 441), and its presence at diagnosis correlated with failure to achieve durable corticosteroid-free remission upon anti-TNF therapy. These results emphasize the limitations of current diagnostic assays and the potential for single-cell mapping tools to identify novel biomarkers of treatment response and tailored therapeutic opportunities.

Mapping Development of the Human Intestinal Niche at Single-Cell Resolution

Cell Stem Cell (IF: 23.45), 2020-11, J Gray Camp et al. DOI: 10.1016/j.stem.2020.11.008 PubMed: 33278341

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

The human intestinal stem cell niche supports self-renewal and epithelial function, but little is known about its development. We used single-cell mRNA sequencing with in situ validation approaches to interrogate human intestinal development from 7-21 weeks post conception, assigning molecular identities and spatial locations to cells and factors that comprise the niche. Smooth muscle cells of the muscularis mucosa, in close proximity to proliferative crypts, are a source of WNT and RSPONDIN ligands, whereas EGF is expressed far from crypts in the villus epithelium. Instead, an PDGFRA/F3/DLL1 mesenchymal population lines the crypt-villus axis and is the source of the epidermal growth factor (EGF) family member NEUREGULIN1 (NRG1). In developing intestine enteroid cultures, NRG1, but not EGF, permitted increased cellular diversity via differentiation of secretory lineages. This work highlights the complexities of intestinal EGF/ERBB signaling and delineates key niche cells and signals of the developing intestine.

Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition

Cell Stem Cell (IF: 23.45), 2018-12, Shinya SugimotoToshiro Sato DOI: 10.1016/j.stem.2018.11.016

PubMed: 30526881

Single Cell Gene Expression, Human, Assay Method, Developmental Biology.

Abstract

Cellular diversity that shapes tissue architecture and function is governed by multiple niche signals. Nonetheless, maintaining cellular diversity in human intestinal organoids has been challenging. Based on niche ligands present in the natural stem cell milieu, we establish a refined organoid culture condition for intestinal epithelia that allows human intestinal organoids to concurrently undergo multi-differentiation and self-renewal. High-throughput screening reveals that the combination of insulin-like growth factor 1 (IGF-1) and fibroblast growth factor 2 (FGF-2) enhances the clonogenic capacity and CRISPR-genome engineering efficiency of human intestinal stem cells. The combination equally enables long-term culture of a range of intestinal organoids, including rat small intestinal organoids. Droplet-based single-cell RNA sequencing further illustrates the conservation of the native cellular diversity in human small intestinal organoids cultured with the refined condition. The modified culture protocol outperforms the conventional method and offers a viable strategy for modeling human intestinal tissues and diseases in an in vivo relevant context.

Memory CD4⁺ T cells are generated in the human fetal intestine

Nature Immunology (IF: 22.3), 2019-1, Boudewijn P F LelieveldtFrits Koning DOI: 10.1038/s41590-018-0294-9

PubMed: 30664737

Single Cell Gene Expression, Human, Immunology.

Abstract

The fetus is thought to be protected from exposure to foreign antigens, yet CD45RO⁺ T cells reside in the fetal intestine. Here we combined functional assays with mass cytometry, single-cell RNA sequencing and high-throughput T cell antigen receptor (TCR) sequencing to characterize the CD4⁺ T cell compartment in the human fetal intestine. We identified 22 CD4⁺ T cell clusters, including naive-like, regulatory-like and memory-like subpopulations, which were confirmed and further characterized at the transcriptional level. Memory-like CD4⁺ T cells had high expression of Ki-67, indicative of cell division, and CD5, a surrogate marker of TCR avidity, and produced the cytokines IFN- γ and IL-2. Pathway analysis revealed a differentiation trajectory associated with cellular activation and proinflammatory effector functions, and TCR repertoire analysis indicated clonal expansions, distinct repertoire characteristics and interconnections between subpopulations of memory-like CD4⁺ T cells. Imaging mass cytometry indicated that memory-like CD4⁺ T cells colocalized with antigen-presenting cells. Collectively, these results provide evidence for the generation of memory-like CD4⁺ T cells in the human fetal intestine that is consistent with exposure to foreign antigens.

Single-cell transcriptome analysis reveals differential nutrient absorption functions in human intestine

Journal of experimental medicine (IF: 11.32), 2020-2, Xuerui Yang et al. DOI: 10.1084/jem.20191130 PubMed: 31753849

Single Cell Gene Expression, Human, Cell Atlas, Cell Biology.

Abstract

The intestine plays an important role in nutrient digestion and absorption, microbe defense, and hormone secretion. Although major cell types have been identified in the mouse intestinal epithelium, cell type-specific markers and functional assignments are largely unavailable for human intestine. Here, our single-cell RNA-seq analyses of 14,537 epithelial cells from human ileum, colon, and rectum reveal different nutrient absorption preferences in the small and large intestine, suggest the existence of Paneth-like cells in the large intestine, and identify potential new marker genes for human transient-amplifying cells and goblet cells. We have validated some of these insights by quantitative PCR, immunofluorescence, and functional analyses. Furthermore, we show both common and differential features of the cellular landscapes between the human and mouse ilea. Therefore, our data provide the basis for detailed characterization of human intestine cell constitution and functions, which would be helpful for a better understanding of human intestine disorders, such as inflammatory bowel disease and intestinal tumorigenesis.

Single-Cell Sequencing of Developing Human Gut Reveals Transcriptional Links to Childhood Crohn's Disease

Developmental Cell (IF: 10.13), 2020-12, Sarah A Teichmann et al. DOI: 10.1016/j.devcel.2020.11.010 PubMed: 33290721

Single Cell Gene Expression, Human, Developmental Biology, Immunology.

Abstract

Human gut development requires the orchestrated interaction of differentiating cell types. Here, we generate an in-depth single-cell map of the developing human intestine at 6–10 weeks post-conception. Our analysis reveals the transcriptional profile of cycling epithelial precursor cells; distinct from LGR5-expressing cells. We propose that these cells may contribute to differentiated cell subsets via the generation of LGR5-expressing stem cells and receive signals from surrounding mesenchymal cells. Furthermore, we draw parallels between the transcriptomes of ex vivo tissues and in vitro fetal organoids, revealing the maturation of organoid cultures in a dish. Lastly, we compare scRNA-seq profiles from pediatric Crohn's disease epithelium alongside matched healthy controls to reveal disease-associated changes in the epithelial composition. Contrasting these with the fetal profiles reveals the re-activation of fetal transcription factors in Crohn's disease. Our study provides a resource available at www.gutcellatlas.org, and underscores the importance of unraveling fetal development in understanding disease.

Single-cell transcriptome analysis reveals differential nutrient absorption functions in human intestine

Journal of experimental medicine (IF: 11.32), 2020-2, Xuerui Yang et al. DOI: 10.1084/jem.20191130 PubMed: 31753849

Single Cell Gene Expression, Human, Cell Atlas, Cell Biology.

Abstract

The intestine plays an important role in nutrient digestion and absorption, microbe defense, and hormone secretion. Although major cell types have been identified in the mouse intestinal epithelium, cell type-specific markers and functional assignments are largely unavailable for human intestine. Here, our single-cell RNA-seq analyses of 14,537 epithelial cells from human ileum, colon, and rectum reveal different nutrient absorption preferences in the small and large intestine, suggest the existence of Paneth-like cells in the large intestine, and identify potential new marker genes for human transient-amplifying cells and goblet cells. We have validated some of these insights by quantitative PCR, immunofluorescence, and functional analyses. Furthermore, we show both common and differential features of the cellular landscapes between the human and mouse ilea. Therefore, our data provide the basis for detailed characterization of human intestine cell constitution and functions, which would be helpful for a better understanding of human intestine disorders, such as inflammatory bowel disease and intestinal tumorigenesis.

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Activation of Hedgehog Signaling Promotes Development of Mouse and Human Enteric Neural Crest Cells, Based on Single-Cell Transcriptome Analyses

Gastroenterology (IF: 18.79), 2019-12, James M Wells et al. DOI: 10.1053/j.gastro.2019.08.019 PubMed: 31442438
Single Cell Gene Expression, Human & Mouse, Developmental Biology, Functional Genomics.

Abstract

It has been a challenge to develop fully functioning cells from human pluripotent stem cells (hPSCs). We investigated how activation of hedgehog signaling regulates derivation of enteric neural crest (NC) cells from hPSCs. We analyzed transcriptomes of mouse and hPSC-derived enteric NCs using single-cell RNA sequencing (scRNA-seq) to identify the changes in expression associated with lineage differentiation. Intestine tissues were collected from Tg(GBS-GFP), *Sufu*, *Ptch1*, and *Gli3* mice and analyzed by flow cytometry and immunofluorescence for levels of messenger RNAs encoding factors in the hedgehog signaling pathway during differentiation of enteric NCs. Human NC cells (HNK-1p75) were derived from IMR90 and UE02302 hPSC lines. hPSCs were incubated with a hedgehog agonist (smoothened agonist [SAG]) and antagonists (cyclopamine) and analyzed for differentiation. hPSC-based innervated colonic organoids were derived from these hPSC lines and analyzed by immunofluorescence and neuromuscular coupling assay for expression of neuronal subtype markers and assessment of the functional maturity of the hPSC-derived neurons, respectively.

Single-cell RNA sequencing analysis showed that neural fate acquisition by human and mouse enteric NC cells requires reduced expression of NC- and cell cycle-specific genes and up-regulation of neuronal or glial lineage-specific genes. Activation of the hedgehog pathway was associated with progression of mouse enteric NCs to the more mature state along the neuronal and glial lineage differentiation trajectories. Activation of the hedgehog pathway promoted development of cultured hPSCs into NCs of greater neurogenic potential by activating expression of genes in the neurogenic lineage. The hedgehog agonist increased differentiation of hPSCs into cells of the neuronal lineage by up-regulating expression of *GLI2* target genes, including *INSM1*, *NHLH1*, and various bHLH family members. The hedgehog agonist increased expression of late neuronal markers and neuronal activities in hPSC-derived neurons. In enteric NCs from humans and mice, activation of hedgehog signaling promotes differentiation into neurons by promoting cell-state transition, expression of genes in the neurogenic lineage, and functional maturity of enteric neurons.

Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell

Nature (IF: 46.49), 2019-4, Jeffrey L Wrana et al. DOI: 10.1038/s41586-019-1154 PubMed: 31019301

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

The turnover of the intestinal epithelium is driven by multipotent LGR5crypt-base columnar cells (CBCs) located at the bottom of crypt zones. However, CBCs are lost following injury, such as irradiation, but the intestinal epithelium is nevertheless able to recover. Thus, a second population of quiescent '+' cells, or reserve stem cells (RSCs), has previously been proposed to regenerate the damaged intestine. Although CBCs and RSCs were thought to be mutually exclusive, subsequent studies have found that LGR5CBCs express RSC markers and that RSCs were dispensable-whereas LGR5cells were essential-for repair of the damaged intestine. In addition, progenitors of absorptive enterocytes, secretory cells and slow cycling LGR5cellshave been shown to contribute to regeneration whereas the transcriptional regulator YAP1, which is important for intestinal regeneration, was suggested to induce a pro-survival phenotype in LGR5cells. Thus, whether cellular plasticity or distinct cell populations are critical for intestinal regeneration remains unknown. Here we applied single-cell RNA sequencing to profile the regenerating mouse intestine and identified a distinct, damage-induced quiescent cell type that we term the revival stem cell (revSC). revSCs are marked by high clusterin expression and are extremely rare under homeostatic conditions, yet give rise-in a temporal hierarchy-to all the major cell types of the intestine, including LGR5CBCs. After intestinal damage by irradiation, targeted ablation of LGR5CBCs, or treatment with dextran sodium sulfate, revSCs undergo a YAP1-dependent transient expansion, reconstitute the LGR5CBC compartment and are required to regenerate a functional intestine. These studies thus define a unique stem cell that is mobilized by damage to revive the homeostatic stem cell compartment and regenerate the intestinal epithelium.

Tracing the origin of adult intestinal stem cells

Nature (IF: 46.49), 2019-5, Benjamin D Simons et al. DOI: 10.1038/s41586-019-1212-5 PubMed: 31092921

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Adult intestinal stem cells are located at the bottom of crypts of Lieberkühn, where they express markers such as LGR5 and fuel the constant replenishment of the intestinal epithelium. Although fetal LGR5-expressing cells can give rise to adult intestinal stem cells, it remains unclear whether this population in the patterned epithelium represents unique intestinal stem-cell precursors. Here we show, using unbiased quantitative lineage-tracing approaches, biophysical modelling and intestinal transplantation, that all cells of the mouse intestinal epithelium-irrespective of their location and pattern of LGR5 expression in the fetal gut tube-contribute actively to the adult intestinal stem cell pool. Using 3D imaging, we find that during fetal development the villus undergoes gross remodelling and fission. This brings epithelial cells from the non-proliferative villus into the proliferative intervillus region, which enables them to contribute to the adult stem-cell niche. Our results demonstrate that large-scale remodelling of the intestinal wall and cell-fate specification are closely linked. Moreover, these findings provide a direct link between the observed plasticity and cellular reprogramming of differentiating cells in adult tissues following damage, revealing that stem-cell identity is an induced rather than a hardwired property.

BCR selection and affinity maturation in Peyer's patch germinal centres

Nature (IF: 46.49), 2020-6, Dan R Littman et al. DOI: 10.1038/s41586-020-2262-4 PubMed: 32499646

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

The antigen-binding variable regions of the B cell receptor (BCR) and of antibodies are encoded by exons that are assembled in developing B cells by V(D)J recombination. The BCR repertoires of primary B cells are vast owing to mechanisms that create diversity at the junctions of V(D)J gene segments that contribute to complementarity-determining region 3 (CDR3), the region that binds antigen. Primary B cells undergo antigen-driven BCR affinity maturation through somatic hypermutation and cellular selection in germinal centres (GCs). Although most GCs are transient, those in intestinal Peyer's patches (PPs)-which depend on the gut microbiota-are chronic, and little is known about their BCR repertoires or patterns of somatic hypermutation. Here, using a high-throughput assay that analyses both V(D)J segment usage and somatic hypermutation profiles, we elucidate physiological BCR repertoires in mouse PP GCs. PP GCs from different mice expand public BCR clonotypes (clonotypes that are shared between many mice) that often have canonical CDR3s in the immunoglobulin heavy chain that, owing to junctional biases during V(D)J recombination, appear much more frequently than predicted in naive B cell repertoires. Some public clonotypes are dependent on the gut microbiota and encode antibodies that are reactive to bacterial glycans, whereas others are independent of gut bacteria. Transfer of faeces from specific-pathogen-free mice to germ-free mice restored germ-dependent clonotypes, directly implicating BCR selection. We identified somatic hypermutations that were recurrently selected in such public clonotypes, indicating that affinity maturation occurs in mouse PP GCs under homeostatic conditions. Thus, persistent gut antigens select recurrent BCR clonotypes to seed chronic PP GC responses.

T Helper Cell Cytokines Modulate Intestinal Stem Cell Renewal and Differentiation

Cell (IF: 38.62), 2018-11, Aviv Regev et al. DOI: 10.1016/j.cell.2018.10.008 PubMed: 30392957

Single Cell Gene Expression, Mouse, Immunology.

Abstract

In the small intestine, a niche of accessory cell types supports the generation of mature epithelial cell types from intestinal stem cells (ISCs). It is unclear, however, if and how immune cells in the niche affect ISC fate or the balance between self-renewal and differentiation. Here, we use single-cell RNA sequencing (scRNA-seq) to identify MHC class II (MHCII) machinery enrichment in two subsets of Lgr5ISCs. We show that MHCII⁺Lgr5ISCs are non-conventional antigen-presenting cells in co-cultures with CD4⁺T helper (Th) cells. Stimulation of intestinal organoids with key Th cytokines affects Lgr5ISC renewal and differentiation in opposing ways: pro-inflammatory signals promote differentiation, while regulatory cells and cytokines reduce it. In vivo genetic perturbation of Th cells or MHCII expression on Lgr5ISCs impacts epithelial cell differentiation and IEC fate during infection. These interactions between Th cells and Lgr5ISCs, thus, orchestrate tissue-wide responses to external signals.

Activity of caspase-8 determines plasticity between cell death pathways

Nature (IF: 46.49), 2019-11, Joshua D Webster et al. DOI: 10.1038/s41586-019-1752-8 PubMed: 31723262

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Caspase-8 is a protease with both pro-death and pro-survival functions: it mediates apoptosis induced by death receptors such as TNFR1, and suppresses necroptosis mediated by the kinase RIPK3 and the pseudokinase MLKL. Mice that lack caspase-8 display MLKL-dependent embryonic lethality, as do mice that express catalytically inactive CASP8(C362A). Casp8Mklmice die during the perinatal period, whereas Casp8Mklmice are viable, which indicates that inactive caspase-8 also has a pro-death scaffolding function. Here we show that mutant CASP8(C362A) induces the formation of ASC (also known as PYCARD) specks, and caspase-1-dependent cleavage of GSDMD and caspases 3 and 7 in MLKL-deficient mouse intestines around embryonic day 18. Caspase-1 and its adaptor ASC contributed to the perinatal lethal phenotype because a number of Casp8MklCasp1 and Casp8MklAsc mice survived beyond weaning. Transfection studies suggest that inactive caspase-8 adopts a distinct conformation to active caspase-8, enabling its prodomain to engage ASC. Upregulation of the lipopolysaccharide sensor caspase-11 in the intestines of both Casp8Mkl and Casp8MklCasp1 mice also contributed to lethality because Casp8MklCasp1Casp11 (Casp11 is also known as Casp4) neonates survived more often than Casp8MklCasp1 neonates. Finally, Casp8Ripk3Casp1Casp11 mice survived longer than Casp8MklCasp1Casp11 mice, indicating that a necroptosis-independent function of RIPK3 also contributes to lethality. Thus, unanticipated plasticity in death pathways is revealed when caspase-8-dependent apoptosis and MLKL-dependent necroptosis are inhibited.

GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells

Nature (IF: 46.49), 2018-6, George Hausmann et al. DOI: 10.1038/s41586-018-0190-3 PubMed: 29875413

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Wnt- β -catenin signalling plays a pivotal role in the homeostasis of the intestinal epithelium by promoting stem cell renewal. In the small intestine, epithelial Paneth cells secrete Wnt ligands and thus adopt the function of the stem cell niche to maintain epithelial homeostasis. It is unclear which cells comprise the stem cell niche in the colon. Here we show that subepithelial mesenchymal GLI1-expressing cells form this essential niche. Blocking Wnt secretion from GLI1-expressing cells prevents colonic stem cell renewal in mice: the stem cells are lost and, as a consequence, the integrity of the colonic epithelium is corrupted, leading to death. GLI1-expressing cells also play an important role in the maintenance of the small intestine, where they serve as a reserve Wnt source that becomes critical when Wnt secretion from epithelial cells is prevented. Our data suggest a mechanism by which the stem cell niche is adjusted to meet the needs of the intestine via adaptive changes in the number of mesenchymal GLI1-expressing cells.

Self-organization and symmetry breaking in intestinal organoid development

Nature (IF: 46.49), 2019-4, Guglielmo Roma et al. DOI: 10.1038/s41586-019-1146 PubMed: 31019299

Single Cell Gene Expression, Mouse, Developmental Biology, Functional Genomics.

Abstract

Intestinal organoids are complex three-dimensional structures that mimic the cell-type composition and tissue organization of the intestine by recapitulating the self-organizing ability of cell populations derived from a single intestinal stem cell. Crucial in this process is a first symmetry-breaking event, in which only a fraction of identical cells in a symmetrical sphere differentiate into Paneth cells, which generate the stem-cell niche and lead to asymmetric structures such as the crypts and villi. Here we combine single-cell quantitative genomic and imaging approaches to characterize the development of intestinal organoids from single cells. We show that their development follows a regeneration process that is driven by transient activation of the transcriptional regulator YAP1. Cell-to-cell variability in YAP1, emerging in symmetrical spheres, initiates Notch and DLL1 activation, and drives the symmetry-breaking event and formation of the first Paneth cell. Our findings reveal how single cells exposed to a uniform growth-promoting environment have the intrinsic ability to generate emergent, self-organized behaviour that results in the formation of complex multicellular asymmetric structures.

