# Single Cell Biology Symposium 2019

**Tuesday, February 12, 2019 – Light Hall 202 and North Lobby**

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|---|---|
| 8:00 am- 8:45 am | **Breakfast & Poster Setup** |
| 8:45 am- 9:00 am | **Introduction by Dr. Vito Quaranta** |
| **Session Chair:** *Darren Tyson, Ph.D.,* Research Assistant Professor<br>Department of Biochemistry | |
| 9:00 am- 9:15 am | **"Identifying neuron-specific determinants of synaptic connectivity by single-cell RNA-Seq"**<br>*David M. Miller, Ph.D.*<br>Professor, Department of Cell and Developmental Biology |
| 9:15 am- 9:30 am | **"Connecting life and death: The BCL-2 family coordinates mitochondrial network dynamics and cell fate"**<br>*Vivian Gama, Ph.D.*<br>Assistant Professor, Department of Cell and Developmental Biology |
| 9:30 am- 10:30 am | **Keynote 1: "Composition and Structure of Human Immune System to Predict and Control Pathobiology"**<br>*Sean Bendall, Ph.D.*<br>Assistant Professor<br>Department of Pathology<br>Stanford University, School of Medicine |
| 10:30 am- 11:00 am | Poster Preview |
| 11:00 am- 11:15 am | **"Cell type specific regulation of mitochondrial genomes"**<br>*Maulik Patel, Ph.D.*<br>Assistant Professor, Department of Cell and Developmental Biology |
| 11:15 am- 11:30 am | **"Implementation of single cell transcriptomics for profiling neurons and glia of the enteric nervous system"**<br>*Michelle Southard- Smith, Ph.D.*<br>Associate Professor, Department of Medicine |
| 11:30 am- 11:45 am | **"A multimodal single-cell platform for multiomic detection of rare phenotypes in intoxicated cancer cell populations"**<br>*Eric Spivey, Ph.D.*<br>Research Assistant Professor, Department of Biomedical Engineering |

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|---|---|
| **Session Chair:** *Amanda Linkous, Ph.D.* Research Associate Professor<br>Department of Biochemistry | |
| 11:45 am- 12:00 pm | **"Single cell analysis in digital pathology: prediction of long-term outcome in urologic oncologies"**<br>*Andries Zijlstra, Ph.D.*<br>Assistant Professor, Department of Pathology, Microbiology and Immunology |
| 12:00 pm- 12:15 pm | **"Defining the Cell Types Targeted by Toxins in Clostridium difficile infection"**<br>*Nick Markham, M.D., Ph.D.*<br>Post-Doctoral Fellow, Lacy Lab, Department of Pathology, Microbiology and Immunology |
| 12:15 pm- 12:30 pm | **"Single Cell Analysis of Circulating Tumor Cells"**<br>*Prasad Kopparapu, Ph.D.*<br>Post-Doctoral Fellow, Lovly Lab, Department of Medicine |
| 12:30 pm- 12:31 pm | Lightning Talk 1: Shristi Shrestha, Post-Doctoral Fellow, Powers Lab<br>Comprehensive transcriptome profiling of single human pancreatic islet cells reveals distinctive α and β cell subpopulations |
| 12:31 pm- 12:32 pm | Lightning Talk 2: Mafi Senosain, Graduate Student, Massion Lab<br>Heterogeneity of lung carcinoma in the microenvironment revealed by mass cytometry |
| 12:32 pm-12:33 pm | Lightning Talk 3: Amrita Banerjee, Graduate Student, Lau lab<br>The role of tuft cell specification and function in inflammatory ileitis |
| 12:35 pm- 1:30 pm | LUNCH |
| 1:30 pm- 2:30 pm | Poster Session |
| 2:30 pm- 2:45 pm | **"Investigating single cells in physiologically relevant environments"**<br>*Gregor Neuert, Ph.D.*<br>Assistant Professor, Department of Molecular Physiology and Biophysics |
| 2:45 pm- 3:45 pm | **Keynote 2: "Mapping lineage histories and differentiation by single-cell analysis"** *Allon Moshe Klein, Ph.D.*<br>Assistant Professor<br>Department of Systems Biology<br>Harvard University |
| 3:45 pm- 4:00 pm | **Final comments by Vito Quaranta, MD** |