A single-cell survey of the small intestinal epithelium

Nature (IF: 46.49), 2017-11, Ramnik J Xavier et al. DOI: 10.1038/nature24489 PubMed: 29144463

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Intestinal epithelial cells absorb nutrients, respond to microbes, function as a barrier and help to coordinate immune responses. Here we report profiling of 53,193 individual epithelial cells from the small intestine and organoids of mice, which enabled the identification and characterization of previously unknown subtypes of intestinal epithelial cell and their gene signatures. We found unexpected diversity in hormone-secreting enteroendocrine cells and constructed the taxonomy of newly identified subtypes, and distinguished between two subtypes of tuft cell, one of which expresses the epithelial cytokine Tslp and the pan-immune marker CD45, which was not previously associated with non-haematopoietic cells. We also characterized the ways in which cell-intrinsic states and the proportions of different cell types respond to bacterial and helminth infections: Salmonella infection caused an increase in the abundance of Paneth cells and enterocytes, and broad activation of an antimicrobial program; Heligmosomoides polygyrus caused an increase in the abundance of goblet and tuft cells. Our survey highlights previously unidentified markers and programs, associates sensory molecules with cell types, and uncovers principles of gut homeostasis and response to pathogens.

Ketone Body Signaling Mediates Intestinal Stem Cell Homeostasis and Adaptation to Diet

Cell (IF: 38.62), 2019-8, Aviv Regev et al. DOI: 10.1016/j.cell.2019.07.048 PubMed: 31442404

Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

Little is known about how metabolites couple tissue-specific stem cell function with physiology. Here we show that, in the mammalian small intestine, the expression of Hmgcs2 (3-hydroxy-3-methylglutaryl-CoA synthetase 2), the gene encoding the rate-limiting enzyme in the production of ketone bodies, including beta-hydroxybutyrate (β OHB), distinguishes self-renewing Lgr5stem cells (ISCs) from differentiated cell types. Hmgcs2 loss depletes β OHB levels in Lgr5ISCs and skews their differentiation toward secretory cell fates, which can be rescued by exogenous β OHB and class I histone deacetylase (HDAC) inhibitor treatment. Mechanistically, β OHB acts by inhibiting HDACs to reinforce Notch signaling, instructing ISC self-renewal and lineage decisions. Notably, although a high-fat ketogenic diet elevates ISC function and post-injury regeneration through β OHB-mediated Notch signaling, a glucose-supplemented diet has the opposite effects. These findings reveal how control of β OHB-activated signaling in ISCs by diet helps to fine-tune stem cell adaptation in homeostasis and injury.

A reinforcing HNF4–SMAD4 feed-forward module stabilizes enterocyte identity

Nature Genetics (IF: 30.33), 2019-4, Ansu O Perekatt et al. DOI: 10.1038/s41588-019-0384-0 PubMed: 30988513

Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

BMP/SMAD signaling is a crucial regulator of intestinal differentiation. However, the molecular underpinnings of the BMP pathway in this context are unknown. Here, we characterize the mechanism by which BMP/SMAD signaling drives enterocyte differentiation. We establish that the transcription factor HNF4A acts redundantly with an intestine-restricted HNF4 paralog, HNF4G, to activate enhancer chromatin and upregulate the majority of transcripts enriched in the differentiated epithelium; cells fail to differentiate on double knockout of both HNF4 paralogs. Furthermore, we show that SMAD4 and HNF4 function via a reinforcing feed-forward loop, activating each other's expression and co-binding to regulatory elements of differentiation genes. This feed-forward regulatory module promotes and stabilizes enterocyte cell identity; disruption of the HNF4-SMAD4 module results in loss of enterocyte fate in favor of progenitor and secretory cell lineages. This intersection of signaling and transcriptional control provides a framework to understand regenerative tissue homeostasis, particularly in tissues with inherent cellular plasticity.

T Cell Receptor Is Required for Differentiation, but Not Maintenance, of Intestinal CD4⁺ Intraepithelial Lymphocytes

Immunity (IF: 25.73), 2020-11, Gabriel D Victora et al. DOI: 10.1016/j.immuni.2020.09.003 PubMed: 33022229

Single Cell Immune Profiling, Mouse, Immunology.

Abstract

The gut epithelium is populated by intraepithelial lymphocytes (IELs), a heterogeneous T cell population with cytotoxic and regulatory properties, which can be acquired at the epithelial layer. However, the role of T cell receptor (TCR) in this process remains unclear. Single-cell transcriptomic analyses revealed distinct clonal expansions between cell states, with CD4CD8 $\alpha\alpha$ IELs being one of the least diverse populations. Conditional deletion of TCR on differentiating CD4T cells or of major histocompatibility complex (MHC) class II on intestinal epithelial cells prevented CD4CD8 $\alpha\alpha$ IEL differentiation. However, TCR ablation on differentiated CD4CD8 $\alpha\alpha$ IELs or long-term cognate antigen withdraw did not affect their maintenance. TCR re-engagement of antigen-specific CD4CD8 $\alpha\alpha$ IELs by *Listeria monocytogenes* did not alter their state but correlated with reduced bacterial invasion. Thus, local antigen recognition is an essential signal for differentiation of CD4T cells at the epithelium, yet differentiated IELs are able to preserve an effector program in the absence of TCR signaling.

Paneth Cell-Derived Lysozyme Defines the Composition of Mucolytic Microbiota and the Inflammatory Tone of the Intestine

Immunity (IF: 25.73), 2020-8, Pawel R Kiela et al. DOI: 10.1016/j.immuni.2020.07.010 PubMed: 32814028

Single Cell Gene Expression, Mouse, Cell Biology, Microbiology.

Abstract

Paneth cells are the primary source of C-type lysozyme, a β -1,4-N-acetylmuramoylhydrolase that enzymatically processes bacterial cell walls. Paneth cells are normally present in human cecum and ascending colon, but are rarely found in descending colon and rectum; Paneth cell metaplasia in this region and aberrant lysozyme production are hallmarks of inflammatory bowel disease (IBD) pathology. Here, we examined the impact of aberrant lysozyme production in colonic inflammation. Targeted disruption of Paneth cell lysozyme (*Lyz1*) protected mice from experimental colitis. *Lyz1*-deficiency diminished intestinal immune responses to bacterial molecular patterns and resulted in the expansion of lysozyme-sensitive mucolytic bacteria, including *Ruminococcus gnavus*, a Crohn's disease-associated pathobiont. Ectopic lysozyme production in colonic epithelium suppressed lysozyme-sensitive bacteria and exacerbated colitis. Transfer of *R. gnavus* into *Lyz1* hosts elicited a type 2 immune response, causing epithelial reprogramming and enhanced anti-colitogenic capacity. In contrast, in lysozyme-intact hosts, processed *R. gnavus* drove pro-inflammatory responses. Thus, Paneth cell lysozyme balances intestinal anti- and pro-inflammatory responses, with implications for IBD.

Heterogenous Populations of Tissue-Resident CD8+ T Cells Are Generated in Response to Infection and Malignancy

Immunity (IF: 25.73), 2020-5, Kyla D Omilusik et al. DOI: 10.1016/j.immuni.2020.04.007 PubMed: 32433949

Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

Tissue-resident memory CD8T cells (Trm) provide host protection through continuous surveillance of non-lymphoid tissues. Using single-cell RNA-sequencing (scRNA-seq) and genetic reporter mice, we identified discrete lineages of intestinal antigen-specific CD8T cells, including a Blimp1^{hi}Id3^{hi}tissue-resident effector cell population most prominent in the early phase of acute viral and bacterial infections and a molecularly distinct Blimp1^{hi}Id3^{hi}tissue-resident memory population that subsequently accumulated at later infection time points. These Trm populations exhibited distinct cytokine production, secondary memory potential, and transcriptional programs including differential roles for transcriptional regulators Blimp1, T-bet, Id2, and Id3 in supporting and maintaining intestinal Trm. Extending our analysis to malignant tissue, we also identified discrete populations of effector-like and memory-like CD8T cell populations with tissue-resident gene-expression signatures that shared features of terminally exhausted and progenitor-exhausted T cells, respectively. Our findings provide insight into the development and functional heterogeneity of Trm cells, which has implications for enhancing vaccination and immunotherapy approaches.

Transcriptional Atlas of Intestinal Immune Cells Reveals that Neuropeptide α -CGRP Modulates Group 2 Innate Lymphoid Cell Responses

Immunity (IF: 25.73), 2019-10, Aviv Regev et al. DOI: 10.1016/j.immuni.2019.09.004 PubMed: 31618654

Single Cell Gene Expression, Mouse, Cell Atlas, Immunology.

Abstract

Signaling abnormalities in immune responses in the small intestine can trigger chronic type 2 inflammation involving interaction of multiple immune cell types. To systematically characterize this response, we analyzed 58,067 immune cells from the mouse small intestine by single-cell RNA sequencing (scRNA-seq) at steady state and after induction of a type 2 inflammatory reaction to ovalbumin (OVA). Computational analysis revealed broad shifts in both cell-type composition and cell programs in response to the inflammation, especially in group 2 innate lymphoid cells (ILC2s). Inflammation induced the expression of exon 5 of Calca, which encodes the alpha-calcitonin gene-related peptide (α -CGRP), in intestinal KLRG1^{hi}ILC2s. α -CGRP antagonized KLRG1^{hi}ILC2s proliferation but promoted IL-5 expression. Genetic perturbation of α -CGRP increased the proportion of intestinal KLRG1^{hi}ILC2s. Our work highlights a model where α -CGRP-mediated neuronal signaling is critical for suppressing ILC2 expansion and maintaining homeostasis of the type 2 immune machinery.

Checkpoint Blockade Immunotherapy Induces Dynamic Changes in PD-1–CD8+ Tumor-Infiltrating T Cells

Immunity (IF: 25.73), 2019-1, Aviv Regev et al. DOI: 10.1016/j.immuni.2018.11.014 PubMed: 30635236

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

An improved understanding of the anti-tumor CD8T cell response after checkpoint blockade would enable more informed and effective therapeutic strategies. Here we examined the dynamics of the effector response of CD8tumor-infiltrating lymphocytes (TILs) after checkpoint blockade therapy. Bulk and single-cell RNA profiles of CD8TILs after combined Tim-3+PD-1 blockade in preclinical models revealed significant changes in the transcriptional profile of PD-1TILs. These cells could be divided into subsets bearing characteristics of naive-, effector-, and memory-precursor-like cells. Effector- and memory-precursor-like TILs contained tumor-antigen-specific cells, exhibited proliferative and effector capacity, and expanded in response to different checkpoint blockade therapies across different tumor models. The memory-precursor-like subset shared features with CD8T cells associated with response to checkpoint blockade in patients and was compromised in the absence of Tcf7. Expression of Tcf7/Tcf1 was requisite for the efficacy of diverse immunotherapies, highlighting the importance of this transcriptional regulator in the development of effective CD8T cell responses upon immunotherapy.

Distinct Mesenchymal Cell Populations Generate the Essential Intestinal BMP Signaling Gradient

Cell Stem Cell (IF: 23.45), 2020-3, Shannon J Turley et al. DOI: 10.1016/j.stem.2020.01.008 PubMed: 32084389

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Intestinal stem cells (ISCs) are confined to crypt bottoms and their progeny differentiate near crypt-villus junctions. Wnt and bone morphogenic protein (BMP) gradients drive this polarity, and colorectal cancer fundamentally reflects disruption of this homeostatic signaling. However, sub-epithelial sources of crucial agonists and antagonists that organize this BMP gradient remain obscure. Here, we couple whole-mount high-resolution microscopy with ensemble and single-cell RNA sequencing (RNA-seq) to identify three distinct PDGFR^{hi} mesenchymal cell types. PDGFR^{hi} telocytes are especially abundant at the villus base and provide a BMP reservoir, and we identified a CD81PDGFR^{lo} population present just below crypts that secretes the BMP antagonist Gremlin1. These cells, referred to as trophocytes, are sufficient to expand ISCs in vitro without additional trophic support and contribute to ISC maintenance in vivo. This study reveals intestinal mesenchymal structure at fine anatomic, molecular, and functional detail and the cellular basis for a signaling gradient necessary for tissue self-renewal.

Astrovirus infects actively secreting goblet cells and alters the gut mucus barrier

Nature Communications (IF: 13.61), 2020-4, Paul G Thomas et al. DOI: 10.1038/s41467-020-15999

PubMed: 32350281

Single Cell Immune Profiling, Mouse, Infectious Disease, Microbiology.

Abstract

Astroviruses are a global cause of pediatric diarrhea, but they are largely understudied, and it is unclear how and where they replicate in the gut. Using an in vivo model, here we report that murine astrovirus preferentially infects actively secreting small intestinal goblet cells, specialized epithelial cells that maintain the mucus barrier. Consequently, virus infection alters mucus production, leading to an increase in mucus-associated bacteria and resistance to enteropathogenic *E. coli* colonization. These studies establish the main target cell type and region of the gut for productive murine astrovirus infection. They further define a mechanism by which an enteric virus can regulate the mucus barrier, induce functional changes to commensal microbial communities, and alter host susceptibility to pathogenic bacteria.

Combinatorial Transcriptional Profiling of Mouse and Human Enteric Neurons Identifies Shared and Disparate Subtypes In Situ

Gastroenterology (IF: 18.79), 2020-9, Ken S Lau et al. DOI: 10.1053/j.gastro.2020.09.032 PubMed: 33010250

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

The enteric nervous system (ENS) coordinates essential intestinal functions through the concerted action of diverse enteric neurons (ENs). However, integrated molecular knowledge of EN subtypes is lacking. To compare human and mouse ENs, we transcriptionally profiled healthy ENS from adult humans and mice. We aimed to identify transcripts marking discrete neuron subtypes and visualize conserved EN subtypes for humans and mice in multiple bowel regions. Human myenteric ganglia and adjacent smooth muscle were isolated by laser-capture microdissection for RNA sequencing. Ganglia-specific transcriptional profiles were identified by computationally subtracting muscle gene signatures. Nuclei from mouse myenteric neurons were isolated and subjected to single-nucleus RNA sequencing, totaling more than 4 billion reads and 25,208 neurons. Neuronal subtypes were defined using mouse single-nucleus RNA sequencing data. Comparative informatics between human and mouse data sets identified shared EN subtype markers, which were visualized in situ using hybridization chain reaction. Several EN subtypes in the duodenum, ileum, and colon are conserved between humans and mice based on orthologous gene expression. However, some EN subtype-specific genes from mice are expressed in completely distinct morphologically defined subtypes in humans. In mice, we identified several neuronal subtypes that stably express gene modules across all intestinal segments, with graded, regional expression of 1 or more marker genes. Our combined transcriptional profiling of human myenteric ganglia and mouse EN provides a rich foundation for developing novel intestinal therapeutics. There is congruency among some EN subtypes, but we note multiple species differences that should be carefully considered when relating findings from mouse ENS research to human gastrointestinal studies.

Replicational Dilution of H3K27me3 in Mammalian Cells and the Role of Poised Promoters

Molecular Cell (IF: 16.13), 2020-4, Megan Barefoot et al. DOI: 10.1016/j.molcel.2020.01.017 PubMed: 32027840
Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

Polycomb repressive complex 2 (PRC2) places H3K27me3 at developmental genes and is causally implicated in keeping bivalent genes silent. It is unclear if that silence requires minimum H3K27me3 levels and how the mark transmits faithfully across mammalian somatic cell generations. Mouse intestinal cells lacking EZH2 methyltransferase reduce H3K27me3 proportionately at all PRC2 target sites, but ~40% uniform residual levels keep target genes inactive. These genes, derepressed in PRC2-null villus cells, remain silent in intestinal stem cells (ISCs). Quantitative chromatin immunoprecipitation and computational modeling indicate that because unmodified histones dilute H3K27me3 by 50% each time DNA replicates, PRC2-deficient ISCs initially retain sufficient H3K27me3 to avoid gene derepression. EZH2 mutant human lymphoma cells also require multiple divisions before H3K27me3 dilution relieves gene silencing. In both cell types, promoters with high basal H3K4me2/3 activate in spite of some residual H3K27me3, compared to less-poised promoters. These findings have implications for PRC2 inhibition in cancer therapy.

TNFAIP8 controls murine intestinal stem cell homeostasis and regeneration by regulating microbiome-induced Akt signaling

Nature Communications (IF: 13.61), 2020-5, Michael V Gonzalez et al. DOI: 10.1038/s41467-020-16379-2 PubMed: 32444641

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The intestine is a highly dynamic environment that requires tight control of the various inputs to maintain homeostasis and allow for proper responses to injury. It was recently found that the stem cell niche and epithelium is regenerated after injury by de-differentiated adult cells, through a process that gives rise to Sca1+ fetal-like cells and is driven by a transient population of Clurevival stem cells (revSCs). However, the molecular mechanisms that regulate this dynamic process have not been fully defined. Here we show that TNFAIP8 (also known as TIPE0) is a regulator of intestinal homeostasis that is vital for proper regeneration. TIPE0 functions through inhibiting basal Akt activation by the commensal microbiota via modulating membrane phospholipid abundance. Loss of TIPE0 in mice results in injury-resistant enterocytes, that are hyperproliferative, yet have regenerative deficits and are shifted towards a de-differentiated state. Tipe0enterocytes show basal induction of the Cluregenerative program and a fetal gene expression signature marked by Sca1, but upon injury are unable to generate Sca1-/ClurevSCs and could not regenerate the epithelium. This work demonstrates the role of TIPE0 in regulating the dynamic signaling that determines the injury response and enables intestinal epithelial cell regenerative plasticity.

Early precursors and molecular determinants of tissue-resident memory CD8⁺ T lymphocytes revealed by single-cell RNA sequencing

Science Immunology (IF: 13.41), 2020-5, Gene W Yeo et al. DOI: 10.1126/sciimmunol.aaz6894 PubMed: 32414833
Single Cell Gene Expression, Mouse, Immunology, Infectious Disease.

Abstract

During an immune response to microbial infection, CD8T cells give rise to distinct classes of cellular progeny that coordinately mediate clearance of the pathogen and provide long-lasting protection against reinfection, including a subset of noncirculating tissue-resident memory (T) cells that mediate potent protection within nonlymphoid tissues. Here, we used single-cell RNA sequencing to examine the gene expression patterns of individual CD8T cells in the spleen and small intestine intraepithelial lymphocyte (siEL) compartment throughout the course of their differentiation in response to viral infection. These analyses revealed previously unknown transcriptional heterogeneity within the siEL CD8T cell population at several stages of differentiation, representing functionally distinct Tcell subsets and a subset of Tcell precursors within the tissue early in infection. Together, these findings may inform strategies to optimize CD8T cell responses to protect against microbial infection and cancer.

Distinct fibroblast subsets regulate lacteal integrity through YAP/TAZ-induced VEGF-C in intestinal villi

Nature Communications (IF: 13.61), 2020-8, Daesik Lim et al. DOI: 10.1038/s41467-020-17886 PubMed: 32796823

Single Cell Gene Expression, Mouse, Cell Atlas.

Abstract

Emerging evidence suggests that intestinal stromal cells (IntSCs) play essential roles in maintaining intestinal homeostasis. However, the extent of heterogeneity within the villi stromal compartment and how IntSCs regulate the structure and function of specialized intestinal lymphatic capillary called lacteal remain elusive. Here we show that selective hyperactivation or depletion of YAP/TAZ in PDGFR β IntSCs leads to lacteal sprouting or regression with junctional disintegration and impaired dietary fat uptake. Indeed, mechanical or osmotic stress regulates IntSC secretion of VEGF-C mediated by YAP/TAZ. Single-cell RNA sequencing delineated novel subtypes of villi fibroblasts that upregulate Vegfc upon YAP/TAZ activation. These populations of fibroblasts were distributed in proximity to lacteal, suggesting that they constitute a peri-lacteal microenvironment. Our findings demonstrate the heterogeneity of IntSCs and reveal that distinct subsets of villi fibroblasts regulate lacteal integrity through YAP/TAZ-induced VEGF-C secretion, providing new insights into the dynamic regulatory mechanisms behind lymphangiogenesis and lymphatic remodeling.

TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes

Science Immunology (IF: 13.41), 2020-5, Sean P J Whelan et al. DOI: 10.1126/sciimmunol.abc3582

PubMed: 32404436

Single Cell Gene Expression, Mouse, Infectious Disease.

Abstract

Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA are frequently observed in COVID-19 patients. However, it is unclear whether SARS-CoV-2 replicates in the human intestine and contributes to possible fecal-oral transmission. Here, we report productive infection of SARS-CoV-2 in ACE2⁺ mature enterocytes in human small intestinal enteroids. Expression of two mucosa-specific serine proteases, TMPRSS2 and TMPRSS4, facilitated SARS-CoV-2 spike fusogenic activity and promoted virus entry into host cells. We also demonstrate that viruses released into the intestinal lumen were inactivated by simulated human colonic fluid, and infectious virus was not recovered from the stool specimens of COVID-19 patients. Our results highlight the intestine as a potential site of SARS-CoV-2 replication, which may contribute to local and systemic illness and overall disease progression.

p53 drives a transcriptional program that elicits a non-cell-autonomous response and alters cell state in vivo

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-9, Xiaoping Su et al. DOI: 10.1073/pnas.2008474117 PubMed: 32900967

Single Cell Gene Expression, Mouse, Cell Biology, Genetic Health.

Abstract

Cell stress and DNA damage activate the tumor suppressor p53, triggering transcriptional activation of a myriad of target genes. The molecular, morphological, and physiological consequences of this activation remain poorly understood in vivo. We activated a p53 transcriptional program in mice by deletion of, a gene that encodes the major p53 inhibitor. By overlaying tissue-specific RNA-sequencing data from pancreas, small intestine, ovary, kidney, and heart with existing p53 chromatin immunoprecipitation (ChIP) sequencing, we identified a large repertoire of tissue-specific p53 genes and a common p53 transcriptional signature of seven genes, which included but not Global p53 activation caused a metaplastic phenotype in the pancreas that was missing in mice with acinar-specific p53 activation, suggesting non-cell-autonomous effects. The p53 cellular response at single-cell resolution in the intestine altered transcriptional cell state, leading to a proximal enterocyte population enriched for genes within oxidative phosphorylation pathways. In addition, a population of active CD8⁺ T cells was recruited. Combined, this study provides a comprehensive profile of the p53 transcriptional response in vivo, revealing both tissue-specific transcriptomes and a unique signature, which were integrated to induce both cell-autonomous and non-cell-autonomous responses and transcriptional plasticity.

Stromal SOX2 Upregulation Promotes Tumorigenesis through the Generation of a SFRP1/2-Expressing Cancer-Associated Fibroblast Population

Developmental Cell (IF: 10.13), 2020-11, Maria T Diaz-Meco et al. DOI: 10.1016/j.devcel.2020.10.014

PubMed: 33207226

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Cancer-associated fibroblasts (CAFs) promote tumor malignancy, but the precise transcriptional mechanisms regulating the acquisition of the CAF phenotype are not well understood. We show that the upregulation of SOX2 is central to this process, which is repressed by protein kinase C ζ (PKC ζ). PKC ζ deficiency activates the reprogramming of colonic fibroblasts to generate a predominant SOX2-dependent CAF population expressing the WNT regulator *Sfrp2* as its top biomarker. SOX2 directly binds the *Sfrp1/2* promoters, and the inactivation of Sox2 or *Sfrp1/2* in CAFs impaired the induction of migration and invasion of colon cancer cells, as well as their tumorigenicity in vivo. Importantly, recurrence-free and overall survival of colorectal cancer (CRC) patients negatively correlates with stromal PKC ζ levels. Also, SOX2 expression in the stroma is associated with CRC T invasion and worse prognosis of recurrence-free survival. Therefore, the PKC ζ -SOX2 axis emerges as a critical step in the control of CAF pro-tumorigenic potential.

Helios is a key transcriptional regulator of outer hair cell maturation

Nature (IF: 46.49), 2018-11, Michael R Bowl et al. DOI: 10.1038/s41586-018-0728-4 PubMed: 30464345

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

The sensory cells that are responsible for hearing include the cochlear inner hair cells (IHCs) and outer hair cells (OHCs), with the OHCs being necessary for sound sensitivity and tuning. Both cell types are thought to arise from common progenitors; however, our understanding of the factors that control the fate of IHCs and OHCs remains limited. Here we identify *Ikzf2* (which encodes Helios) as an essential transcription factor in mice that is required for OHC functional maturation and hearing. Helios is expressed in postnatal mouse OHCs, and in the cello mouse model a point mutation in *Ikzf2* causes early-onset sensorineural hearing loss. *Ikzf2*OHCs have greatly reduced prestin-dependent electromotile activity, a hallmark of OHC functional maturation, and show reduced levels of crucial OHC-expressed genes such as *Slc26a5* (which encodes prestin) and *Ocm*. Moreover, we show that ectopic expression of *Ikzf2* in IHCs: induces the expression of OHC-specific genes; reduces the expression of canonical IHC genes; and confers electromotility to IHCs, demonstrating that *Ikzf2* can partially shift the IHC transcriptome towards an OHC-like identity.

Hair Cell Mechanotransduction Regulates Spontaneous Activity and Spiral Ganglion Subtype Specification in the Auditory System

Cell (IF: 38.62), 2018-8, Joseph C Burns et al. DOI: 10.1016/j.cell.2018.07.008 PubMed: 30078710

Single Cell Gene Expression, Mouse, Neuroscience.

Abstract

Type I spiral ganglion neurons (SGNs) transmit sound information from cochlear hair cells to the CNS. Using transcriptome analysis of thousands of single neurons, we demonstrate that murine type I SGNs consist of subclasses that are defined by the expression of subsets of transcription factors, cell adhesion molecules, ion channels, and neurotransmitter receptors. Subtype specification is initiated prior to the onset of hearing during the time period when auditory circuits mature. Gene mutations linked to deafness that disrupt hair cell mechanotransduction or glutamatergic signaling perturb the firing behavior of SGNs prior to hearing onset and disrupt SGN subtype specification. We thus conclude that an intact hair cell mechanotransduction machinery is critical during the pre-hearing period to regulate the firing behavior of SGNs and their segregation into subtypes. Because deafness is frequently caused by defects in hair cells, our findings have significant ramifications for the etiology of hearing loss and its treatment.

Characterization of the development of the mouse cochlear epithelium at the single cell level

Nature Communications (IF: 13.61), 2020-5, Elizabeth C Driver et al. DOI: 10.1038/s41467-020-16113

PubMed: 32404924

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Mammalian hearing requires the development of the organ of Corti, a sensory epithelium comprising unique cell types. The limited number of each of these cell types, combined with their close proximity, has prevented characterization of individual cell types and/or their developmental progression. To examine cochlear development more closely, we transcriptionally profile approximately 30,000 isolated mouse cochlear cells collected at four developmental time points. Here we report on the analysis of those cells including the identification of both known and unknown cell types. Trajectory analysis for OHCs indicates four phases of gene expression while fate mapping of progenitor cells suggests that OHCs and their surrounding supporting cells arise from a distinct (lateral) progenitor pool. *Tgfr1* is identified as being expressed in lateral progenitor cells and a *Tgfr1* antagonist inhibits OHC development. These results provide insights regarding cochlear development and demonstrate the potential value and application of this data set.

Heterogeneity in old fibroblasts is linked to variability in reprogramming and wound healing

Nature (IF: 46.49), 2019-10, Michael P Snyder et al. DOI: 10.1038/s41586-019-1658-5 PubMed: 31645721

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Age-associated chronic inflammation (inflammageing) is a central hallmark of ageing, but its influence on specific cells remains largely unknown. Fibroblasts are present in most tissues and contribute to wound healing. They are also the most widely used cell type for reprogramming to induced pluripotent stem (iPS) cells, a process that has implications for regenerative medicine and rejuvenation strategies. Here we show that fibroblast cultures from old mice secrete inflammatory cytokines and exhibit increased variability in the efficiency of iPS cell reprogramming between mice. Variability between individuals is emerging as a feature of old age, but the underlying mechanisms remain unknown. To identify drivers of this variability, we performed multi-omics profiling of fibroblast cultures from young and old mice that have different reprogramming efficiencies. This approach revealed that fibroblast cultures from old mice contain 'activated fibroblasts' that secrete inflammatory cytokines, and that the proportion of activated fibroblasts in a culture correlates with the reprogramming efficiency of that culture. Experiments in which conditioned medium was swapped between cultures showed that extrinsic factors secreted by activated fibroblasts underlie part of the variability between mice in reprogramming efficiency, and we have identified inflammatory cytokines, including TNF, as key contributors. Notably, old mice also exhibited variability in wound healing rate in vivo. Single-cell RNA-sequencing analysis identified distinct subpopulations of fibroblasts with different cytokine expression and signalling in the wounds of old mice with slow versus fast healing rates. Hence, a shift in fibroblast composition, and the ratio of inflammatory cytokines that they secrete, may drive the variability between mice in reprogramming in vitro and influence wound healing rate in vivo. This variability may reflect distinct stochastic ageing trajectories between individuals, and could help in developing personalized strategies to improve iPS cell generation and wound healing in elderly individuals.

On the design of CRISPR-based single-cell molecular screens

Nature Methods (IF: 36.15), 2018-4, Jay Shendure Cole Trapnell DOI: 10.1038/nmeth.4604 PubMed: 29457792

Single Cell Gene Expression, Human, Assay Method.

Abstract

Several groups recently coupled CRISPR perturbations and single-cell RNA-seq for pooled genetic screens. We demonstrate that vector designs of these studies are susceptible to ~50% swapping of guide RNA-barcode associations because of lentiviral template switching. We optimized a published alternative, CROP-seq, in which the guide RNA also serves as the barcode, and here confirm that this strategy performs robustly and doubled the rate at which guides are assigned to cells to 94%.

Genetic and transcriptional evolution alters cancer cell line drug response

Nature (IF: 46.49), 2018-8, Rameen Beroukhi, Todd R Golub DOI: 10.1038/s41586-018-0409-3

PubMed: 30089904

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Human cancer cell lines are the workhorse of cancer research. Although cell lines are known to evolve in culture, the extent of the resultant genetic and transcriptional heterogeneity and its functional consequences remain understudied. Here we use genomic analyses of 106 human cell lines grown in two laboratories to show extensive clonal diversity. Further comprehensive genomic characterization of 27 strains of the common breast cancer cell line MCF7 uncovered rapid genetic diversification. Similar results were obtained with multiple strains of 13 additional cell lines. Notably, genetic changes were associated with differential activation of gene expression programs and marked differences in cell morphology and proliferation. Barcoding experiments showed that cell line evolution occurs as a result of positive clonal selection that is highly sensitive to culture conditions. Analyses of single-cell-derived clones demonstrated that continuous instability quickly translates into heterogeneity of the cell line. When the 27 MCF7 strains were tested against 321 anti-cancer compounds, we uncovered considerably different drug responses: at least 75% of compounds that strongly inhibited some strains were completely inactive in others. This study documents the extent, origins and consequences of genetic variation within cell lines, and provides a framework for researchers to measure such variation in efforts to support maximally reproducible cancer research.

Chromosomal instability drives metastasis through a cytosolic DNA response

Nature (IF: 46.49), 2018-1, Charles Swanton, Lewis C Cantley DOI: 10.1038/nature25432 PubMed: 29342134

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Chromosomal instability is a hallmark of cancer that results from ongoing errors in chromosome segregation during mitosis. Although chromosomal instability is a major driver of tumour evolution, its role in metastasis has not been established. Here we show that chromosomal instability promotes metastasis by sustaining a tumour cell-autonomous response to cytosolic DNA. Errors in chromosome segregation create a preponderance of micronuclei whose rupture spills genomic DNA into the cytosol. This leads to the activation of the cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) cytosolic DNA-sensing pathway and downstream noncanonical NF- κ B signalling. Genetic suppression of chromosomal instability markedly delays metastasis even in highly aneuploid tumour models, whereas continuous chromosome segregation errors promote cellular invasion and metastasis in a STING-dependent manner. By subverting lethal epithelial responses to cytosolic DNA, chromosomally unstable tumour cells co-opt chronic activation of innate immune pathways to spread to distant organs.

Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment

Cell (IF: 38.62), 2018-8, Alexander Y Rudensky Dana Pe'er DOI: 10.1016/j.cell.2018.05.060 PubMed: 29961579
Single Cell Immune Profiling, Human, Cancer Research, Cell Atlas.

Abstract

Knowledge of immune cell phenotypes in the tumor microenvironment is essential for understanding mechanisms of cancer progression and immunotherapy response. We profiled 45,000 immune cells from eight breast carcinomas, as well as matched normal breast tissue, blood, and lymph nodes, using single-cell RNA-seq. We developed a preprocessing pipeline, SEQC, and a Bayesian clustering and normalization method, Biscuit, to address computational challenges inherent to single-cell data. Despite significant similarity between normal and tumor tissue-resident immune cells, we observed continuous phenotypic expansions specific to the tumor microenvironment. Analysis of paired single-cell RNA and T cell receptor (TCR) sequencing data from 27,000 additional T cells revealed the combinatorial impact of TCR utilization on phenotypic diversity. Our results support a model of continuous activation in T cells and do not comport with the macrophage polarization model in cancer. Our results have important implications for characterizing tumor-infiltrating immune cells.

Single-Cell Analysis Reveals Fibroblast Clusters Linked to Immunotherapy Resistance in Cancer

Cancer Discovery (IF: 28.3), 2020-9, Anne Vincent-Salomon et al. DOI: 10.1158/2159-8290.CD-19-1384
PubMed: 32434947

Single Cell Gene Expression, Human, Cancer Research, Immuno-oncology.

Abstract

A subset of cancer-associated fibroblasts (CAF-S1) mediates immunosuppression in breast cancers, but its heterogeneity and its impact on immunotherapy response remain unknown. Here, we identify 8 CAF-S1 clusters by analyzing more than 19,000 single CAF-S1 fibroblasts from breast cancer. We validate the five most abundant clusters by flow cytometry and analyses in other cancer types, highlighting their relevance. Myofibroblasts from clusters 0 and 3, characterized by extracellular matrix proteins and TGF β signaling, respectively, are indicative of primary resistance to immunotherapies. Cluster 0/ecm-myCAF upregulates PD-1 and CTLA4 protein levels in regulatory T lymphocytes (Tregs), which, in turn, increases CAF-S1 cluster 3/TGF β -myCAF cellular content. Thus, our study highlights a positive feedback loop between specific CAF-S1 clusters and Tregs and uncovers their role in immunotherapy resistance. SIGNIFICANCE: Our work provides a significant advance in characterizing and understanding FAPCAF in cancer. We reached a high resolution at single-cell level, which enabled us to identify specific clusters associated with immunosuppression and immunotherapy resistance. Identification of cluster-specific signatures paves the way for therapeutic options in combination with immunotherapies..

High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer

Nature Genetics (IF: 30.33), 2019-5, Céline Vallot et al. DOI: 10.1038/s41588-019-0424-9 PubMed: 31152164
Single Cell Gene Expression, Human & Mouse, Assay Method, Cancer Research.

Abstract

Modulation of chromatin structure via histone modification is a major epigenetic mechanism and regulator of gene expression. However, the contribution of chromatin features to tumor heterogeneity and evolution remains unknown. Here we describe a high-throughput droplet microfluidics platform to profile chromatin landscapes of thousands of cells at single-cell resolution. Using patient-derived xenograft models of acquired resistance to chemotherapy and targeted therapy in breast cancer, we found that a subset of cells within untreated drug-sensitive tumors share a common chromatin signature with resistant cells, undetectable using bulk approaches. These cells, and cells from the resistant tumors, have lost chromatin marks-H3K27me3, which is associated with stable transcriptional repression-for genes known to promote resistance to treatment. This single-cell chromatin immunoprecipitation followed by sequencing approach paves the way to study the role of chromatin heterogeneity, not just in cancer but in other diseases and healthy systems, notably during cellular differentiation and development.

A pooled single-cell genetic screen identifies regulatory checkpoints in the continuum of the epithelial-to-mesenchymal transition

Nature Genetics (IF: 30.33), 2019-9, Jay Shendure et al. DOI: 10.1038/s41588-019-0489-5 PubMed: 31477929
Single Cell Gene Expression, Human, Assay Method, Developmental Biology.

Abstract

Integrating single-cell trajectory analysis with pooled genetic screening could reveal the genetic architecture that guides cellular decisions in development and disease. We applied this paradigm to probe the genetic circuitry that controls epithelial-to-mesenchymal transition (EMT). We used single-cell RNA sequencing to profile epithelial cells undergoing a spontaneous spatially determined EMT in the presence or absence of transforming growth factor- β . Pseudospacial trajectory analysis identified continuous waves of gene regulation as opposed to discrete 'partial' stages of EMT. KRAS was connected to the exit from the epithelial state and the acquisition of a fully mesenchymal phenotype. A pooled single-cell CRISPR-Cas9 screen identified EMT-associated receptors and transcription factors, including regulators of KRAS, whose loss impeded progress along the EMT. Inhibiting the KRAS effector MEK and its upstream activators EGFR and MET demonstrates that interruption of key signaling events reveals regulatory "checkpoints" in the EMT continuum that mimic discrete stages, and reconciles opposing views of the program that controls EMT.

Multiplexed activation of endogenous genes by CRISPRa elicits potent antitumor immunity

Nature Immunology (IF: 22.3), 2019-10, Charles S Fuchs et al. DOI: 10.1038/s41590-019-0500-4

PubMed: 31611701

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

Immunotherapy has transformed cancer treatment. However, current immunotherapy modalities face various limitations. In the present study, we developed multiplexed activation of endogenous genes as an immunotherapy (MAEGI), a new form of immunotherapy that elicits antitumor immunity through multiplexed activation of endogenous genes in tumors. We leveraged CRISPR activation (CRISPRa) to directly augment the in situ expression of endogenous genes, and thereby the presentation of tumor antigens, leading to dramatic antitumor immune responses. Deploying this as a cell-based vaccination strategy showed efficacy in both prophylactic and therapeutic settings. Intratumoral adeno-associated virus delivery of CRISPRa libraries elicited strong antitumor immunity across multiple cancer types. Precision targeting of mutated gene sets eradicated a large fraction of established tumors at both local and distant sites. This treatment modality led to alterations in the tumor microenvironment, marked by enhanced T cell infiltration and antitumor immune signatures. Multiplexed endogenous gene activation is a versatile and highly scalable strategy to elicit potent immune responses against cancer, distinct from all existing cancer therapies.

Integrating spatial gene expression and breast tumour morphology via deep learning

Nature Biomedical Engineering (IF: 18.95), 2020-8, Joakim Lundeberg et al. DOI: 10.1038/s41551-020-0578

PubMed: 32572199

Spatial Gene Expression, Human, Computational Method.

Abstract

Spatial transcriptomics allows for the measurement of RNA abundance at a high spatial resolution, making it possible to systematically link the morphology of cellular neighbourhoods and spatially localized gene expression. Here, we report the development of a deep learning algorithm for the prediction of local gene expression from haematoxylin-and-eosin-stained histopathology images using a new dataset of 30,612 spatially resolved gene expression data matched to histopathology images from 23 patients with breast cancer. We identified over 100 genes, including known breast cancer biomarkers of intratumoral heterogeneity and the co-localization of tumour growth and immune activation, the expression of which can be predicted from the histopathology images at a resolution of 100 μm . We also show that the algorithm generalizes well to The Cancer Genome Atlas and to other breast cancer gene expression datasets without the need for re-training. Predicting the spatially resolved transcriptome of a tissue directly from tissue images may enable image-based screening for molecular biomarkers with spatial variation.