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## Abstracts

1. **Corey Hayford,** ***Graduate Student, Quaranta Lab***

Tumor heterogeneity has been known to contribute to diverse patient outcomes in response to targeted therapies. It has primarily been studied in the context of genetic mutations that are either pre-existing or acquired in response to drug treatment. Here, we have discovered signs of pre-existing differences in isogenic oncogene-addicted cancer cell sublines, both on the proliferative and molecular levels. This pre-existing plasticity has led us to hypothesize that cancer cell populations hedge their bets amongst multiple phenotypic states in order to ensure population survival. In addition, we have evidence that these populations have begun to diversify into several proliferative states over time in drug-naïve conditions, which we think is due to subtle changes in the molecular fingerprint of single cells. Using a novel gRNA barcoding technology, in conjunction with scRNAseq, we have begun to unravel distinct transitions between molecular states which we plan to correlate to proliferative phenotypes.

2. **Todd Bartkowiak,** ***Post-Doctoral Fellow, Irish Lab***

Single Cell Systems Neuroimmunology Reveals Immunosuppressive Correlates with Ventricular Stem Cell Niche Contact in Human Glioblastoma

Todd Bartkowiak, Allison R. Greenplate, Justine Sinnaeve, Akshitkumar M. Mistry, Caroline Roe, Bret C. Mobley, Lola B. Chambless, Reid C. Thompson, Kyle D. Weaver, Rebecca A. Ihrie, and Jonathan M. Irish

Glioblastoma (GBM) is an aggressive brain cancer accounting for ~60% of all tumors originating in the brain tissue, and for which median survival remains ~15 months post-diagnosis, even when administered standard of care. The capacity for new immunotherapeutic strategies, however, to enhance anti-tumor immunity and treat once intractable peripheral solid tumors may offer hope for GBM patients. Yet, the brain is restrictive of immune responses compared to peripheral tissue. In fact, regional position of GBM nodules within the brain environment may pose an additional hurdle to the establishment of effective anti-tumor immunity, as distinct brain regions may differentially modulate immune responses, thus impacting treatment outcomes. One region of interest, the ventricular-subventricular zone (V-SVZ), is the largest neurogenic region that harbors stem cells in the adult brain, is enriched in factors that support tumor growth, and is thought to act as a site of gliomagenesis. Further, patients whose tumors show radiographic contact with the V-SVZ have diminished survival outcomes compared to patients whose tumors do not contact the V-SVZ. We hypothesized that this is due, in part, to the ability of the V-SVZ niche to establish a uniquely immunosuppressive microenvironment, aiding tumor growth by suppressing anti-tumor immunity. Using high-dimensional mass cytometry, we interrogated >35 parameters in nine immune populations infiltrating human GBM tumors. We found that immune cells infiltrating V-SVZ-contacting tumors possessed an activated and suppressive immune signature compared to immune infiltrates from V-SVZ non-contacting tumors. Several clinically actionable immunotherapeutic targets were also uncovered that may be used to optimize treatment strategies for GBM.

3. **Christian Meyer,** ***Graduate Student, Quaranta Lab***

A consensus framework for calculating drug synergy

Christian T. Meyer[1], David J. Wooten[2], Joshua Bauer[3], Darren R. Tyson[1], Vito Quaranta[1]

[1]Center for Cancer Systems Biology, Vanderbilt University, [2]Department of Physics, Pennsylvania State University, [3]Institute of Chemical Biology, High-Throughput Screening Facility, Vanderbilt University