Single-cell transcriptomics reveals multi-step adaptations to endocrine therapy

Nature Communications (IF: 13.61), 2019-9, Iros Barozzi et al. DOI: 10.1038/s41467-019-11721-9

PubMed: 31477698

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Resistant tumours are thought to arise from the action of Darwinian selection on genetically heterogeneous cancer cell populations. However, simple clonal selection is inadequate to describe the late relapses often characterising luminal breast cancers treated with endocrine therapy (ET), suggesting a more complex interplay between genetic and non-genetic factors. Here, we dissect the contributions of clonal genetic diversity and transcriptional plasticity during the early and late phases of ET at single-cell resolution. Using single-cell RNA-sequencing and imaging we disentangle the transcriptional variability of plastic cells and define a rare subpopulation of pre-adapted (PA) cells which undergoes further transcriptomic reprogramming and copy number changes to acquire full resistance. We find evidence for sub-clonal expression of a PA signature in primary tumours and for dominant expression in clustered circulating tumour cells. We propose a multi-step model for ET resistance development and advocate the use of stage-specific biomarkers.

SREBP1 drives Keratin-80-dependent cytoskeletal changes and invasive behavior in endocrine-resistant ER α breast cancer

Nature Communications (IF: 13.61), 2019-5, Fernando CalvoLuca Magnani DOI: 10.1038/s41467-019-09676

PubMed: 31073170

Single Cell Gene Expression, Human, Cancer Research.

Abstract

Approximately 30% of ER α breast cancer patients relapse with metastatic disease following adjuvant endocrine therapies. The connection between acquisition of drug resistance and invasive potential is poorly understood. In this study, we demonstrate that the type II keratin topological associating domain undergoes epigenetic reprogramming in aromatase inhibitors (AI)-resistant cells, leading to Keratin-80 (KRT80) upregulation. KRT80 expression is driven by de novo enhancer activation by sterol regulatory element-binding protein 1 (SREBP1). KRT80 upregulation directly promotes cytoskeletal rearrangements at the leading edge, increased focal adhesion and cellular stiffening, collectively promoting cancer cell invasion. Shearwave elasticity imaging performed on prospectively recruited patients confirms KRT80 levels correlate with stiffer tumors. Immunohistochemistry showed increased KRT80-positive cells at relapse and, using several clinical endpoints, KRT80 expression associates with poor survival. Collectively, our data uncover an unpredicted and potentially targetable direct link between epigenetic and cytoskeletal reprogramming promoting cell invasion in response to chronic AI treatment.

Single-cell landscape in mammary epithelium reveals bipotent-like cells associated with breast cancer risk and outcome

Communications Biology (IF: 11.2), 2019-8, Kerstin B Meyer et al. DOI: 10.1038/s42003-019-0554-8

PubMed: 31428694

Single Cell Gene Expression, Human, Cancer Research, Computational Method.

Abstract

Adult stem-cells may serve as the cell-of-origin for cancer, yet their unbiased identification in single cell RNA sequencing data is challenging due to the high dropout rate. In the case of breast, the existence of a bipotent stem-like state is also controversial. Here we apply a marker-free algorithm to scRNA-Seq data from the human mammary epithelium, revealing a high-potency cell-state enriched for an independent mammary stem-cell expression module. We validate this stem-like state in independent scRNA-Seq data. Our algorithm further predicts that the stem-like state is bipotent, a prediction we are able to validate using FACS sorted bulk expression data. The bipotent stem-like state correlates with clinical outcome in basal breast cancer and is characterized by overexpression of and, two modulators of basal breast cancer risk. This study illustrates the power of a marker-free computational framework to identify a novel bipotent stem-like state in the mammary epithelium.

Myeloid cell – synthesized coagulation factor X dampens antitumor immunity

Science Immunology (IF: 13.41), 2019-9, Hartmut Weiler et al. DOI: 10.1126/sciimmunol.aaw8405

PubMed: 31541031

Single Cell Gene Expression, Mouse, Functional Genomics, Immuno-oncology.

Abstract

Immune evasion in the tumor microenvironment (TME) is a crucial barrier for effective cancer therapy, and plasticity of innate immune cells may contribute to failures of targeted immunotherapies. Here, we show that rivaroxaban, a direct inhibitor of activated coagulation factor X (FX), promotes antitumor immunity by enhancing infiltration of dendritic cells and cytotoxic T cells at the tumor site. Profiling FX expression in the TME identifies monocytes and macrophages as crucial sources of extracellular FX. By generating mice with immune cells lacking the ability to produce FX, we show that myeloid cell-derived FX plays a pivotal role in promoting tumor immune evasion. In mouse models of cancer, we report that the efficacy of rivaroxaban is comparable with anti-programmed cell death ligand 1 (PD-L1) therapy and that rivaroxaban synergizes with anti-PD-L1 in improving antitumor immunity. Mechanistically, we demonstrate that FXa promotes immune evasion by signaling through protease-activated receptor 2 and that rivaroxaban specifically targets this cell-autonomous signaling pathway to reprogram tumor-associated macrophages. Collectively, our results have uncovered the importance of FX produced in the TME as a regulator of immune cell activation and suggest translational potential of direct oral anticoagulants to remove persisting roadblocks for immunotherapy and provide extravascular benefits in other diseases.

Stromal cell diversity associated with immune evasion in human triple-negative breast cancer

EMBO Journal (IF: 10.16), 2020-10, X Shirley Liu et al. DOI: 10.15252/emboj.2019104063 PubMed: 32790115

Single Cell Gene Expression, Human, Cancer Research.

Abstract

The tumour stroma regulates nearly all stages of carcinogenesis. Stromal heterogeneity in human triple-negative breast cancers (TNBCs) remains poorly understood, limiting the development of stromal-targeted therapies. Single-cell RNA sequencing of five TNBCs revealed two cancer-associated fibroblast (CAF) and two perivascular-like (PVL) subpopulations. CAFs clustered into two states: the first with features of myofibroblasts and the second characterised by high expression of growth factors and immunomodulatory molecules. PVL cells clustered into two states consistent with a differentiated and immature phenotype. We showed that these stromal states have distinct morphologies, spatial relationships and functional properties in regulating the extracellular matrix. Using cell signalling predictions, we provide evidence that stromal-immune crosstalk acts via a diverse array of immunoregulatory molecules. Importantly, the investigation of gene signatures from inflammatory-CAFs and differentiated-PVL cells in independent TNBC patient cohorts revealed strong associations with cytotoxic T-cell dysfunction and exclusion, respectively. Such insights present promising candidates to further investigate for new therapeutic strategies in the treatment of TNBCs.

Colonic epithelial cell diversity in health and inflammatory bowel disease

Nature (IF: 46.49), 2019-2, Hashem KoohyAlison Simmons DOI: 10.1038/s41586-019-0992 PubMed: 30814735

Single Cell Gene Expression, Human, Cell Atlas, Cell Biology.

Abstract

The colonic epithelium facilitates host-microorganism interactions to control mucosal immunity, coordinate nutrient recycling and form a mucus barrier. Breakdown of the epithelial barrier underpins inflammatory bowel disease (IBD). However, the specific contributions of each epithelial-cell subtype to this process are unknown. Here we profile single colonic epithelial cells from patients with IBD and unaffected controls. We identify previously unknown cellular subtypes, including gradients of progenitor cells, colonocytes and goblet cells within intestinal crypts. At the top of the crypts, we find a previously unknown absorptive cell, expressing the proton channel OTO2 and the satiety peptide uroguanylin, that senses pH and is dysregulated in inflammation and cancer. In IBD, we observe a positional remodelling of goblet cells that coincides with downregulation of WFDC2-an antiprotease molecule that we find to be expressed by goblet cells and that inhibits bacterial growth. In vivo, WFDC2 preserves the integrity of tight junctions between epithelial cells and prevents invasion by commensal bacteria and mucosal inflammation. We delineate markers and transcriptional states, identify a colonic epithelial cell and uncover fundamental determinants of barrier breakdown in IBD.

Massively parallel single-cell mitochondrial DNA genotyping and chromatin profiling

Nature Biotechnology (IF: 42.3), 2020-8, Aviv Regev et al. DOI: 10.1038/s41587-020-0645-6 PubMed: 32788668
Single Cell Immune Profiling & Single Cell ATAC, Human, Assay Method, Cancer Research.

Abstract

Natural mitochondrial DNA (mtDNA) mutations enable the inference of clonal relationships among cells. mtDNA can be profiled along with measures of cell state, but has not yet been combined with the massively parallel approaches needed to tackle the complexity of human tissue. Here, we introduce a high-throughput, droplet-based mitochondrial single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq), a method that combines high-confidence mtDNA mutation calling in thousands of single cells with their concomitant high-quality accessible chromatin profile. This enables the inference of mtDNA heteroplasmy, clonal relationships, cell state and accessible chromatin variation in individual cells. We reveal single-cell variation in heteroplasmy of a pathologic mtDNA variant, which we associate with intra-individual chromatin variability and clonal evolution. We clonally trace thousands of cells from cancers, linking epigenomic variability to subclonal evolution, and infer cellular dynamics of differentiating hematopoietic cells in vitro and in vivo. Taken together, our approach enables the study of cellular population dynamics and clonal properties in vivo.

Macrophages Maintain Epithelium Integrity by Limiting Fungal Product Absorption

Cell (IF: 38.62), 2020-10, Danijela Matic Vignjevic et al. DOI: 10.1016/j.cell.2020.08.048 PubMed: 32970988
Single Cell Gene Expression, Mouse, Immunology.

Abstract

The colon is primarily responsible for absorbing fluids. It contains a large number of microorganisms including fungi, which are enriched in its distal segment. The colonic mucosa must therefore tightly regulate fluid influx to control absorption of fungal metabolites, which can be toxic to epithelial cells and lead to barrier dysfunction. How this is achieved remains unknown. Here, we describe a mechanism by which the innate immune system allows rapid quality check of absorbed fluids to avoid intoxication of colonocytes. This mechanism relies on a population of distal colon macrophages that are equipped with “balloon-like” protrusions (BLPs) inserted in the epithelium, which sample absorbed fluids. In the absence of macrophages or BLPs, epithelial cells keep absorbing fluids containing fungal products, leading to their death and subsequent loss of epithelial barrier integrity. These results reveal an unexpected and essential role of macrophages in the maintenance of colon-microbiota interactions in homeostasis. VIDEO ABSTRACT.

The Human and Mouse Enteric Nervous System at Single-Cell Resolution

Cell (IF: 38.62), 2020-9, Ramnik J Xavier et al. DOI: 10.1016/j.cell.2020.08.003 PubMed: 32888429

Single Cell Gene Expression, Human & Mouse, Cell Atlas, Neuroscience.

Abstract

The enteric nervous system (ENS) coordinates diverse functions in the intestine but has eluded comprehensive molecular characterization because of the rarity and diversity of cells. Here we develop two methods to profile the ENS of adult mice and humans at single-cell resolution: RAISIN RNA-seq for profiling intact nuclei with ribosome-bound mRNA and MIRACL-seq for label-free enrichment of rare cell types by droplet-based profiling. The 1,187,535 nuclei in our mouse atlas include 5,068 neurons from the ileum and colon, revealing extraordinary neuron diversity. We highlight circadian expression changes in enteric neurons, show that disease-related genes are dysregulated with aging, and identify differences between the ileum and proximal/distal colon. In humans, we profile 436,202 nuclei, recovering 1,445 neurons, and identify conserved and species-specific transcriptional programs and putative neuro-epithelial, neuro-stromal, and neuro-immune interactions. The human ENS expresses risk genes for neuropathic, inflammatory, and extra-intestinal diseases, suggesting neuronal contributions to disease.

Molecular Pathways of Colon Inflammation Induced by Cancer Immunotherapy

Cell (IF: 38.62), 2020-8, Guo-Cheng Yuan et al. DOI: 10.1016/j.cell.2020.06.001 PubMed: 32603654

Single Cell Immune Profiling, Human, Immuno-oncology, Immunology.

Abstract

Checkpoint blockade with antibodies specific for the PD-1 and CTLA-4 inhibitory receptors can induce durable responses in a wide range of human cancers. However, the immunological mechanisms responsible for severe inflammatory side effects remain poorly understood. Here we report a comprehensive single-cell analysis of immune cell populations in colitis, a common and severe side effect of checkpoint blockade. We observed a striking accumulation of CD8 T cells with highly cytotoxic and proliferative states and no evidence of regulatory T cell depletion. T cell receptor (TCR) sequence analysis demonstrated that a substantial fraction of colitis-associated CD8 T cells originated from tissue-resident populations, explaining the frequently early onset of colitis symptoms following treatment initiation. Our analysis also identified cytokines, chemokines, and surface receptors that could serve as therapeutic targets for colitis and potentially other inflammatory side effects of checkpoint blockade.

Mucosal Profiling of Pediatric-Onset Colitis and IBD Reveals Common Pathogenics and Therapeutic Pathways

Cell (IF: 38.62), 2019-11, Min Yang et al. DOI: 10.1016/j.cell.2019.10.027 PubMed: 31730855

Single Cell Immune Profiling, Human, Immunology.

Abstract

Pediatric-onset colitis and inflammatory bowel disease (IBD) have significant effects on the growth of infants and children, but the etiopathogenesis underlying disease subtypes remains incompletely understood. Here, we report single-cell clustering, immune phenotyping, and risk gene analysis for children with undifferentiated colitis, Crohn's disease, and ulcerative colitis. We demonstrate disease-specific characteristics, as well as common pathogenesis marked by impaired cyclic AMP (cAMP)-response signaling. Specifically, infiltration of PDE4B- and TNF-expressing macrophages, decreased abundance of CD39-expressing intraepithelial T cells, and platelet aggregation and release of 5-hydroxytryptamine at the colonic mucosae were common in colitis and IBD patients. Targeting these pathways by using the phosphodiesterase inhibitor dipyridamole restored immune homeostasis and improved colitis symptoms in a pilot study. In summary, comprehensive analysis of the colonic mucosae has uncovered common pathogenesis and therapeutic targets for children with colitis and IBD.

Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis

Cell (IF: 38.62), 2019-7, Ramnik J Xavier et al. DOI: 10.1016/j.cell.2019.06.029 PubMed: 31348891

Single Cell Gene Expression, Human, Cell Atlas, Immunology.

Abstract

Genome-wide association studies (GWAS) have revealed risk alleles for ulcerative colitis (UC). To understand their cell type specificities and pathways of action, we generate an atlas of 366,650 cells from the colon mucosa of 18 UC patients and 12 healthy individuals, revealing 51 epithelial, stromal, and immune cell subsets, including BEST4enterocytes, microfold-like cells, and IL13RA2IL11inflammatory fibroblasts, which we associate with resistance to anti-TNF treatment. Inflammatory fibroblasts, inflammatory monocytes, microfold-like cells, and T cells that co-express CD8 and IL-17 expand with disease, forming intercellular interaction hubs. Many UC risk genes are cell type specific and co-regulated within relatively few gene modules, suggesting convergence onto limited sets of cell types and pathways. Using this observation, we nominate and infer functions for specific risk genes across GWAS loci. Our work provides a framework for interrogating complex human diseases and mapping risk variants to cell types and pathways.

Single-cell atlas of colonic CD8⁺ T cells in ulcerative colitis

Nature Medicine (IF: 36.23), 2020-9, Hashem Koohy et al. DOI: 10.1038/s41591-020-1003-4 PubMed: 32747828
Single Cell Immune Profiling, Human, Immunology.

Abstract

Colonic antigen-experienced lymphocytes such as tissue-resident memory CD8⁺T cells can respond rapidly to repeated antigen exposure. However, their cellular phenotypes and the mechanisms by which they drive immune regulation and inflammation remain unclear. Here we compiled an unbiased atlas of human colonic CD8⁺T cells in health and ulcerative colitis (UC) using single-cell transcriptomics with T-cell receptor repertoire analysis and mass cytometry. We reveal extensive heterogeneity in CD8⁺T-cell composition, including expanded effector and post-effector terminally differentiated CD8⁺T cells. While UC-associated CD8⁺effector T cells can trigger tissue destruction and produce tumor necrosis factor (TNF)- α , post-effector cells acquire innate signatures to adopt regulatory functions that may mitigate excessive inflammation. Thus, we identify colonic CD8⁺T-cell phenotypes in health and UC, define their clonal relationships and characterize terminally differentiated dysfunctional UC CD8⁺T cells expressing IL-26, which attenuate acute colitis in a humanized IL-26 transgenic mouse model.

Lineage-dependent gene expression programs influence the immune landscape of colorectal cancer

Nature Genetics (IF: 30.33), 2020-6, Sabine Tejpar et al. DOI: 10.1038/s41588-020-0636 PubMed: 32451460
Single Cell Gene Expression, Human, Cancer Research, Immuno-oncology.

Abstract

Immunotherapy for metastatic colorectal cancer is effective only for mismatch repair-deficient tumors with high microsatellite instability that demonstrate immune infiltration, suggesting that tumor cells can determine their immune microenvironment. To understand this cross-talk, we analyzed the transcriptome of 91,103 unsorted single cells from 23 Korean and 6 Belgian patients. Cancer cells displayed transcriptional features reminiscent of normal differentiation programs, and genetic alterations that apparently fostered immunosuppressive microenvironments directed by regulatory T cells, myofibroblasts and myeloid cells. Inter-cellular network reconstruction supported the association between cancer cell signatures and specific stromal or immune cell populations. Our collective view of the cellular landscape and inter-cellular interactions in colorectal cancer provide mechanistic information for the design of efficient immuno-oncology treatment strategies.

Ascl2-Dependent Cell Dedifferentiation Drives Regeneration of Ablated Intestinal Stem Cells

Cell Stem Cell (IF: 23.45), 2020-3, Hans Clevers et al. DOI: 10.1016/j.stem.2019.12.011 PubMed: 32084390

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Ablation of LGR5 intestinal stem cells (ISCs) is associated with rapid restoration of the ISC compartment. Different intestinal crypt populations dedifferentiate to provide new ISCs, but the transcriptional and signaling trajectories that guide this process are unclear, and a large body of work suggests that quiescent reserve ISCs contribute to regeneration. By timing the interval between LGR5 lineage tracing and lethal injury, we show that ISC regeneration is explained nearly completely by dedifferentiation, with contributions from absorptive and secretory progenitors. The ISC-restricted transcription factor ASCL2 confers measurable competitive advantage to resting ISCs and is essential to restore the ISC compartment. Regenerating cells re-express Ascl2 days before Lgr5, and single-cell RNA sequencing (scRNA-seq) analyses reveal transcriptional paths underlying dedifferentiation. ASCL2 target genes include the interleukin-11 (IL-11) receptor Il11ra1, and recombinant IL-11 enhances crypt cell regenerative potential. These findings reveal cell dedifferentiation as the principal means for ISC restoration and highlight an ASCL2-regulated signal that enables this adaptive response.

Transdifferentiation of tumor infiltrating innate lymphoid cells during progression of colorectal cancer

Cell Research (IF: 20.4), 2020-7, Jianming Ying et al. DOI: 10.1038/s41422-020-0312 PubMed: 32367039

Single Cell Gene Expression, Human & Mouse, Cancer Research.

Abstract

Innate lymphoid cells (ILCs) reside in mucosal surfaces to potentiate immune responses, sustain mucosal integrity and maintain tissue homeostasis. However, how tumor infiltrating ILCs modulate tumor development and progression is unclear. Here we profiled tumor infiltrating ILCs during colorectal cancer (CRC) progression by single-cell RNA sequencing. We identified six clusters of tumor infiltrating ILCs with unique features. ILC1s expressed inhibitory receptors and underwent inhibitory functional conversion at the late stage of CRC. ILC2s were classified into three subsets (called ILC2-A, -B, -C), of which ILC2-C subset could facilitate tumor progression. HS3ST1 and PD1 were highly expressed in ILC2s of late stage CRC tumors and deficiency of HS3ST1 or PD1 in ILC2s suppressed tumor growth. Moreover, ILC3s transdifferentiated into ILCregs during CRC progression and ILCregs promoted tumor growth. Of note, TGF- β signaling initiated the conversion of ILC3s to ILCregs and blockade of TGF- β signaling could disrupt the ILCreg transdifferentiation and inhibited tumor growth. Thus, intervention of ILC conversions might be a potential strategy for CRC immunotherapy.

Distinct microbial and immune niches of the human colon

Nature Immunology (IF: 22.3), 2020-3, Trevor D Lawley et al. DOI: 10.1038/s41590-020-0602 PubMed: 32066951
Single Cell Gene Expression & Single Cell Immune Profiling, Human, Immunology.

Abstract

Gastrointestinal microbiota and immune cells interact closely and display regional specificity; however, little is known about how these communities differ with location. Here, we simultaneously assess microbiota and single immune cells across the healthy, adult human colon, with paired characterization of immune cells in the mesenteric lymph nodes, to delineate colonic immune niches at steady state. We describe distinct helper T cell activation and migration profiles along the colon and characterize the transcriptional adaptation trajectory of regulatory T cells between lymphoid tissue and colon. Finally, we show increasing B cell accumulation, clonal expansion and mutational frequency from the cecum to the sigmoid colon and link this to the increasing number of reactive bacterial species.

Sympathetic Input to Multiple Cell Types in Mouse and Human Colon Produces Region-Specific Responses

Gastroenterology (IF: 18.79), 2020-9, Robert O Heuckeroth et al. DOI: 10.1053/j.gastro.2020.09.030
PubMed: 32980343
Single Cell Gene Expression, Human, Neuroscience.

Abstract

The colon is innervated by intrinsic and extrinsic neurons that coordinate functions necessary for digestive health. Sympathetic input suppresses colon motility by acting on intrinsic myenteric neurons, but the extent of sympathetic-induced changes on large-scale network activity in myenteric circuits has not been determined. Compounding the complexity of sympathetic function, there is evidence that sympathetic transmitters can regulate activity in non-neuronal cells (such as enteric glia and innate immune cells). We performed anatomical tracing, immunohistochemistry, optogenetic (GCaMP calcium imaging, channelrhodopsin), and colon motility studies in mice and single-cell RNA sequencing in human colon to investigate how sympathetic postganglionic neurons modulate colon function. Individual neurons in each sympathetic prevertebral ganglion innervated the proximal or distal colon, with processes closely opposed to multiple cell types. Calcium imaging in semi-intact mouse colon preparations revealed changes in spontaneous and evoked neural activity, as well as activation of non-neuronal cells, induced by sympathetic nerve stimulation. The overall pattern of response to sympathetic stimulation was unique to the proximal or distal colon. Region-specific changes in cellular activity correlated with motility patterns produced by electrical and optogenetic stimulation of sympathetic pathways. Pharmacology experiments (mouse) and RNA sequencing (human) indicated that appropriate receptors were expressed on different cell types to account for the responses to sympathetic stimulation. Regional differences in expression of α -1 adrenoceptors in human colon emphasize the translational relevance of our mouse findings. Sympathetic neurons differentially regulate activity of neurons and non-neuronal cells in proximal and distal colon to promote distinct changes in motility patterns, likely reflecting the distinct roles played by these 2 regions.

Interleukin 1 beta and Matrix Metalloproteinase 3 Contribute to Development of Epidermal Growth Factor Receptor – Dependent Serrated Polyps in Mouse Cecum

Gastroenterology (IF: 18.79), 2019-12, Glaucia C Furtado et al. DOI: 10.1053/j.gastro.2019.08.025

PubMed: 31470007

Single Cell Gene Expression, Mouse, Functional Genomics, Immunology.

Abstract

Transgenic mice (HBUS) that express the epidermal growth factor receptor (EGFR) ligand HBEGF (heparin-binding epidermal growth factor-like growth factor) and a constitutively active G protein-coupled receptor (US28) in intestinal epithelial cells develop serrated polyps in the cecum. Development of serrated polyps depends on the composition of the gut microbiota and is associated with bacterial invasion of the lamina propria, accompanied by induction of inflammation and up-regulation of interleukin 1 beta (IL1B) and matrix metalloproteinase (MMP) 3 in the cecum. We investigated the mechanisms by which these changes contribute to development of serrated polyps. We performed studies with C57BL/6 (control) and HBUS mice. To accelerate polyp development, we increased the exposure of the bacteria to the lamina propria by injecting HBUS mice with diphtheria toxin, which binds transgenic HBEGF expressed by the epithelial cells and causes apoptosis. Mice were given injections of IL1B-neutralizing antibody and the MMP inhibitor N-isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid. Intestinal tissues were collected from mice and analyzed by histology, reverse-transcription polymerase chain reaction, enzyme-linked immunosorbent assay, immunofluorescence, and flow cytometry. We examined fibroblast subsets in polyps using single-cell RNA sequencing. Administration of diphtheria toxin to HBUS mice accelerated development of serrated polyps (95% of treated mice developed polyps before 100 days of age, compared with 53% given vehicle). IL1B stimulated subsets of platelet-derived growth factor receptor alpha(PDGFRFA) fibroblasts isolated from cecum, resulting in increased expression of MMP3. Neutralizing antibodies against IL1B or administration of the MMP inhibitor reduced the number of serrated polyps that formed in the HBUS mice. Single-cell RNA sequencing analysis showed subsets of fibroblasts in serrated polyps that express genes that regulate matrix fibroblasts and inflammation. In studies of mice, we found that barrier breakdown and expression of inflammatory factors contribute to development of serrated polyps. Subsets of cecal PDGFRFAfibroblasts are activated by release of IL1B from myeloid cells during the early stages of serrated polyp development. MMP3 produced by PDGFRFAfibroblasts is important for serrated polyp development. Our findings confirm the functions of previously identified serrated polyp-associated molecules and indicate roles for immune and stromal cells in serrated polyp development.

CellTag Indexing: genetic barcode-based sample multiplexing for single-cell genomics

Genome Biology (IF: 19.04), 2019-12, Yuhei Kiritani et al. DOI: 10.1186/s13059-019-1699 PubMed: 31072405

Single Cell Gene Expression, Mouse, Assay Method.

Abstract

High-throughput single-cell assays increasingly require special consideration in experimental design, sample multiplexing, batch effect removal, and data interpretation. Here, we describe a lentiviral barcode-based multiplexing approach, CellTag Indexing, which uses predefined genetic barcodes that are heritable, enabling cell populations to be tagged, pooled, and tracked over time in the same experimental replicate. We demonstrate the utility of CellTag Indexing by sequencing transcriptomes using a variety of cell types, including long-term tracking of cell engraftment and differentiation *in vivo*. Together, this presents CellTag Indexing as a broadly applicable genetic multiplexing tool that is complementary with existing single-cell technologies.

High-dimensional cytometric analysis of colorectal cancer reveals novel mediators of antitumour immunity

Gut (IF: 17.75), 2020-4, Frits Koning et al. DOI: 10.1136/gutjnl-2019-318672 PubMed: 31270164

Single Cell Gene Expression, Human, Cancer Research, Immuno-oncology.

Abstract

A comprehensive understanding of anticancer immune responses is paramount for the optimal application and development of cancer immunotherapies. We unravelled local and systemic immune profiles in patients with colorectal cancer (CRC) by high-dimensional analysis to provide an unbiased characterisation of the immune contexture of CRC. Thirty-six immune cell markers were simultaneously assessed at the single-cell level by mass cytometry in 35 CRC tissues, 26 tumour-associated lymph nodes, 17 colorectal healthy mucosa and 19 peripheral blood samples from 31 patients with CRC. Additionally, functional, transcriptional and spatial analyses of tumour-infiltrating lymphocytes were performed by flow cytometry, single-cell RNA-sequencing and multispectral immunofluorescence. We discovered that a previously unappreciated innate lymphocyte population (LinCD7CD127CD56CD45RO) was enriched in CRC tissues and displayed cytotoxic activity. This subset demonstrated a tissue-resident (CD103CD69) phenotype and was most abundant in immunogenic mismatch repair (MMR)-deficient CRCs. Their presence in tumours was correlated with the infiltration of tumour-resident cytotoxic, helper and $\gamma\delta$ T cells with highly similar activated (HLA-DRCD38PD-1) phenotypes. Remarkably, activated $\gamma\delta$ T cells were almost exclusively found in MMR-deficient cancers. Non-activated counterparts of tumour-resident cytotoxic and $\gamma\delta$ T cells were present in CRC and healthy mucosa tissues, but not in lymph nodes, with the exception of tumour-positive lymph nodes. This work provides a blueprint for the understanding of the heterogeneous and intricate immune landscape of CRC, including the identification of previously unappreciated immune cell subsets. The concomitant presence of tumour-resident innate and adaptive immune cell populations suggests a multitargeted exploitation of their antitumour properties in a therapeutic setting.

Epithelial-derived gasdermin D mediates nonlytic IL-1 β release during experimental colitis

Journal of clinical investigation, The (IF: 12.81), 2020-8, Theresa T Pizarro et al. DOI: 10.1172/JCI138103

PubMed: 32597834

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

Gasdermin D (GSDMD) induces pyroptosis via the pore-forming activity of its N-terminal domain, cleaved by activated caspases associated with the release of IL-1 β . Here, we report a nonpyroptotic role of full-length GSDMD in guiding the release of IL-1 β -containing small extracellular vesicles (sEVs) from intestinal epithelial cells (IECs). In response to caspase-8 inflammasome activation, GSDMD, chaperoned by Cdc37/Hsp90, recruits the E3 ligase, NEDD4, to catalyze polyubiquitination of pro-IL-1 β , serving as a signal for cargo loading into secretory vesicles. GSDMD and IL-1 β colocalize with the exosome markers CD63 and ALIX intracellularly, and GSDMD and NEDD4 are required for release of CD63+ sEVs containing IL-1 β , GSDMD, NEDD4, and caspase-8. Importantly, increased expression of epithelial-derived GSDMD is observed both in patients with inflammatory bowel disease (IBD) and those with experimental colitis. While GSDMD-dependent release of IL-1 β -containing sEVs is detected in cultured colonic explants from colitic mice, GSDMD deficiency substantially attenuates disease severity, implicating GSDMD-mediated release of IL-1 β sEVs in the pathogenesis of intestinal inflammation, such as that observed in IBD.

Loss of the branched-chain amino acid transporter CD98hc alters the development of colonic macrophages in mice

Communications Biology (IF: 11.2), 2020-3, Julien Roux et al. DOI: 10.1038/s42003-020-0842-3

PubMed: 32188932

Single Cell Gene Expression, Mouse, Developmental Biology, Immunology.

Abstract

Comprehensive development is critical for gut macrophages being essential for the intestinal immune system. However, the underlying mechanisms of macrophage development in the colon remain elusive. To investigate the function of branched-chain amino acids in the development of gut macrophages, an inducible knock-out mouse model for the branched-chain amino acid transporter CD98hc in CX3CR1 macrophages was generated. The relatively selective deletion of CD98hc in macrophage populations leads to attenuated severity of chemically-induced colitis that we assessed by clinical, endoscopic, and histological scoring. Single-cell RNA sequencing of colonic lamina propria macrophages revealed that conditional deletion of CD98hc alters the monocyte waterfall development to MHC II macrophages. The change in the macrophage development after deletion of CD98hc is associated with increased apoptotic gene expression. Our results show that CD98hc deletion changes the development of colonic macrophages.

Reprogramming roadmap reveals route to human induced trophoblast stem cells

Nature (IF: 46.49), 2020-10, Owen J L Rackham et al. DOI: 10.1038/s41586-020-2734-6 PubMed: 32939092

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

The reprogramming of human somatic cells to primed or naive induced pluripotent stem cells recapitulates the stages of early embryonic development. The molecular mechanism that underpins these reprogramming processes remains largely unexplored, which impedes our understanding and limits rational improvements to reprogramming protocols. Here, to address these issues, we reconstruct molecular reprogramming trajectories of human dermal fibroblasts using single-cell transcriptomics. This revealed that reprogramming into primed and naive pluripotency follows diverging and distinct trajectories. Moreover, genome-wide analyses of accessible chromatin showed key changes in the regulatory elements of core pluripotency genes, and orchestrated global changes in chromatin accessibility over time. Integrated analysis of these datasets revealed a role for transcription factors associated with the trophectoderm lineage, and the existence of a subpopulation of cells that enter a trophectoderm-like state during reprogramming. Furthermore, this trophectoderm-like state could be captured, which enabled the derivation of induced trophoblast stem cells. Induced trophoblast stem cells are molecularly and functionally similar to trophoblast stem cells derived from human blastocysts or first-trimester placentas. Our results provide a high-resolution roadmap for the transcription-factor-mediated reprogramming of human somatic cells, indicate a role for the trophectoderm-lineage-specific regulatory program during this process, and facilitate the direct reprogramming of somatic cells into induced trophoblast stem cells.

Controlled modelling of human epiblast and amnion development using stem cells

Nature (IF: 46.49), 2019-9, Deborah L Gumucio et al. DOI: 10.1038/s41586-019-1535-2 PubMed: 31511693

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Early human embryonic development involves extensive lineage diversification, cell-fate specification and tissue patterning. Despite its basic and clinical importance, early human embryonic development remains relatively unexplained owing to interspecies divergence and limited accessibility to human embryo samples. Here we report that human pluripotent stem cells (hPSCs) in a microfluidic device recapitulate, in a highly controllable and scalable fashion, landmarks of the development of the epiblast and amniotic ectoderm parts of the conceptus, including lumenogenesis of the epiblast and the resultant pro-amniotic cavity, formation of a bipolar embryonic sac, and specification of primordial germ cells and primitive streak cells. We further show that amniotic ectoderm-like cells function as a signalling centre to trigger the onset of gastrulation-like events in hPSCs. Given its controllability and scalability, the microfluidic model provides a powerful experimental system to advance knowledge of human embryology and reproduction. This model could assist in the rational design of differentiation protocols of hPSCs for disease modelling and cell therapy, and in high-throughput drug and toxicity screens to prevent pregnancy failure and birth defects.

Modeling neural tube development by differentiation of human embryonic stem cells in a microfluidic WNT gradient

Nature Biotechnology (IF: 42.3), 2020-11, Thomas Laurell et al. DOI: 10.1038/s41587-020-0525-0

PubMed: 32451506

Single Cell Gene Expression, Human & Mouse, Developmental Biology, Neuroscience.

Abstract

The study of brain development in humans is limited by the lack of tissue samples and suitable in vitro models. Here, we model early human neural tube development using human embryonic stem cells cultured in a microfluidic device. The approach, named microfluidic-controlled stem cell regionalization (MiSTR), exposes pluripotent stem cells to signaling gradients that mimic developmental patterning. Using a WNT-activating gradient, we generated a neural tissue exhibiting progressive caudalization from forebrain to midbrain to hindbrain, including formation of isthmus organizer characteristics. Single-cell transcriptomics revealed that rostro-caudal organization was already established at 24 h of differentiation, and that the first markers of a neural-specific transcription program emerged in the rostral cells at 48 h. The transcriptomic hallmarks of rostro-caudal organization recapitulated gene expression patterns of the early rostro-caudal neural plate in mouse embryos. Thus, MiSTR will facilitate research on the factors and processes underlying rostro-caudal neural tube patterning.

Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells

Nature Biotechnology (IF: 42.3), 2020-4, Leonardo Velazco-Cruz et al. DOI: 10.1038/s41587-020-0430-6

PubMed: 32094658

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Generation of pancreatic β cells from human pluripotent stem cells (hPSCs) holds promise as a cell replacement therapy for diabetes. In this study, we establish a link between the state of the actin cytoskeleton and the expression of pancreatic transcription factors that drive pancreatic lineage specification. Bulk and single-cell RNA sequencing demonstrated that different degrees of actin polymerization biased cells toward various endodermal lineages and that conditions favoring a polymerized cytoskeleton strongly inhibited neurogenin 3-induced endocrine differentiation. Using latrunculin A to depolymerize the cytoskeleton during endocrine induction, we developed a two-dimensional differentiation protocol for generating human pluripotent stem-cell-derived β (SC- β) cells with improved in vitro and in vivo function. SC- β cells differentiated from four hPSC lines exhibited first- and second-phase dynamic glucose-stimulated insulin secretion. Transplantation of islet-sized aggregates of these cells rapidly reversed severe preexisting diabetes in mice at a rate close to that of human islets and maintained normoglycemia for at least 9 months.

Visualizing structure and transitions in high-dimensional biological data

Nature Biotechnology (IF: 42.3), 2019-12, Guy Wolf et al. DOI: 10.1038/s41587-019-0336-3 PubMed: 31796933
Single Cell Gene Expression, Human, Computational Method.

Abstract

The high-dimensional data created by high-throughput technologies require visualization tools that reveal data structure and patterns in an intuitive form. We present PHATE, a visualization method that captures both local and global nonlinear structure using an information-geometric distance between data points. We compare PHATE to other tools on a variety of artificial and biological datasets, and find that it consistently preserves a range of patterns in data, including continual progressions, branches and clusters, better than other tools. We define a manifold preservation metric, which we call denoised embedding manifold preservation (DEMaP), and show that PHATE produces lower-dimensional embeddings that are quantitatively better denoised as compared to existing visualization methods. An analysis of a newly generated single-cell RNA sequencing dataset on human germ-layer differentiation demonstrates how PHATE reveals unique biological insight into the main developmental branches, including identification of three previously undescribed subpopulations. We also show that PHATE is applicable to a wide variety of data types, including mass cytometry, single-cell RNA sequencing, Hi-C and gut microbiome data.

Self-organizing neuruloids model developmental aspects of Huntington's disease in the ectodermal compartment

Nature Biotechnology (IF: 42.3), 2019-9, Fred Etoc et al. DOI: 10.1038/s41587-019-0237-5 PubMed: 31501559
Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Harnessing the potential of human embryonic stem cells to mimic normal and aberrant development with standardized models is a pressing challenge. Here we use micropattern technology to recapitulate early human neurulation in large numbers of nearly identical structures called neuruloids. Dual-SMAD inhibition followed by bone morphogenetic protein 4 stimulation induced self-organization of neuruloids harboring neural progenitors, neural crest, sensory placode and epidermis. Single-cell transcriptomics unveiled the precise identities and timing of fate specification. Investigation of the molecular mechanism of neuruloid self-organization revealed a pulse of pSMAD1 at the edge that induced epidermis, whose juxtaposition to central neural fates specifies neural crest and placodes, modulated by fibroblast growth factor and Wnt. Neuruloids provide a unique opportunity to study the developmental aspects of human diseases. Using isogenic Huntington's disease human embryonic stem cells and deep neural network analysis, we show how specific phenotypic signatures arise in our model of early human development as a consequence of mutant huntingtin protein, outlining an approach for phenotypic drug screening.

Defining the Teratoma as a Model for Multi-lineage Human Development

Cell (IF: 38.62), 2020-11, Kun Zhang et al. DOI: 10.1016/j.cell.2020.10.018 PubMed: 33152263

Single Cell Gene Expression, Human & Mouse, Developmental Biology.

Abstract

We propose that the teratoma, a recognized standard for validating pluripotency in stem cells, could be a promising platform for studying human developmental processes. Performing single-cell RNA sequencing (RNA-seq) of 179,632 cells across 23 teratomas from 4 cell lines, we found that teratomas reproducibly contain approximately 20 cell types across all 3 germ layers, that inter-teratoma cell type heterogeneity is comparable with organoid systems, and teratoma gut and brain cell types correspond well to similar fetal cell types. Furthermore, cellular barcoding confirmed that injected stem cells robustly engraft and contribute to all lineages. Using pooled CRISPR-Cas9 knockout screens, we showed that teratomas can enable simultaneous assaying of the effects of genetic perturbations across all germ layers. Additionally, we demonstrated that teratomas can be sculpted molecularly via microRNA (miRNA)-regulated suicide gene expression to enrich for specific tissues. Taken together, teratomas are a promising platform for modeling multi-lineage development, pan-tissue functional genetic screening, and tissue engineering.

Generation of Blastocyst-like Structures from Mouse Embryonic and Adult Cell Cultures

Cell (IF: 38.62), 2019-10, Jun Wu et al. DOI: 10.1016/j.cell.2019.09.029 PubMed: 31626770

Single Cell Gene Expression, Mouse, Assay Method, Developmental Biology.