Two goals motivate treating diseases with drug combinations: reduce off-target toxicity by minimizing dose (synergistic potency) and improve outcomes by escalating effect (synergistic efficacy). Surprisingly, current drug synergy frameworks do not distinguish between these types of synergy, failing to harness the potential of chemical libraries. We therefore developed Multidimensional Synergy of Combinations (MuSyC), a framework based on a multi-dimensional Hill-equation which recasts the synergistic potency and efficacy of a combination as extensions of classic pharmacology measures of potency and efficacy for a single drug. By grounding drug synergy in the mathematics of Hill kinetics, MuSyC reveals the previously obscured connection between Bliss Independence and Loewe Additivity, subsuming each into a more general model. MuSyC therefore provides a consensus framework for interpreting combination pharmacology and bridges the theoretical void between the often contradictory, traditional drug-synergy paradigms. We applied MuSyC to high-throughput, drug-combination screens in mutant-EGFR lung cancer where we find co-targeting the MAPK pathway only results in synergistic potency, whereas synergistic efficacy is achieved by targeting independent pathways, such as MAPK with epigenetic regulators or microtubule stability. In contrast, we find the combination therapy targeting BRAF and MEK in BRAF-mutant melanoma to be synergistically efficacious, highlighting MuSyC's utility for investigating disease-specific, drug-class trends in synergy. Finally, we employ MuSyC to decipher a functional genomic screen in combination with a targeted inhibitor in BRAF-mutant melanoma. MuSyC distinguishes the contribution of each molecular species in the kinome to the potency and efficacy of mutant-BRAF inhibition providing a systems view of the critical junctures in BRAF oncogene signaling. These findings showcase MuSyC's potential to transform the enterprise of drug-combination screens by precisely guiding translation of combinations towards dose reduction, improved efficacy, or both.

**4. Amanda Johnson, *Post-Doctoral Fellow, Neuert Lab***

Pathway stimulation threshold rates regulate cell signaling response

Amanda N. Johnson, Guoliang Li, Hossein Jashnsaz, Benjamin K. Kesler, Alexander Thiemicke, Dustin C. Rogers and Gregor Neuert

Department of Molecular Physiology and Biophysics, School of Medicine, Vanderbilt University, Nashville, TN 37232, USA

All cells may experience varying rates of environmental change, yet very little is known about how signaling pathways consider these rates in deciding cell behavior. To address this fundamental question, we monitored the activation of the conserved budding yeast high osmolarity sensing kinase Hog1 in response to varying rates of osmotic stress at the single cell level in real time. A rate condition required for signaling activation was discovered. The set point for this condition is determined by the phosphatase Protein Tyrosine Phosphatase 2 (Ptp2), which is known to act on Hog1. Altering Ptp2 expression levels specifically changes the rate of osmotic stress stimulation required to activate signaling, affecting the range of stress addition rates that trigger a cellular response. These findings show how dynamic perturbations can be used to uncover important new signaling regulation mechanisms.

**5. Jashnaz Hossein, *Post-Doctoral Fellow, Neuert Lab***

Combining theory with experiments to identify predictive models of signal transduction

Hossein Jashnsaz[1], Zachary Fox[2], Guoliang Li[1], Brian Munsky[2], and Gregor Neuert[1]
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Predicting cell signaling response upon environmental changes over time or upon genetic mutations is currently an unsolved problem. The reason for this predicament is the fact that the mechanisms of how cells sense, process, and respond to extracellular stress are not fully understood. Here, we present our combined theoretical and experimental framework based on a network motif approach to develop a general framework to quantitatively compare predictive models of signal transduction networks. To demonstrate the feasibility of this approach, we used the High Osmolarity Glycerol (HOG) Stress Activated Protein Kinase (SAPK) signaling pathway in the yeast Saccharomyces cerevisiae model organism. Experimental conditions consist of different types of extracellular osmotic stress gradients such as sudden activation (step, pulse), gradual increasing concentrations over time (linear gradient), and nonlinear increasing concentrations over time (quadratic gradient). Under these conditions, we quantify in single cells Hog1 nuclear localization as a measure of signal transduction.. Hog1 shows distinct activation dynamics under different stimulus inputs. Our general model framework is based on a prior proposed signal transduction pathway topology. At its core it is an n-node circuit motif (a network with n nodes) where each node represents a signaling protein, and the linkages between each pair of nodes represent interactions of those (activation, deactivation, none). Model complexity could increase by increasing the number of the nodes and their linkages as well as by introducing feedforward and feedback loops (FFL and FBL). By combining this approach with information theory criteria, we supplement intuitive with rationally experimental design to select biologically meaningful models. Our ultimate goal is to use the combined experimental and theoretical framework to develop models that are maximally predictive of signaling responses for any pathway, in any organism and in any environmental condition.

**6. Shristi Shrestha, *Post-Doctoral Fellow*, *Powers Lab***

Comprehensive transcriptome profiling of single human pancreatic islet cells reveals distinctive α and β cell subpopulations

Shristi Shrestha[1], Diane C. Saunders[1], John T. Walker[2], Rachana Haliyur[2], Greg Poffenberger1, Radhika Aramandla[1], Nripesh Prasad[4], Angela Jones[4], Shawn E. Levy[4], Alvin C. Powers[1,2,3], Marcela Brissova[1]
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Pancreatic islets are micro-organs composed of multiple different cell types including endocrine, vascular and immune cells that work in concert to maintain glucose homeostasis. Unraveling the phenotypes of these islet cellular components will aid our understanding towards function of islet micro-organ and how islet cells respond to aging or diabetes. To investigate possible heterogeneity of islet cell subsets, we used single cell RNA-sequencing to transcriptionally profile all islet cell types simultaneously. In this study, we focused particularly on heterogeneity in two major islet cell types, glucagon-producing α cells, and insulin-producing β cells. Using ChromiumTM from 10x Genomics, we analyzed approximately 79,881 single cells from pancreatic islets of five normal human donors (ages 14-66 years) generating approximately

17 billion reads and obtaining 2316 median genes per cell. In the context of the same donor (n=2), parallel single-cell transcriptome analysis of flow cytometry purified α and β cells showed high correlation (r=0.99) with the Seurat identified α and β subsets originating from dispersed whole islets. In addition, Seurat-guided dispersed islet analysis identified cell types that segregated into eight different clusters (17% β, 66% α, 4%, 4% ductal, 4% mesenchymal, 2% acinar, 2% endothelial, and 1% immune cells). Examining α and β cell clusters, we found that the α cell subset contained a subpopulation with higher cell cycle regulation marker expression (i.e., TOP2A, MKi67, CDK1). Interestingly, the β cell cluster had a cell subpopulation (~2%) that highly expressed genes associated with ER stress and unfolded protein response (UPR) (i.e., HERPUD1, HSPA5, DDIT3). We further investigated β cells expressing functionally important MAFA and MAFB genes, which are expressed only in a fraction of β cells by histological analysis, and found that PERK-mediated UPR transcripts were enriched in the MAFB-expressing β cell subset, while the MAFA-expressing β cells differentially expressed genes associated with β cell function (i.e., INS, IGFBP5, TFF3). This study indicates heterogeneity in both α and β cell populations with increased UPR in some β cells and proliferation markers in some α cells. We are now applying this approach to define the transcriptomic landscape of the islet micro-organ at different ages and in diabetes.

7. **Benjamin Kesler, *Graduate Student, Neuert Lab***

Determining long noncoding RNA interactions in mammalian cells using automated image analysis and single molecule RNA-Fluorescent In-Situ Hybridization