Abstract

A single mouse blastomere from an embryo until the 8-cell stage can generate an entire blastocyst. Whether laboratory-cultured cells retain a similar generative capacity remains unknown. Starting from a single stem cell type, extended pluripotent stem (EPS) cells, we established a 3D differentiation system that enabled the generation of blastocyst-like structures (EPS-blastoids) through lineage segregation and self-organization. EPS-blastoids resembled blastocysts in morphology and cell-lineage allocation and recapitulated key morphogenetic events during preimplantation and early postimplantation development in vitro. Upon transfer, some EPS-blastoids underwent implantation, induced decidualization, and generated live, albeit disorganized, tissues in utero. Single-cell and bulk RNA-sequencing analysis revealed that EPS-blastoids contained all three blastocyst cell lineages and shared transcriptional similarity with natural blastocysts. We also provide proof of concept that EPS-blastoids can be generated from adult cells via cellular reprogramming. EPS-blastoids provide a unique platform for studying early embryogenesis and pave the way to creating viable synthetic embryos by using cultured cells.

YY1 Is a Structural Regulator of Enhancer-Promoter Loops

Cell (IF: 38.62), 2017-12, Nathanael S Gray et al. DOI: 10.1016/j.cell.2017.11.008 PubMed: 29224777

Single Cell Gene Expression, Mouse, Cell Biology.

Abstract

There is considerable evidence that chromosome structure plays important roles in gene control, but we have limited understanding of the proteins that contribute to structural interactions between gene promoters and their enhancer elements. Large DNA loops that encompass genes and their regulatory elements depend on CTCF-CTCF interactions, but most enhancer-promoter interactions do not employ this structural protein. Here, we show that the ubiquitously expressed transcription factor Yin Yang 1 (YY1) contributes to enhancer-promoter structural interactions in a manner analogous to DNA interactions mediated by CTCF. YY1 binds to active enhancers and promoter-proximal elements and forms dimers that facilitate the interaction of these DNA elements. Deletion of YY1 binding sites or depletion of YY1 protein disrupts enhancer-promoter looping and gene expression. We propose that YY1-mediated enhancer-promoter interactions are a general feature of mammalian gene control.

Targeted Perturb-seq enables genome-scale genetic screens in single cells

Nature Methods (IF: 36.15), 2020-6, Lars Velten et al. DOI: 10.1038/s41592-020-0837-5 PubMed: 32483332

Single Cell Gene Expression, Human & Mouse, Assay Method.

Abstract

The transcriptome contains rich information on molecular, cellular and organismal phenotypes. However, experimental and statistical limitations constrain sensitivity and throughput of genetic screening with single-cell transcriptomics readout. To overcome these limitations, we introduce targeted Perturb-seq (TAP-seq), a sensitive, inexpensive and platform-independent method focusing single-cell RNA-seq coverage on genes of interest, thereby increasing the sensitivity and scale of genetic screens by orders of magnitude. TAP-seq permits routine analysis of thousands of CRISPR-mediated perturbations within a single experiment, detects weak effects and lowly expressed genes, and decreases sequencing requirements by up to 50-fold. We apply TAP-seq to generate perturbation-based enhancer-target gene maps for 1,778 enhancers within 2.5% of the human genome. We thereby show that enhancer-target association is jointly determined by three-dimensional contact frequency and epigenetic states, allowing accurate prediction of enhancer targets throughout the genome. In addition, we demonstrate that TAP-seq can identify cell subtypes with only 100 sequencing reads per cell.

Souporcell: robust clustering of single-cell RNA-seq data by genotype without reference genotypes

Nature Methods (IF: 36.15), 2020-6, Martin Hemberg et al. DOI: 10.1038/s41592-020-0820-1 PubMed: 32366989
Single Cell Gene Expression, Human, Plasmodium, Computational Method.

Abstract

Methods to deconvolve single-cell RNA-sequencing (scRNA-seq) data are necessary for samples containing a mixture of genotypes, whether they are natural or experimentally combined. Multiplexing across donors is a popular experimental design that can avoid batch effects, reduce costs and improve doublet detection. By using variants detected in scRNA-seq reads, it is possible to assign cells to their donor of origin and identify cross-genotype doublets that may have highly similar transcriptional profiles, precluding detection by transcriptional profile. More subtle cross-genotype variant contamination can be used to estimate the amount of ambient RNA. Ambient RNA is caused by cell lysis before droplet partitioning and is an important confounder of scRNA-seq analysis. Here we develop souporcell, a method to cluster cells using the genetic variants detected within the scRNA-seq reads. We show that it achieves high accuracy on genotype clustering, doublet detection and ambient RNA estimation, as demonstrated across a range of challenging scenarios.

Identification of a LIF-Responsive, Replication-Competent Subpopulation of Human β Cells

Cell Metabolism (IF: 24.29), 2020-2, Jennifer H Kenty et al. DOI: 10.1016/j.cmet.2019.12.009 PubMed: 31928884
Single Cell Gene Expression, Human, Cell Biology, Functional Genomics.

Abstract

The beta (β)-cell mass formed during embryogenesis is amplified by cell replication during fetal and early postnatal development. Thereafter, β cells become functionally mature, and their mass is maintained by a low rate of replication. For those few β cells that replicate in adult life, it is not known how replication is initiated nor whether this occurs in a specialized subset of β cells. We capitalized on a YAP overexpression system to induce replication of stem-cell-derived β cells and, by single-cell RNA sequencing, identified an upregulation of the leukemia inhibitory factor (LIF) pathway. Activation of the LIF pathway induces replication of human β cells in vitro and in vivo. The expression of the LIF receptor is restricted to a subset of transcriptionally distinct human β cells with increased proliferative capacity. This study delineates novel genetic networks that control the replication of LIF-responsive, replication-competent human β cells.

Naive Pluripotent Stem Cells Exhibit Phenotypic Variability that Is Driven by Genetic Variation

Cell Stem Cell (IF: 23.45), 2020-9, Laura G Reinholdt et al. DOI: 10.1016/j.stem.2020.07.019 PubMed: 32795399
Single Cell Gene Expression, Mouse, Developmental Biology, Genetic Health.

Abstract

Variability among pluripotent stem cell (PSC) lines is a prevailing issue that hampers not only experimental reproducibility but also large-scale applications and personalized cell-based therapy. This variability could result from epigenetic and genetic factors that influence stem cell behavior. Naive culture conditions minimize epigenetic fluctuation, potentially overcoming differences in PSC line differentiation potential. Here we derived PSCs from distinct mouse strains under naive conditions and show that lines from distinct genetic backgrounds have divergent differentiation capacity, confirming a major role for genetics in PSC phenotypic variability. This is explained in part through inconsistent activity of extra-cellular signaling, including the Wnt pathway, which is modulated by specific genetic variants. Overall, this study shows that genetic background plays a dominant role in driving phenotypic variability of PSCs.

A Multiplexed Barcodelet Single-Cell RNA-Seq Approach Elucidates Combinatorial Signaling Pathways that Drive ESC Differentiation

Cell Stem Cell (IF: 23.45), 2020-6, David K Gifford et al. DOI: 10.1016/j.stem.2020.04.020 PubMed: 32459995
Single Cell Gene Expression, Mouse, Assay Method, Developmental Biology.

Abstract

Empirical optimization of stem cell differentiation protocols is time consuming, is laborintensive, and typically does not comprehensively interrogate all relevant signaling pathways. Here we describe barcodelet single-cell RNA sequencing (barRNA-seq), which enables systematic exploration of cellular perturbations by tagging individual cells with RNA “barcodelets” to identify them on the basis of the treatments they receive. We apply barRNA-seq to simultaneously manipulate up to seven developmental pathways and study effects on embryonic stem cell (ESC) germ layer specification and mesodermal specification, uncovering combinatorial effects of signaling pathway activation on gene expression. We further develop a data-driven framework for identifying combinatorial signaling perturbations that drive cells toward specific fates, including several annotated in an existing scRNA-seq gastrulation atlas, and use this approach to guide ESC differentiation into a notochord-like population. We expect that barRNA-seq will have broad utility for investigating and understanding how cooperative signaling pathways drive cell fate acquisition.

Wnt Activation and Reduced Cell-Cell Contact Synergistically Induce Massive Expansion of Functional Human iPSC-Derived Cardiomyocytes

Cell Stem Cell (IF: 23.45), 2020-7, Joost P G Sluijter et al. DOI: 10.1016/j.stem.2020.06.001 PubMed: 32619518
Single Cell Gene Expression, Human, Cell Biology, Developmental Biology.

Abstract

Modulating signaling pathways including Wnt and Hippo can induce cardiomyocyte proliferation in vivo. Applying these signaling modulators to human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in vitro can expand CMs modestly (<5-fold). Here, we demonstrate massive expansion of hiPSC-CMs in vitro (i.e., 100- to 250-fold) by glycogen synthase kinase-3 β (GSK-3 β) inhibition using CHIR99021 and concurrent removal of cell-cell contact. We show that GSK-3 β inhibition suppresses CM maturation, while contact removal prevents CMs from cell cycle exit. Remarkably, contact removal enabled 10 to 25 times greater expansion beyond GSK-3 β inhibition alone. Mechanistically, persistent CM proliferation required both LEF/TCF activity and AKT phosphorylation but was independent from yes-associated protein (YAP) signaling. Engineered heart tissues from expanded hiPSC-CMs showed comparable contractility to those from unexpanded hiPSC-CMs, demonstrating uncompromised cellular functionality after expansion. In summary, we uncovered a molecular interplay that enables massive hiPSC-CM expansion for large-scale drug screening and tissue engineering applications.

Reconstructed Single-Cell Fate Trajectories Define Lineage Plasticity Windows during Differentiation of Human PSC-Derived Distal Lung Progenitors

Cell Stem Cell (IF: 23.45), 2020-4, Ziv Bar-Joseph et al. DOI: 10.1016/j.stem.2019.12.009 PubMed: 32004478
Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Alveolar epithelial type 2 cells (AEC2s) are the facultative progenitors responsible for maintaining lung alveoli throughout life but are difficult to isolate from patients. Here, we engineer AEC2s from human pluripotent stem cells (PSCs) in vitro and use time-series single-cell RNA sequencing with lentiviral barcoding to profile the kinetics of their differentiation in comparison to primary fetal and adult AEC2 benchmarks. We observe bifurcating cell-fate trajectories as primordial lung progenitors differentiate in vitro, with some progeny reaching their AEC2 fate target, while others diverge to alternative non-lung endodermal fates. We develop a Continuous State Hidden Markov model to identify the timing and type of signals, such as overexuberant Wnt responses, that induce some early multipotent NKX2-1 progenitors to lose lung fate. Finally, we find that this initial developmental plasticity is regulatable and subsides over time, ultimately resulting in PSC-derived AEC2s that exhibit a stable phenotype and nearly limitless self-renewal capacity.

Proneural factors Ascl1 and Neurog2 contribute to neuronal subtype identities by establishing distinct chromatin landscapes

Nature Neuroscience (IF: 21.5), 2019-5, Shaun Mahony et al. DOI: 10.1038/s41593-019-0399 PubMed: 31086315
Single Cell Gene Expression, Mouse, Developmental Biology, Neuroscience.

Abstract

Developmental programs that generate the astonishing neuronal diversity of the nervous system are not completely understood and thus present a major challenge for clinical applications of guided cell differentiation strategies. Using direct neuronal programming of embryonic stem cells, we found that two main vertebrate proneural factors, Ascl1 and neurogenin 2 (Neurog2), induce different neuronal fates by binding to largely different sets of genomic sites. Their divergent binding patterns are not determined by the previous chromatin state, but are distinguished by enrichment of specific E-box sequences that reflect the binding preferences of the DNA-binding domains. The divergent Ascl1 and Neurog2 binding patterns result in distinct chromatin accessibility and enhancer activity profiles that differentially shape the binding of downstream transcription factors during neuronal differentiation. This study provides a mechanistic understanding of how transcription factors constrain terminal cell fates, and it delineates the importance of choosing the right proneural factor in neuronal reprogramming strategies.

A Tppp3+Pdgfra+ tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis

Nature cell biology (IF: 20.96), 2019-11, Sara Flamenco et al. DOI: 10.1038/s41556-019-0417 PubMed: 31768046
Single Cell Gene Expression, Mouse, Cell Biology, Developmental Biology.

Abstract

Tendon injuries cause prolonged disability and never recover completely. Current mechanistic understanding of tendon regeneration is limited. Here, we use single-cell transcriptomics to identify a tubulin polymerization-promoting protein family member 3-expressing (Tppp3) cell population as potential tendon stem cells. Through inducible lineage tracing, we demonstrate that these cells can generate new tenocytes and self-renew upon injury. A fraction of Tppp3 cells expresses platelet-derived growth factor receptor alpha (Pdgfra). Ectopic platelet-derived growth factor-AA (PDGF-AA) protein induces new tenocyte production while inactivation of Pdgfra in Tppp3 cells blocks tendon regeneration. These results support Tppp3Pdgfra cells as tendon stem cells. Unexpectedly, Tppp3Pdgfra fibro-adipogenic progenitors coexist in the tendon stem cell niche and give rise to fibrotic cells, revealing a clandestine origin of fibrotic scars in healing tendons. Our results explain why fibrosis occurs in injured tendons and present clinical challenges to enhance tendon regeneration without a concurrent increase in fibrosis by PDGF application.

Multipotent RAG1⁺ progenitors emerge directly from haemogenic endothelium in human pluripotent stem cell-derived haematopoietic organoids

Nature cell biology (IF: 20.96), 2020-1, Andrew G Elefanty et al. DOI: 10.1038/s41556-019-0445-8

PubMed: 31907413

Single Cell Gene Expression, Human, Developmental Biology, Immunology.

Abstract

Defining the ontogeny of the human adaptive immune system during embryogenesis has implications for understanding childhood diseases including leukaemias and autoimmune conditions. Using RAG1:GFP human pluripotent stem cell reporter lines, we examined human T-cell genesis from pluripotent-stem-cell-derived haematopoietic organoids. Under conditions favouring T-cell development, RAG1⁺ cells progressively upregulated a cohort of recognized T-cell-associated genes, arresting development at the CD4⁺CD8⁺ stage. Sort and re-culture experiments showed that early RAG1⁺ cells also possessed B-cell, myeloid and erythroid potential. Flow cytometry and single-cell-RNA-sequencing data showed that early RAG1⁺ cells co-expressed the endothelial/haematopoietic progenitor markers CD34, VECAD and CD90, whereas imaging studies identified RAG1⁺ cells within CD31⁺ endothelial structures that co-expressed SOX17⁺ or the endothelial marker CAV1. Collectively, these observations provide evidence for a wave of human T-cell development that originates directly from haemogenic endothelium via a RAG1⁺ intermediate with multilineage potential.

Histone H3 lysine 4 monomethylation modulates long-range chromatin interactions at enhancers

Cell Research (IF: 20.4), 2018-2, Joanna Wysocka et al. DOI: 10.1038/cr.2018.1 PubMed: 29313530

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Long-range chromatin interactions between enhancers and promoters are essential for transcription of many developmentally controlled genes in mammals and other metazoans. Currently, the exact mechanisms that connect distal enhancers to their specific target promoters remain to be fully elucidated. Here, we show that the enhancer-specific histone H3 lysine 4 monomethylation (H3K4me1) and the histone methyltransferases MLL3 and MLL4 (MLL3/4) play an active role in this process. We demonstrate that in differentiating mouse embryonic stem cells, MLL3/4-dependent deposition of H3K4me1 at enhancers correlates with increased levels of chromatin interactions, whereas loss of this histone modification leads to reduced levels of chromatin interactions and defects in gene activation during differentiation. H3K4me1 facilitates recruitment of the Cohesin complex, a known regulator of chromatin organization, to chromatin in vitro and in vivo, providing a potential mechanism for MLL3/4 to promote chromatin interactions between enhancers and promoters. Taken together, our results support a role for MLL3/4-dependent H3K4me1 in orchestrating long-range chromatin interactions at enhancers in mammalian cells.

Effects of 3D culturing conditions on the transcriptomic profile of stem-cell-derived neurons

Nature Biomedical Engineering (IF: 18.95), 2018-7, Joshua Z LevinFeng Zhang DOI: 10.1038/s41551-018-0219-9 PubMed: 30271673

Single Cell Gene Expression, Human, Developmental Biology, Neuroscience.

Abstract

Understanding neurological diseases requires tractable genetic systems. Engineered 3D neural tissues are an attractive choice, but how the cellular transcriptomic profiles in these tissues are affected by the encapsulating materials and are related to the human-brain transcriptome is not well understood. Here, we report the characterization of the effects of culturing conditions on the transcriptomic profiles of induced neuronal cells, as well as a method for the rapid generation of 3D co-cultures of neuronal and astrocytic cells from the same pool of human embryonic stem cells. By comparing the gene-expression profiles of neuronal cells in culture conditions relevant to the developing human brain, we found that modifying the degree of crosslinking of composite hydrogels can tune expression patterns so they correlate with those of specific brain regions and developmental stages. Moreover, by using single-cell sequencing, we show that our engineered tissues recapitulate transcriptional patterns of cell types in the human brain. The analysis of culturing conditions will inform the development of 3D neural tissues for use as tractable models of brain diseases.

RNA Interactions Are Essential for CTCF-Mediated Genome Organization

Molecular Cell (IF: 16.13), 2019-11, Jane Skok et al. DOI: 10.1016/j.molcel.2019.08.015 PubMed: 31522988

Single Cell Gene Expression, Mouse, Functional Genomics.

Abstract

The function of the CCCTC-binding factor (CTCF) in the organization of the genome has become an important area of investigation, but the mechanisms by which CTCF dynamically contributes to genome organization are not clear. We previously discovered that CTCF binds to large numbers of endogenous RNAs, promoting its self-association. In this regard, we now report two independent features that disrupt CTCF association with chromatin: inhibition of transcription and disruption of CTCF-RNA interactions through mutations of 2 of its 11 zinc fingers that are not required for CTCF binding to its cognate DNA site: zinc finger 1 (ZF1) or zinc finger 10 (ZF10). These mutations alter gene expression profiles as CTCF mutants lose their ability to form chromatin loops and thus the ability to insulate chromatin domains and to mediate CTCF long-range genomic interactions. Our results point to the importance of CTCF-mediated RNA interactions as a structural component of genome organization.

Gene-edited human stem cell-derived β cells from a patient with monogenic diabetes reverse preexisting diabetes in mice

Science Translational Medicine (IF: 18.56), 2020-4, Fumihiko Urano et al. DOI: 10.1126/scitranslmed.aax9106
PubMed: 32321868

Single Cell Gene Expression, Human, Immunology.

Abstract

Differentiation of insulin-producing pancreatic β cells from induced pluripotent stem cells (iPSCs) derived from patients with diabetes promises to provide autologous cells for diabetes cell replacement therapy. However, current approaches produce patient iPSC-derived β (SC- β) cells with poor function in vitro and in vivo. Here, we used CRISPR-Cas9 to correct a diabetes-causing pathogenic variant in Wolfram syndrome 1 () in iPSCs derived from a patient with Wolfram syndrome (WS). After differentiation to β cells with our recent six-stage differentiation strategy, corrected WS SC- β cells performed robust dynamic insulin secretion in vitro in response to glucose and reversed preexisting streptozocin-induced diabetes after transplantation into mice. Single-cell transcriptomics showed that corrected SC- β cells displayed increased insulin and decreased expression of genes associated with endoplasmic reticulum stress. CRISPR-Cas9 correction of a diabetes-inducing gene variant thus allows for robust differentiation of autologous SC- β cells that can reverse severe diabetes in an animal model.

CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons

Neuron (IF: 16), 2019-10, Michael E Ward et al. DOI: 10.1016/j.neuron.2019.07.014 PubMed: 31422865

Single Cell Gene Expression, Human, Assay Method.

Abstract

CRISPR/Cas9-based functional genomics have transformed our ability to elucidate mammalian cell biology. However, most previous CRISPR-based screens were conducted in cancer cell lines rather than healthy, differentiated cells. Here, we describe a CRISPR interference (CRISPRi)-based platform for genetic screens in human neurons derived from induced pluripotent stem cells (iPSCs). We demonstrate robust and durable knockdown of endogenous genes in such neurons and present results from three complementary genetic screens. First, a survival-based screen revealed neuron-specific essential genes and genes that improved neuronal survival upon knockdown. Second, a screen with a single-cell transcriptomic readout uncovered several examples of genes whose knockdown had strikingly cell-type-specific consequences. Third, a longitudinal imaging screen detected distinct consequences of gene knockdown on neuronal morphology. Our results highlight the power of unbiased genetic screens in iPSC-derived differentiated cell types and provide a platform for systematic interrogation of normal and disease states of neurons. VIDEO ABSTRACT.