Benjamin Kesler, Guoliang Li, Alexander Thiemicke, Gregor Neuert

Human diseases are often caused by abnormal biological processes within specific cell types or individual cells within a tissue, yet most studies have focused on cell populations and ignored possible differences between individual cells. One reason is a lack of easily implemented and broadly applicable experimental and computational approaches for microscopy image acquisition and analysis. We propose an approach in which we optimize the experimental data acquisition prior to analysis to generate high quality images that are easier to analyze computationally. The images are then processed and analyzed in parallel to determine cellular and nuclear boundaries, which allows for single-cell quantification of features of interest. We applied our approach to investigate mammalian antisense long noncoding RNAs (lncRNAs) Tsix and Xist. By utilizing two-color, single-molecule RNA-FISH and our image acquisition and analysis pipeline, we were able to quantify Tsix and Xist at single-cell and single-molecule resolution in more than 2000 cells. We discovered mutual inhibition and thresholds of inhibition for Xist and Tsix in terms of both single transcripts and transcription sites, which provides evidence for mutual inhibition and suggests a switch-like inhibition mechanism. This data and generalizable pipeline set the stage for further quantitative investigation of this locus as well as other features across diverse species, cell types, and imaging modalities.

8. **Rohit Venkat, *Graduate Student, Neuert Lab***

In the pursuit of understanding epigenetic regulation by the noncoding genome, thousands of long noncoding RNAs (lncRNAs) have been cataloged in humans and model organisms alike, yet the functions of the overwhelming majority of lncRNAs and their regulation remain unclear. Recent findings suggest that lncRNA transcription serves an intrinsic role as local regulators of gene expression, but our present understanding of lncRNAs is still limited in part because current approaches are insensitive to distinguishing between different mechanisms of transcriptional

regulation and fail to account for the stochastic nature of gene expression. To overcome these limitations to understanding lncRNA function, we will study lncRNA-mRNA gene networks in budding yeast whose temporal expression patterns are altered in response to osmotic stress. Candidate lncRNA-mRNA gene networks will be identified for investigation by assessing temporal changes in nascent transcription and TFIIB transcription factor occupancy during osmotic stress conditions. To interrogate these candidate gene networks in detail, lncRNA and mRNA transcripts will be spatiotemporally resolved in individual cells using single-molecule RNA FISH microscopy. Combined with genetic gain- and loss-of-function strategies, this approach will provide the ability to distinguish between different mechanisms of transcriptional regulation that could not otherwise be achieved by static population studies using global gene deletion mutants. By illuminating how lncRNA dynamics and genomic context coordinate different modes of transcriptional regulation, our work will lay the foundation for systematic lncRNA characterization and improved mechanistic understanding of the noncoding genome.

**9. Alexander Thiemicke, *Graduate Student*, *Neuert Lab***

Cellular stress that changes over time differentially regulates MAPK signaling and cell phenotype in human immune cells

Alexander Thiemicke and Gregor Neuert, PhD

Immune cells are frequently exposed to changes in their environment such as different stresses or changes in cytokine concentrations over time. Such kinetic environmental changes may result in differential regulation of cell signaling, cell fate and cellular phenotype. Yet, studies on how the kinetics of extracellular perturbations influences these signaling dynamics are in its infancy. To demonstrate the importance of kinetic changes in the environment, we choose hyperosmotic stress by NaCl that has been shown to be critically involved in physiological and pathophysiological processes in several human tissues. Resident immune cells can get activated by NaCl in such tissues and contribute to inflammation. We demonstrate that kinetic NaCl concentration gradients have a differentiating effect on cell signaling, cell fate and cellular phenotype. Upon instant addition of 300 mosmol/kg NaCl, viability decreases strongly (to below 5%) in human T cell and monocytic cell lines. When the same concentration is reached gradually over a period of six hour, cell viability remains at 30%. To investigate molecular mechanisms, we used fluorescent cell barcoding for flow cytometry of intracellular processes such as apoptosis, proliferation, DNA damage, inflammation, MAPK signaling, transcription, translation, factor activation and RNA expression. This analysis reveals a significant difference in the activation of markers of apoptosis and p38 signaling between instant and kinetic linear gradient NaCl application. Markers for inflammation and for proliferation show a dependence on the cumulative exposure to NaCl, but not on the temporal gradient of application. These results demonstrate that cells differentially regulate cell signaling, cell fate and cellular phenotype upon different temporally changing environments. Determining their response to gradually increasing concentrations of NaCl over time will help to better understand diseases resulting from such inflammation.

**10. Aaron May-Zhang, *Post-Doctoral Fellow*, *Southard-Smith Lab***

The enteric nervous system (ENS) or "second brain" is the network of neurons and glia in the intestinal wall that coordinates the essential functions of the bowel. To expand our limited understanding of cellular diversity within the ENS, we profiled mouse enteric neurons and glia by single-cell RNASeq. Phox2b-CFP reporter mice were used to isolate enteric neurons and glia by FACS. After encapsulation on the 10x platform and sequencing, enteric neurons were

unexpectedly absent from our data set. To remedy the loss of enteric neurons, we modified our single-cell RNASeq procedure to capture nuclei. Here, we demonstrate that our method retains nuclear RNA during isolation and that the expression profiles of enteric neurons and glia can be determined solely using nuclear RNA. Our sequencing data offers unique insights into the identities of cells within the ENS.

**11. Mafi Senosain,** ***Graduate Student*****,** ***Massion Lab***

Lung adenocarcinoma (ADC) is a heterogeneous group of tumors associated with dramatically different survival rates, even when detected at an early stage. The overarching goal of our research is to identify the cellular and molecular predictors of indolent and aggressive behavior of early lung ADCs. We hypothesized that mass cytometry, a single cell proteomic approach, would allow the discovery of cellular determinants of early lung ADC behavior. To test this hypothesis, we prepared a mass cytometry panel of 34 labeled antibodies and validated their performance in four lung ADC cell lines (A459, H23, PC9 and H3211) and in peripheral blood mononuclear cells (PBMCs). We then tested our panel in a set of 11 early stage lung ADCs. Based on radiomics features, 4 of these ADCs had long-, one intermediate- and 6 short-predicted survival, establishing the rationale for the comparisons. Tumors were disaggregated into a single cell suspension within one hour after resection and cryopreserved before mass cytometry analysis. We used unsupervised clustering algorithm FlowSOM to identify cellular subpopulations and analyze differences in their distribution both within the tumor microenvironment (TME) and the epithelial compartment. We found that long-predicted survival tumors had a higher proportion of immune cells, whereas some short-predicted survival tumors had a higher proportion of fibroblasts/mesenchymal cells. Additionally, tumors show high degree of heterogeneity with distinct protein expression profiles among epithelial subpopulations, and some subsets with high HLA-DR expression were positively correlated with CD4+ and CD8+ T cells. Our results of mass cytometry in early lung ADCs suggest a distinct cellular profile of epithelial and stromal cells among indolent vs aggressive ADCs. This work deserves further validation at the cellular and molecular level to gain further insights into tumor behavior.

12. **Chris Habermann,** ***Lab Staff*****,** ***Kropski Lab***

Examining Normal and Fibrotic Explant Lung Differences at a Single-Cell Resolution
Habermann AC, Calvi CL, Winters NI, Blackwell TS, Banovich NE, Kropski JA

Background: Despite decades of research, the fundamental mechanisms of the pathogenesis of pulmonary fibrosis are still unsettled. Though many histopathologic patterns of pulmonary fibrosis have been identified with their own different patterns of risk factors, it is unclear which mechanisms are conserved between the different forms of pulmonary fibrosis and which drive distinct pathologies and outcomes. In order to decipher both the conserved and distinct mechanisms driving pulmonary fibrosis phenotypes, we aim to create a large dataset of single-cell transcriptomic data from the explant lungs of healthy and diseased lungs. Methods: At the time of lung transplantation, single-cell suspensions were generated from the lung parenchyma of patients receiving lung transplants (diseased) and from declined donor lungs (controls). Single-cell suspensions with red blood cell fraction depleted and CD45+ fraction reduced were used for scRNA-seq. Samples from more and less fibrotic regions of idiopathic pulmonary fibrosis (IPF) lungs were tagged separately for processing. Single-cell RNA-sequencing library preparation was performed using the 10X Genomics 3' or 5' assay, and sequencing was performed on an Illumina