Resolving Cell Fate Decisions during Somatic Cell Reprogramming by Single-Cell RNA-Seq

Molecular Cell (IF: 16.13), 2019-2, Duanqing Pei et al. DOI: 10.1016/j.molcel.2019.01.042 PubMed: 30772174

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs), which is a highly heterogeneous process. Here we report the cell fate continuum during somatic cell reprogramming at single-cell resolution. We first develop SOT to analyze cell fate continuum from Oct4/Sox2/Klf4- or OSK-mediated reprogramming and show that cells bifurcate into two categories, reprogramming potential (RP) or non-reprogramming (NR). We further show that Klf4 contributes to Cd34+/Fxyd5+/Psc+ keratinocyte-like NR fate and that IFN- γ impedes the final transition to chimera-competent pluripotency along the RP cells. We analyze more than 150,000 single cells from both OSK and chemical reprogramming and identify additional NR/RP bifurcation points. Our work reveals a generic bifurcation model for cell fate decisions during somatic cell reprogramming that may be applicable to other systems and inspire further improvements for reprogramming.

A Single-Cell Transcriptomics CRISPR-Activation Screen Identifies Epigenetic Regulators of the Zygotic Genome Activation Program

Cell systems (IF: 11.45), 2020-7, Oliver Stegle et al. DOI: 10.1016/j.cels.2020.06.004 PubMed: 32634384

Single Cell Gene Expression, Mouse, Developmental Biology, Functional Genomics.

Abstract

Zygotic genome activation (ZGA) is an essential transcriptional event in embryonic development that coincides with extensive epigenetic reprogramming. Complex manipulation techniques and maternal stores of proteins preclude large-scale functional screens for ZGA regulators within early embryos. Here, we combined pooled CRISPR activation (CRISPRa) with single-cell transcriptomics to identify regulators of ZGA-like transcription in mouse embryonic stem cells, which serve as a tractable, in vitro proxy of early mouse embryos. Using multi-omics factor analysis (MOFA+) applied to ~200,000 single-cell transcriptomes comprising 230 CRISPRa perturbations, we characterized molecular signatures of ZGA and uncovered 24 factors that promote a ZGA-like response. Follow-up assays validated top screen hits, including the DNA-binding protein Dppa2, the chromatin remodeler Smarca5, and the transcription factor Patz1, and functional experiments revealed that Smarca5's regulation of ZGA-like transcription is dependent on Dppa2. Together, our single-cell transcriptomic profiling of CRISPRa-perturbed cells provides both system-level and molecular insights into the mechanisms that orchestrate ZGA.

Isolation of Cardiomyocytes Undergoing Mitosis With Complete Cytokinesis

Circulation Research (IF: 15.63), 2019-12, Eric J Kort et al. DOI: 10.1161/CIRCRESAHA.119.314908

PubMed: 31648614

Single Cell Gene Expression, Human, Cell Biology.

Abstract

Adult human cardiomyocytes do not complete cytokinesis despite passing through the S-phase of the cell cycle. As a result, polyploidization and multinucleation occur. To get a deeper understanding of the mechanisms surrounding division of cardiomyocytes, there is a crucial need for a technique to isolate cardiomyocytes that complete cell division/cytokinesis. Markers of cell cycle progression based on DNA content cannot distinguish between mitotic cardiomyocytes that fail to complete cytokinesis from those cells that undergo true cell division. With the use of molecular beacons (MBs) targeting specific mRNAs, we aimed to identify truly proliferative cardiomyocytes derived from human induced pluripotent stem cells. Fluorescence-activated cell sorting combined with MBs was performed to sort cardiomyocyte populations enriched for mitotic cells. Expressions of cell cycle specific genes were confirmed by means of reverse transcription-quantitative polymerase chain reaction and single-cell RNA sequencing (scRNA-seq) combined with gene signatures of cell cycle progression. We characterized the sorted groups by proliferation assays and time-lapse microscopy which confirmed the proliferative advantage of MB-positive cell populations relative to MB-negative and G2/M populations. Gene expression analysis revealed that the MB-positive cardiomyocyte subpopulation exhibited patterns consistent with the processes of nuclear division, chromosome segregation, and transition from M to G1 phase. The use of dual-MBs targeting and mRNAs enabled the enrichment of cytokinetic events (). Interestingly, cells that did not complete cytokinesis and remained binucleated were found to be while polyploid cardiomyocytes that replicated DNA but failed to complete karyokinesis were found to be. This study demonstrates a novel alternative to existing DNA content-based approaches for sorting cardiomyocytes with true mitotic potential that can be used to study the unique dynamics of cardiomyocyte nuclei during mitosis. Our technique for sorting live cardiomyocytes undergoing cytokinesis would provide a basis for future studies to uncover mechanisms underlying the development and regeneration of heart tissue.

Large-Scale Single-Cell RNA-Seq Reveals Molecular Signatures of Heterogeneous Populations of Human Induced Pluripotent Stem Cell-Derived Endothelial Cells

Circulation Research (IF: 15.63), 2018-8, Thomas Quertermous Joseph C Wu et al.

DOI: 10.1161/CIRCRESAHA.118.312913 PubMed: 29986945

Single Cell Gene Expression, Human, Cell Atlas, Developmental Biology.

Abstract

Human-induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) have risen as a useful tool in cardiovascular research, offering a wide gamut of translational and clinical applications. However, inefficiency of the currently available iPSC-EC differentiation protocol and underlying heterogeneity of derived iPSC-ECs remain as major limitations of iPSC-EC technology. Here, we performed droplet-based single-cell RNA sequencing (scRNA-seq) of the human iPSCs after iPSC-EC differentiation. Droplet-based scRNA-seq enables analysis of thousands of cells in parallel, allowing comprehensive analysis of transcriptional heterogeneity. Bona fide iPSC-EC cluster was identified by scRNA-seq, which expressed high levels of endothelial-specific genes. iPSC-ECs, sorted by CD144 antibody-conjugated magnetic sorting, exhibited standard endothelial morphology and function including tube formation, response to inflammatory signals, and production of NO. Nonendothelial cell populations resulting from the differentiation protocol were identified, which included immature cardiomyocytes, hepatic-like cells, and vascular smooth muscle cells. Furthermore, scRNA-seq analysis of purified iPSC-ECs revealed transcriptional heterogeneity with 4 major subpopulations, marked by robust enrichment of CLDN5, APLNR, GJA5, and ESM1 genes, respectively. Massively parallel, droplet-based scRNA-seq allowed meticulous analysis of thousands of human iPSCs subjected to iPSC-EC differentiation. Results showed inefficiency of the differentiation technique, which can be improved with further studies based on identification of molecular signatures that inhibit expansion of nonendothelial cell types. Subtypes of bona fide human iPSC-ECs were also identified, allowing us to sort for iPSC-ECs with specific biological function and identity.

A method for the generation of human stem cell-derived alpha cells

Nature Communications (IF: 13.61), 2020-5, Patrik Rorsman et al. DOI: 10.1038/s41467-020-16049-3

PubMed: 32382023

Single Cell Gene Expression, Human, Assay Method, Developmental Biology.

Abstract

The generation of pancreatic cell types from renewable cell sources holds promise for cell replacement therapies for diabetes. Although most effort has focused on generating pancreatic beta cells, considerable evidence indicates that glucagon secreting alpha cells are critically involved in disease progression and proper glucose control. Here we report on the generation of stem cell-derived human pancreatic alpha (SC-alpha) cells from pluripotent stem cells via a transient pre-alpha cell intermediate. These pre-alpha cells exhibit a transcriptional profile similar to mature alpha cells and although they produce proinsulin protein, they do not secrete significant amounts of processed insulin. Compound screening identified a protein kinase c activator that promotes maturation of pre-alpha cells into SC-alpha cells. The resulting SC-alpha cells do not express insulin, share an ultrastructure similar to cadaveric alpha cells, express and secrete glucagon in response to glucose and some glucagon secretagogues, and elevate blood glucose upon transplantation in mice.

Single cell analyses and machine learning define hematopoietic progenitor and HSC-like cells derived from human PSCs

Blood (IF: 14.82), 2020-7, Nicola Romanò et al. DOI: 10.1182/blood.2020006229 PubMed: 32614947

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Haematopoietic stem and progenitor cells (HSPCs) develop through distinct waves at various anatomical sites during embryonic development. The in vitro differentiation of human pluripotent stem cells (hPSCs) is able to recapitulate some of these processes, however, it has proven difficult to generate functional haematopoietic stem cells (HSCs). To define the dynamics and heterogeneity of HSPCs that can be generated in vitro from hPSCs, we exploited single cell RNA sequencing (scRNAseq) in combination with single cell protein expression analysis. Bioinformatics analyses and functional validation defined the transcriptomes of naïve progenitors as well as erythroid, megakaryocyte and leukocyte-committed progenitors and we identified CD44, CD326, ICAM2/CD9 and CD18 as markers of these progenitors, respectively. Using an artificial neural network (ANN), that we trained on a scRNAseq derived from human fetal liver, we were able to identify a wide range of hPSCs-derived HPSC phenotypes, including a small group classified as HSCs. This transient HSC-like population decreased as differentiation proceeded and was completely missing in the dataset that had been generated using cells selected on the basis of CD43 expression. By comparing the single cell transcriptome of in vitro-generated HSC-like cells with those generated within the fetal liver we identified transcription factors and molecular pathways that can be exploited in the future to improve the in vitro production of HSCs.

Loss of the transcription factor MAFB limits β -cell derivation from human PSCs

Nature Communications (IF: 13.61), 2020-6, Roland Stein et al. DOI: 10.1038/s41467-020-16550-9

PubMed: 32488111

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

Next generation sequencing studies have highlighted discrepancies in β -cells which exist between mice and men. Numerous reports have identified MAF BZIP Transcription Factor B (MAFB) to be present in human β -cells postnatally, while its expression is restricted to embryonic and neo-natal β -cells in mice. Using CRISPR/Cas9-mediated gene editing, coupled with endocrine cell differentiation strategies, we dissect the contribution of MAFB to β -cell development and function specifically in humans. Here we report that MAFB knockout hPSCs have normal pancreatic differentiation capacity up to the progenitor stage, but favor somatostatin- and pancreatic polypeptide-positive cells at the expense of insulin- and glucagon-producing cells during endocrine cell development. Our results describe a requirement for MAFB late in the human pancreatic developmental program and identify it as a distinguishing transcription factor within islet cell subtype specification. We propose that hPSCs represent a powerful tool to model human pancreatic endocrine development and associated disease pathophysiology.

Identification of cell surface markers and establishment of monolayer differentiation to retinal pigment epithelial cells

Nature Communications (IF: 13.61), 2020-12, Anders Kvanta et al. DOI: 10.1038/s41467-020-15326-5

PubMed: 32231223

Single Cell Gene Expression, Human, Developmental Biology.

Abstract

In vitro differentiation of human pluripotent stem cells into functional retinal pigment epithelial (RPE) cells provides a potentially unlimited source for cell based reparative therapy of age-related macular degeneration. Although the inherent pigmentation of the RPE cells have been useful to grossly evaluate differentiation efficiency and allowed manual isolation of pigmented structures, accurate quantification and automated isolation has been challenging. To address this issue, here we perform a comprehensive antibody screening and identify cell surface markers for RPE cells. We show that these markers can be used to isolate RPE cells during in vitro differentiation and to track, quantify and improve differentiation efficiency. Finally, these surface markers aided to develop a robust, direct and scalable monolayer differentiation protocol on human recombinant laminin-111 and -521 without the need for manual isolation.

Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells

Nature Communications (IF: 13.61), 2020-1, Finn Hawkins et al. DOI: 10.1038/s41467-019-13916-6

PubMed: 31924806

Single Cell Gene Expression, Human, Developmental Biology, Genetic Health.

Abstract

Efficient generation of human induced pluripotent stem cell (hiPSC)-derived human intestinal organoids (HIOs) would facilitate the development of in vitro models for a variety of diseases that affect the gastrointestinal tract, such as inflammatory bowel disease or Cystic Fibrosis. Here, we report a directed differentiation protocol for the generation of mesenchyme-free HIOs that can be primed towards more colonic or proximal intestinal lineages in serum-free defined conditions. Using a CDX2iPSC knock-in reporter line to track the emergence of hindgut progenitors, we follow the kinetics of CDX2 expression throughout directed differentiation, enabling the purification of intestinal progenitors and robust generation of mesenchyme-free organoids expressing characteristic markers of small intestinal or colonic epithelium. We employ HIOs generated in this way to measure CFTR function using cystic fibrosis patient-derived iPSC lines before and after correction of the CFTR mutation, demonstrating their future potential for disease modeling and therapeutic screening applications.

Immune-evasive human islet-like organoids ameliorate diabetes

Nature (IF: 46.49), 2020-8, Michael Downes et al. DOI: 10.1038/s41586-020-2631 PubMed: 32814902

Single Cell Gene Expression, Human, Immunology.

Abstract

Islets derived from stem cells hold promise as a therapy for insulin-dependent diabetes, but there remain challenges towards achieving this goal. Here we generate human islet-like organoids (HILOs) from induced pluripotent stem cells and show that non-canonical WNT4 signalling drives the metabolic maturation necessary for robust ex vivo glucose-stimulated insulin secretion. These functionally mature HILOs contain endocrine-like cell types that, upon transplantation, rapidly re-establish glucose homeostasis in diabetic NOD/SCID mice. Overexpression of the immune checkpoint protein programmed death-ligand 1 (PD-L1) protected HILO xenografts such that they were able to restore glucose homeostasis in immune-competent diabetic mice for 50 days. Furthermore, ex vivo stimulation with interferon- γ induced endogenous PD-L1 expression and restricted T cell activation and graft rejection. The generation of glucose-responsive islet-like organoids that are able to avoid immune detection provides a promising alternative to cadaveric and device-dependent therapies in the treatment of diabetes.

Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas

Nature Biotechnology (IF: 42.3), 2020-1, Diane M Simeone et al. DOI: 10.1038/s41587-019-0392-8

PubMed: 31932730

Spatial Gene Expression, Human, Cancer Research, Cell Atlas.

Abstract

Single-cell RNA sequencing (scRNA-seq) enables the systematic identification of cell populations in a tissue, but characterizing their spatial organization remains challenging. We combine a microarray-based spatial transcriptomics method that reveals spatial patterns of gene expression using an array of spots, each capturing the transcriptomes of multiple adjacent cells, with scRNA-Seq generated from the same sample. To annotate the precise cellular composition of distinct tissue regions, we introduce a method for multimodal intersection analysis. Applying multimodal intersection analysis to primary pancreatic tumors, we find that subpopulations of ductal cells, macrophages, dendritic cells and cancer cells have spatially restricted enrichments, as well as distinct coenrichments with other cell types. Furthermore, we identify colocalization of inflammatory fibroblasts and cancer cells expressing a stress-response gene module. Our approach for mapping the architecture of scRNA-seq-defined subpopulations can be applied to reveal the interactions inherent to complex tissues.

Endocrine-Exocrine Signaling Drives Obesity-Associated Pancreatic Ductal Adenocarcinoma

Cell (IF: 38.62), 2020-5, Charles S Fuchs et al. DOI: 10.1016/j.cell.2020.03.062 PubMed: 32304665

Single Cell Gene Expression, Mouse, Cancer Research, Genetic Health.

Abstract

Obesity is a major modifiable risk factor for pancreatic ductal adenocarcinoma (PDAC), yet how and when obesity contributes to PDAC progression is not well understood. Leveraging an autochthonous mouse model, we demonstrate a causal and reversible role for obesity in early PDAC progression, showing that obesity markedly enhances tumorigenesis, while genetic or dietary induction of weight loss intercepts cancer development. Molecular analyses of human and murine samples define microenvironmental consequences of obesity that foster tumorigenesis rather than new driver gene mutations, including significant pancreatic islet cell adaptation in obesity-associated tumors. Specifically, we identify aberrant beta cell expression of the peptide hormone cholecystokinin (Cck) in response to obesity and show that islet Cck promotes oncogenic Kras-driven pancreatic ductal tumorigenesis. Our studies argue that PDAC progression is driven by local obesity-associated changes in the tumor microenvironment and implicate endocrine-exocrine signaling beyond insulin in PDAC development.

Long-Term Expansion of Pancreatic Islet Organoids from Resident Procr+ Progenitors

Cell (IF: 38.62), 2020-3, Hans Clevers et al. DOI: 10.1016/j.cell.2020.02.048 PubMed: 32200801

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

It has generally proven challenging to produce functional β cells in vitro. Here, we describe a previously unidentified protein C receptor positive (Procr) cell population in adult mouse pancreas through single-cell RNA sequencing (scRNA-seq). The cells reside in islets, do not express differentiation markers, and feature epithelial-to-mesenchymal transition characteristics. By genetic lineage tracing, Procrislet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long periods by serial passaging, while differentiation can be induced at any time point in culture. β cells dominate in differentiated islet organoids, while α , δ , and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, these organoids reverse disease. These findings demonstrate that the adult mouse pancreatic islet contains a population of Procr endocrine progenitors.

A Multiscale Map of the Stem Cell State in Pancreatic Adenocarcinoma

Cell (IF: 38.62), 2019-4, Peter D Adams et al. DOI: 10.1016/j.cell.2019.03.010 PubMed: 30955884

Single Cell Gene Expression, Human & Mouse, Cancer Research.

Abstract

Drug resistance and relapse remain key challenges in pancreatic cancer. Here, we have used RNA sequencing (RNA-seq), chromatin immunoprecipitation (ChIP)-seq, and genome-wide CRISPR analysis to map the molecular dependencies of pancreatic cancer stem cells, highly therapy-resistant cells that preferentially drive tumorigenesis and progression. This integrated genomic approach revealed an unexpected utilization of immuno-regulatory signals by pancreatic cancer epithelial cells. In particular, the nuclear hormone receptor retinoic-acid-receptor-related orphan receptor gamma (ROR γ), known to drive inflammation and T cell differentiation, was upregulated during pancreatic cancer progression, and its genetic or pharmacologic inhibition led to a striking defect in pancreatic cancer growth and a marked improvement in survival. Further, a large-scale retrospective analysis in patients revealed that ROR γ expression may predict pancreatic cancer aggressiveness, as it positively correlated with advanced disease and metastasis. Collectively, these data identify an orthogonal co-option of immuno-regulatory signals by pancreatic cancer stem cells, suggesting that autoimmune drugs should be evaluated as novel treatment strategies for pancreatic cancer patients.

Regulatory T-cell Depletion Alters the Tumor Microenvironment and Accelerates Pancreatic Carcinogenesis

Cancer Discovery (IF: 28.3), 2020-3, Filip Bednar et al. DOI: 10.1158/2159-8290.CD-19-0958 PubMed: 31911451

Single Cell Gene Expression, Mouse, Cancer Research, Immunology.

Abstract

Regulatory T cells (Treg) are abundant in human and mouse pancreatic cancer. To understand the contribution to the immunosuppressive microenvironment, we depleted Tregs in a mouse model of pancreatic cancer. Contrary to our expectations, Treg depletion failed to relieve immunosuppression and led to accelerated tumor progression. We show that Tregs are a key source of TGF β ligands and, accordingly, their depletion reprogramed the fibroblast population, with loss of tumor-restraining, smooth muscle actin-expressing fibroblasts. Conversely, we observed an increase in chemokines, and leading to increased myeloid cell recruitment, restoration of immune suppression, and promotion of carcinogenesis, an effect that was inhibited by blockade of the common CCL3/6/8 receptor CCR1. Further, Treg depletion unleashed pathologic CD4T-cell responses. Our data point to new mechanisms regulating fibroblast differentiation in pancreatic cancer and support the notion that fibroblasts are a heterogeneous population with different and opposing functions in pancreatic carcinogenesis. SIGNIFICANCE: Here, we describe an unexpected cross-talk between Tregs and fibroblasts in pancreatic cancer. Treg depletion resulted in differentiation of inflammatory fibroblast subsets, in turn driving infiltration of myeloid cells through CCR1, thus uncovering a potentially new therapeutic approach to relieve immunosuppression in pancreatic cancer.