Novaseq. Alignment and demultiplexing were performed using Cell Ranger. Graph-based clustering and scRNA-seq analysis was performed using the Seurat package in R. Diagnoses were assigned based on clinical interpretation of explant pathology. Results: Joint graph-based clustering and canonical correlation analysis of scRNA-seq profiles from >60,000 cells from control (n=5), IPF (n=4), chronic hypersensitivity pneumonitis (n=1), sarcoidosis (n=1), Hermansky-Pudlak syndrome (n=1), and nonspecific interstitial pneumonia (n=1) samples identified 30 distinct clusters including canonical cell types, as well as numerous intermediate/transitional cell types and/or states. Compared to control lungs, fibrotic lungs demonstrated a proximalized distribution of lung epithelial cells. Cell-type specific differential expression analysis comparing all diseased to control lungs identified >490 differentially expressed genes (DEG) in alveolar type II epithelial (AT2) cells, >780 DEG in T cells, and >720 DEG in endothelial cells (adjusted $p<0.05$). Fibrotic AT2 cells expressed increased mediators of matrix remodeling, senescence, and immune activation. Fibroblast gene expression programs across pathologies were largely conserved. Conclusions: The cell-type specific transcriptional programs of fibrotic lungs exhibit large scale changes from control lungs and suggest a direct role of epithelial cells in fibrotic remodeling. Shared fibroblast gene expression programs across pathologies suggest therapies targeting these pathways should be broadly effective across forms of pulmonary fibrosis.

**13. Seth Taylor, *Post-Doctoral Fellow*, *Miller Lab***

A gene expression map of an entire nervous system at single cell resolution.

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Brains are built from a fundamental structural motif comprised of a single neuron and its synapses. Because distinct genetic programs specify each type of neuron, the goal of elucidating the molecular pathways that define the brain must include a gene expression map at single cell resolution. As a participating laboratory in the C. elegans Neuronal Gene Expression Map and Network (CeNGEN) consortium, we are using 10X Genomics droplet-based sequencing to produce a gene expression fingerprint of the C. elegans nervous system at the single cell level. C. elegans is particularly amenable to this goal as the adult nervous system contains exactly 302 neurons of 118 classes, each defined by a known developmental lineage, detailed anatomy and synaptic connectivity. Thus, our approach will provide an invaluable gene expression catalog for delineating the underlying mechanisms that define each of these fundamental characteristics. In an initial experiment, we used a fluorescent pan-neural marker (rab-3::tagRFP) for FACS-isolation of all neuron types. From 9,268 neurons, we identified 84 distinct clusters of single-cell profiles. We used existing gene expression data in WormBase to assign known neuron types to > 30 clusters. To ensure capture of all neuron types and to facilitate their identification, we are performing a series of single-cell profiling experiments on known subgroups of C. elegans neurons. Preliminary evidence suggests that this approach is likely to reveal even greater diversity in the C. elegans nervous system than the 118 neuron types predicted from anatomical data. For example, the VA class of motor neurons is defined by 12 individual neurons (VA1 - VA12) distributed along the length of the ventral nerve cord. Single cell sequencing of the VA class,

however, has identified putative subtypes of VA neurons in separate clusters expressing specific HOX genes, Wnt pathway components and peptide neurotransmitters. We are using a combination of promoter-based GFP reporters and single molecule in situ hybridization (smFISH) to validate these results in vivo and to assign distinct clusters to specific VA neurons. We plan to extend this approach to additional neuron types as they are revealed by single cell sequencing to produce the first high resolution and complete gene expression map of an entire nervous system.

**14. Amrita Banerjee, *Graduate Student*, *Lau lab***

THE ROLE OF TUFT CELL SPECIFICATION AND FUNCTION IN INFLAMMATORY ILEITIS

Amrita Banerjee, Charles A. Herring, Alan J. Simmons, Hyeyon Kim, Bob Chen, Paige N. Vega, Eliot T. McKinley, Qi Liu, Robert J. Coffey, and Ken S. Lau

BACKGROUND: Inflammatory Bowel Disease (IBD) and its subtype Crohn's disease (CD) arise due to a loss of tolerance to environmental antigens in genetically susceptible individuals. Longitudinal analysis of CD incidence has identified an inverse correlation between rates of communicable disease and autoimmune disorders, particularly in countries endemic for helminth infestation. A case report published by Broadhurst et al described the use of helminth eggs to treat an IBD patient with refractory disease. Induction of a type 2 immune response following helminth colonization promoted mucosal healing and achieved clinical remission. Epithelial tuft cells are responsible for orchestrating the type 2 immune response following helminth colonization via the release of the type 2 cytokine IL-25. In acute infection, tuft cells drive their own specification and tuft cell hyperplasia is a critical host response for worm extrusion. Therefore, modulation of tuft cell function may prove efficacious in CD treatment. RESULTS: In a well-established mouse model (TNFΔARE) of Crohn's-like ileitis, we observed highly inflamed regions with lower tuft cell numbers, while less inflamed regions had more Dclk1+ tuft cells, suggesting an inverse correlation between inflammation and tuft cell specification. We applied p-Creode, a novel trajectory mapping algorithm, to single-cell RNA sequencing datasets in order to investigate tuft cell specification in wildtype and TNFΔARE animals. Contrary to previously published literature, p-Creode demonstrated that epithelial tuft cells are specified outside of the canonical secretory lineage. We then developed a novel, genetically-inducible model of tuft cell hyperplasia (Lrig1CreERT2/+; Atoh1fl/fl - AtohKO), where the loss of the master secretory regulator Atonal Homolog 1 (Atoh1) drove increased tuft cell numbers in the in vivo small intestine. However, recombination of Atoh1 in ex vivo small intestinal enteroids did not induce tuft cell hyperplasia. Similarly, broad-spectrum antibiotic treatment of AtohKO animals suppressed tuft cell hyperplasia, implicating a role for the microbiome in driving tuft cell specification in the absence of eukaryotic infection. Our novel findings suggest that commensal microbial-derived metabolites in the AtohKO model are capable of driving tuft cell hyperplasia, independent of helminth colonization. FUTURE DIRECTIONS & IMPACT: We will identify species and metabolite changes that are responsible for driving tuft cell hyperplasia independent of helminth colonization. Understanding tuft cell specification and function could enable us to better leverage this rare and elusive cell type to modulate inflammatory symptoms in IBD.

# Single Cell Biology Symposium 2019

**15. Sarah Maddox, *Graduate Student, Quaranta Lab***

Network Control of Phenotypic State Transitions in Small Cell Lung Cancer

Small cell lung cancer (SCLC) is an aggressive tumor type that relapses at high rates and metastasizes early. Despite these poor outcomes, the standard of care for SCLC has not changed in decades, because the mechanisms of acquired resistance are not well understood. Within the past decade, the role of intratumoral heterogeneity in tumor aggressiveness has become more apparent, as cooperation between subtypes of SCLC can potentiate metastasis [Calbo, 2011] and treatment evasion [Jahchan, 2016]. The discovery of multiple SCLC phenotypes necessitates further analysis to map the phenotypic space of SCLC. Consensus clustering and weighted gene co-expression network analysis (WGCNA) reveal 4 clusters, including a novel neuroendocrine variant (NEv2) distinguished by gene modules and drug response, and a transcriptional factor network is able to explain these four steady states. Analysis of human and mouse tumors suggests that SCLC phenotype is plastic, both stochastically and in response to drug. We use RNA velocity of single cell RNA-seq data to understand and quantify movement through the phenotypic landscape, and well as make predictions of perturbations that may induce those transitions. Characterization of the transitions between core SCLC phenotypes and predictions of perturbation strategies may suggest new methods of personalized treatment.

# Single Cell Biology Symposium 2019

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