Transcription phenotypes of pancreatic cancer are driven by genomic events during tumor evolution

Nature Genetics (IF: 30.33), 2020-1, Steven Gallinger et al. DOI: 10.1038/s41588-019-0566-9 PubMed: 31932696
Single Cell Gene Expression, Human, Cancer Research, Genome Analysis.

Abstract

Pancreatic adenocarcinoma presents as a spectrum of a highly aggressive disease in patients. The basis of this disease heterogeneity has proved difficult to resolve due to poor tumor cellularity and extensive genomic instability. To address this, a dataset of whole genomes and transcriptomes was generated from purified epithelium of primary and metastatic tumors. Transcriptome analysis demonstrated that molecular subtypes are a product of a gene expression continuum driven by a mixture of intratumoral subpopulations, which was confirmed by single-cell analysis. Integrated whole-genome analysis uncovered that molecular subtypes are linked to specific copy number aberrations in genes such as mutant KRAS and GATA6. By mapping tumor genetic histories, tetraploidization emerged as a key mutational process behind these events. Taken together, these data support the premise that the constellation of genomic aberrations in the tumor gives rise to the molecular subtype, and that disease heterogeneity is due to ongoing genomic instability during progression.

Single-Cell RNA Sequencing Reveals Stromal Evolution into LRRC15+ Myofibroblasts as a Determinant of Patient Response to Cancer Immunotherapy

Cancer Discovery (IF: 28.3), 2020-2, Richard Bourgon et al. DOI: 10.1158/2159-8290.CD-19-0644
PubMed: 31699795

Single Cell Gene Expression, Mouse, Immuno-oncology.

Abstract

With only a fraction of patients responding to cancer immunotherapy, a better understanding of the entire tumor microenvironment is needed. Using single-cell transcriptomics, we chart the fibroblastic landscape during pancreatic ductal adenocarcinoma (PDAC) progression in animal models. We identify a population of carcinoma-associated fibroblasts (CAF) that are programmed by TGF β and express the leucine-rich repeat containing 15 (LRRC15) protein. These LRRC15CAFs surround tumor islets and are absent from normal pancreatic tissue. The presence of LRRC15CAFs in human patients was confirmed in >80,000 single cells from 22 patients with PDAC as well as by using IHC on samples from 70 patients. Furthermore, immunotherapy clinical trials comprising more than 600 patients across six cancer types revealed elevated levels of the LRRC15CAF signature correlated with poor response to anti-PD-L1 therapy. This work has important implications for targeting nonimmune elements of the tumor microenvironment to boost responses of patients with cancer to immune checkpoint blockade therapy. SIGNIFICANCE: This study describes the single-cell landscape of CAFs in pancreatic cancer during tumor evolution. A TGF β -driven, LRRC15CAF lineage is associated with poor outcome in immunotherapy trial data comprising multiple solid-tumor entities and represents a target for combinatorial therapy..

Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts

Cancer Discovery (IF: 28.3), 2019-8, Paul Robson et al. DOI: 10.1158/2159-8290.CD-19-0094 PubMed: 31197017
Single Cell Gene Expression, Human & Mouse, Cancer Research.

Abstract

Cancer-associated fibroblasts (CAF) are major players in the progression and drug resistance of pancreatic ductal adenocarcinoma (PDAC). CAFs constitute a diverse cell population consisting of several recently described subtypes, although the extent of CAF heterogeneity has remained undefined. Here we use single-cell RNA sequencing to thoroughly characterize the neoplastic and tumor microenvironment content of human and mouse PDAC tumors. We corroborate the presence of myofibroblastic CAFs and inflammatory CAFs and define their unique gene signatures. Moreover, we describe a new population of CAFs that express MHC class II and CD74, but do not express classic costimulatory molecules. We term this cell population antigen-presenting CAFs and find that they activate CD4T cells in an antigen-specific fashion in a model system, confirming their putative immunomodulatory capacity. Our cross-species analysis paves the way for investigating distinct functions of CAF subtypes in PDAC immunity and progression. SIGNIFICANCE: Appreciating the full spectrum of fibroblast heterogeneity in pancreatic ductal adenocarcinoma is crucial to developing therapies that specifically target tumor-promoting CAFs. This work identifies MHC class II-expressing CAFs with a capacity to present antigens to CD4T cells, and potentially to modulate the immune response in pancreatic tumors...

Beta Cell Dedifferentiation Induced by IRE1 α Deletion Prevents Type 1 Diabetes

Cell Metabolism (IF: 24.29), 2020-4, Rupa Sridharan et al. DOI: 10.1016/j.cmet.2020.03.002 PubMed: 32220307
Single Cell Gene Expression, Mouse, Immunology.

Abstract

Immune-mediated destruction of insulin-producing β cells causes type 1 diabetes (T1D). However, how β cells participate in their own destruction during the disease process is poorly understood. Here, we report that modulating the unfolded protein response (UPR) in β cells of non-obese diabetic (NOD) mice by deleting the UPR sensor IRE1 α prior to insulinitis induced a transient dedifferentiation of β cells, resulting in substantially reduced islet immune cell infiltration and β cell apoptosis. Single-cell and whole-islet transcriptomics analyses of immature β cells revealed remarkably diminished expression of β cell autoantigens and MHC class I components, and upregulation of immune inhibitory markers. IRE1 α -deficient mice exhibited significantly fewer cytotoxic CD8T cells in their pancreata, and adoptive transfer of their total T cells did not induce diabetes in Rag1 mice. Our results indicate that inducing β cell dedifferentiation, prior to insulinitis, allows these cells to escape immune-mediated destruction and may be used as a novel preventive strategy for T1D in high-risk individuals.

IL1-Induced JAK/STAT Signaling Is Antagonized by TGF β to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma

Cancer Discovery (IF: 28.3), 2019-2, Jonathan Preall et al. DOI: 10.1158/2159-8290.CD-18-0710

PubMed: 30366930

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is poorly responsive to therapies and histologically contains a paucity of neoplastic cells embedded within a dense desmoplastic stroma. Within the stroma, cancer-associated fibroblasts (CAF) secrete tropic factors and extracellular matrix components, and have been implicated in PDAC progression and chemotherapy resistance. We recently identified two distinct CAF subtypes characterized by either myofibroblastic or inflammatory phenotypes; however, the mechanisms underlying their diversity and their roles in PDAC remain unknown. Here, we use organoid and mouse models to identify TGF β and IL1 as tumor-secreted ligands that promote CAF heterogeneity. We show that IL1 induces LIF expression and downstream JAK/STAT activation to generate inflammatory CAFs and demonstrate that TGF β antagonizes this process by downregulating IL1R1 expression and promoting differentiation into myofibroblasts. Our results provide a mechanism through which distinct fibroblast niches are established in the PDAC microenvironment and illuminate strategies to selectively target CAFs that support tumor growth. SIGNIFICANCE: Understanding the mechanisms that determine CAF heterogeneity in PDAC is a prerequisite for the rational development of approaches that selectively target tumor-promoting CAFs. Here, we identify an IL1-induced signaling cascade that leads to JAK/STAT activation and promotes an inflammatory CAF state, suggesting multiple strategies to target these cells..

Single-cell RNA-seq with spike-in cells enables accurate quantification of cell-specific drug effects in pancreatic islets

Genome Biology (IF: 19.04), 2020-5, Christoph Bock et al. DOI: 10.1186/s13059-020-02006-2 PubMed: 32375897

Single Cell Gene Expression, Human & Mouse, Assay Method, Immunology.

Abstract

Single-cell RNA-seq (scRNA-seq) is emerging as a powerful tool to dissect cell-specific effects of drug treatment in complex tissues. This application requires high levels of precision, robustness, and quantitative accuracy-beyond those achievable with existing methods for mainly qualitative single-cell analysis. Here, we establish the use of standardized reference cells as spike-in controls for accurate and robust dissection of single-cell drug responses. We find that contamination by cell-free RNA can constitute up to 20% of reads in human primary tissue samples, and we show that the ensuing biases can be removed effectively using a novel bioinformatics algorithm. Applying our method to both human and mouse pancreatic islets treated ex vivo, we obtain an accurate and quantitative assessment of cell-specific drug effects on the transcriptome. We observe that FOXO inhibition induces dedifferentiation of both alpha and beta cells, while artemether treatment upregulates insulin and other beta cell marker genes in a subset of alpha cells. In beta cells, dedifferentiation and insulin repression upon artemether treatment occurs predominantly in mouse but not in human samples. This new method for quantitative, error-correcting, scRNA-seq data normalization using spike-in reference cells helps clarify complex cell-specific effects of pharmacological perturbations with single-cell resolution and high quantitative accuracy.

Mesenchymal Plasticity Regulated by Prrx1 Drives Aggressive Pancreatic Cancer Biology

Gastroenterology (IF: 18.79), 2020-9, Dieter Saur et al. DOI: 10.1053/j.gastro.2020.09.010 PubMed: 33007300
Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a fibroblast-rich desmoplastic stroma. Cancer-associated fibroblasts (CAFs) have been shown to display a high degree of interconvertible states including quiescent, inflammatory, and myofibroblastic phenotypes; however, the mechanisms by which this plasticity is achieved are poorly understood. Here, we aim to elucidate the role of CAF plasticity and its impact on PDAC biology. To investigate the role of mesenchymal plasticity in PDAC progression, we generated a PDAC mouse model in which CAF plasticity is modulated by genetic depletion of the transcription factor Prrx1. Primary pancreatic fibroblasts from this mouse model were further characterized by functional in vitro assays. To characterize the impact of CAFs on tumor differentiation and response to chemotherapy, various coculture experiments were performed. In vivo, tumors were characterized by morphology, extracellular matrix composition, and tumor dissemination and metastasis. Our in vivo findings showed that Prrx1-deficient CAFs remain constitutively activated. Importantly, this CAF phenotype determines tumor differentiation and disrupts systemic tumor dissemination. Mechanistically, coculture experiments of tumor organoids and CAFs showed that CAFs shape the epithelial-to-mesenchymal phenotype and confer gemcitabine resistance of PDAC cells induced by CAF-derived hepatocyte growth factor. Furthermore, gene expression analysis showed that patients with pancreatic cancer with high stromal expression of Prrx1 display the squamous, most aggressive, subtype of PDAC. Here, we define that the Prrx1 transcription factor is critical for tuning CAF activation, allowing a dynamic switch between a dormant and an activated state. This work shows that Prrx1-mediated CAF plasticity has significant impact on PDAC biology and therapeutic resistance.

Targeting TGF β R2-mutant tumors exposes vulnerabilities to stromal TGF β blockade in pancreatic cancer

EMBO Molecular Medicine (IF: 10.37), 2019-11, Kyla E Driscoll et al. DOI: 10.15252/emmm.201910515
PubMed: 31609088

Single Cell Gene Expression, Mouse, Cancer Research.

Abstract

TGF β is important during pancreatic ductal adenocarcinoma (PDA) progression. Canonical TGF β signaling suppresses epithelial pancreatic cancer cell proliferation; as a result, inhibiting TGF β has not been successful in PDA. In contrast, we demonstrate that inhibition of stromal TGF β R2 reduces IL-6 production from cancer-associated fibroblasts, resulting in a reduction of STAT3 activation in tumor cells and reversion of the immunosuppressive landscape. Up to 7% of human PDA have tumor cell-specific deficiency in canonical TGF β signaling via loss of TGF β R2. We demonstrate that in PDA that harbors epithelial loss of TGF β R2, inhibition of TGF β signaling is selective for stromal cells and results in a therapeutic benefit. Our study highlights the potential benefit of TGF β blockade in PDA and the importance of stratifying PDA patients who might benefit from such therapy.

Lineage dynamics of murine pancreatic development at single-cell resolution

Nature Communications (IF: 13.61), 2018-9, Chun J Ye et al. DOI: 10.1038/s41467-018-06176-3

PubMed: 30254276

Single Cell Gene Expression, Mouse, Developmental Biology.

Abstract

Organogenesis requires the complex interactions of multiple cell lineages that coordinate their expansion, differentiation, and maturation over time. Here, we profile the cell types within the epithelial and mesenchymal compartments of the murine pancreas across developmental time using a combination of single-cell RNA sequencing, immunofluorescence, in situ hybridization, and genetic lineage tracing. We identify previously underappreciated cellular heterogeneity of the developing mesenchyme and reconstruct potential lineage relationships among the pancreatic mesothelium and mesenchymal cell types. Within the epithelium, we find a previously undescribed endocrine progenitor population, as well as an analogous population in both human fetal tissue and human embryonic stem cells differentiating toward a pancreatic beta cell fate. Further, we identify candidate transcriptional regulators along the differentiation trajectory of this population toward the alpha or beta cell lineages. This work establishes a roadmap of pancreatic development and demonstrates the broad utility of this approach for understanding lineage dynamics in developing organs.

Sel1L-Hrd1 ER-associated degradation maintains β cell identity via TGF- β signaling

Journal of clinical investigation, The (IF: 12.81), 2020-7, Peter Arvan et al. DOI: 10.1172/JCI134874

PubMed: 32182217

Single Cell Gene Expression, Mouse, Immunology.

Abstract

β Cell apoptosis and dedifferentiation are 2 hotly debated mechanisms underlying β cell loss in type 2 diabetes; however, the molecular drivers underlying such events remain largely unclear. Here, we performed a side-by-side comparison of mice carrying β cell-specific deletion of ER-associated degradation (ERAD) and autophagy. We reported that, while autophagy was necessary for β cell survival, the highly conserved Sel1L-Hrd1 ERAD protein complex was required for the maintenance of β cell maturation and identity. Using single-cell RNA-Seq, we demonstrated that Sel1L deficiency was not associated with β cell loss, but rather loss of β cell identity. Sel1L-Hrd1 ERAD controlled β cell identity via TGF- β signaling, in part by mediating the degradation of TGF- β receptor 1. Inhibition of TGF- β signaling in Sel1L-deficient β cells augmented the expression of β cell maturation markers and increased the total insulin content. Our data revealed distinct pathogenic effects of 2 major proteolytic pathways in β cells, providing a framework for therapies targeting distinct mechanisms of protein quality control.

SAME-clustering: Single-cell Aggregated Clustering via Mixture Model Ensemble

Nucleic Acids Research (IF: 11.8), 2020-1, Yin Shen et al. DOI: 10.1093/nar/gkz959 PubMed: 31777938

Single Cell Gene Expression, Human & Mouse, Computational Method.

Abstract

Clustering is an essential step in the analysis of single cell RNA-seq (scRNA-seq) data to shed light on tissue complexity including the number of cell types and transcriptomic signatures of each cell type. Due to its importance, novel methods have been developed recently for this purpose. However, different approaches generate varying estimates regarding the number of clusters and the single-cell level cluster assignments. This type of unsupervised clustering is challenging and it is often times hard to gauge which method to use because none of the existing methods outperform others across all scenarios. We present SAME-clustering, a mixture model-based approach that takes clustering solutions from multiple methods and selects a maximally diverse subset to produce an improved ensemble solution. We tested SAME-clustering across 15 scRNA-seq datasets generated by different platforms, with number of clusters varying from 3 to 15, and number of single cells from 49 to 32 695. Results show that our SAME-clustering ensemble method yields enhanced clustering, in terms of both cluster assignments and number of clusters. The mixture model ensemble clustering is not limited to clustering scRNA-seq data and may be useful to a wide range of clustering applications.

Single-cell RNA sequencing of murine islets shows high cellular complexity at all stages of autoimmune diabetes

Journal of experimental medicine (IF: 11.32), 2020-6, Xiaoxiao Wan et al. DOI: 10.1084/jem.20192362

PubMed: 32251514

Single Cell Gene Expression, Mouse, Immunology.

Abstract

Tissue-specific autoimmune diseases are driven by activation of diverse immune cells in the target organs. However, the molecular signatures of immune cell populations over time in an autoimmune process remain poorly defined. Using single-cell RNA sequencing, we performed an unbiased examination of diverse islet-infiltrating cells during autoimmune diabetes in the nonobese diabetic mouse. The data revealed a landscape of transcriptional heterogeneity across the lymphoid and myeloid compartments. Memory CD4 and cytotoxic CD8 T cells appeared early in islets, accompanied by regulatory cells with distinct phenotypes. Surprisingly, we observed a dramatic remodeling in the islet microenvironment, in which the resident macrophages underwent a stepwise activation program. This process resulted in polarization of the macrophage subpopulations into a terminal proinflammatory state. This study provides a single-cell atlas defining the staging of autoimmune diabetes and reveals that diabetic autoimmunity is driven by transcriptionally distinct cell populations specialized in divergent biological functions.

Single-cell resolution analysis of the human pancreatic ductal progenitor cell niche

Proceedings of the National Academy of Sciences of the United States of America (IF: 10.62), 2020-5, Ricardo Luis Pastori et al. DOI: 10.1073/pnas.1918314117 PubMed: 32354994

Single Cell Gene Expression, Human, Cell Atlas, Developmental Biology.

Abstract

We have described multipotent progenitor-like cells within the major pancreatic ducts (MPDs) of the human pancreas. They express PDX1, its surrogate surface marker P2RY1, and the bone morphogenetic protein (BMP) receptor 1A (BMPR1A)/activin-like kinase 3 (ALK3), but not carbonic anhydrase II (CAII). Here we report the single-cell RNA sequencing (scRNA-seq) of ALK3-sorted ductal cells, a fraction that harbors BMP-responsive progenitor-like cells. Our analysis unveiled the existence of multiple subpopulations along two major axes, one that encompasses a gradient of ductal cell differentiation stages, and another featuring cells with transitional phenotypes toward acinar tissue. A third potential ducto-endocrine axis is revealed upon integration of the ALK3 dataset with a single-cell whole-pancreas transcriptome. When transplanted into immunodeficient mice, P2RY1/ALK3 populations (enriched in PDX1/ALK3/CAII cells) differentiate into all pancreatic lineages, including functional β -cells. This process is accelerated when hosts are treated systemically with an ALK3 agonist. We found PDX1/ALK3/CAII progenitor-like cells in the MPDs of types 1 and 2 diabetes donors, regardless of the duration of the disease. Our findings open the door to the pharmacological activation of progenitor cells in situ.

Accumulation of long-chain fatty acids in the tumor microenvironment drives dysfunction in intrapancreatic CD8+ T cells

Journal of experimental medicine (IF: 11.32), 2020-8, Giulio Draetta et al. DOI: 10.1084/jem.20191920 PubMed: 32491160

Single Cell Gene Expression, Human, Immunology.

Abstract

CD8+ T cells are master effectors of antitumor immunity, and their presence at tumor sites correlates with favorable outcomes. However, metabolic constraints imposed by the tumor microenvironment (TME) can dampen their ability to control tumor progression. We describe lipid accumulation in the TME areas of pancreatic ductal adenocarcinoma (PDA) populated by CD8+ T cells infiltrating both murine and human tumors. In this lipid-rich but otherwise nutrient-poor TME, access to using lipid metabolism becomes particularly valuable for sustaining cell functions. Here, we found that intrapancreatic CD8+ T cells progressively accumulate specific long-chain fatty acids (LCFAs), which, rather than provide a fuel source, impair their mitochondrial function and trigger major transcriptional reprogramming of pathways involved in lipid metabolism, with the subsequent reduction of fatty acid catabolism. In particular, intrapancreatic CD8+ T cells specifically exhibit down-regulation of the very-long-chain acyl-CoA dehydrogenase (VLCAD) enzyme, which exacerbates accumulation of LCFAs and very-long-chain fatty acids (VLCFAs) that mediate lipotoxicity. Metabolic reprogramming of tumor-specific T cells through enforced expression of ACADVL enabled enhanced intratumoral T cell survival and persistence in an engineered mouse model of PDA, overcoming one of the major hurdles to immunotherapy for PDA.



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