

## Sanjeev Kumar Memorial Lecture

### Mitochondrial calcium uniporter in cancer development and invasion

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Cancer presents a major health crisis in the US today. Over a third of Americans will be diagnosed with cancer in their lifetimes, and it is second only to heart disease in annual lethality. A stronger understanding of disease mechanisms and new therapeutic modalities are desperately needed to improve outcomes for many patients. We have previously shown that cancer cell lines are 'addicted' to constitutive Ca<sup>2+</sup> flow into mitochondria through the mitochondrial calcium uniporter (MCU) at endoplasmic reticulum-mitochondria contact sites. We hypothesized that mitochondrial Ca<sup>2+</sup> influx through the MCU contributes to cancer cell development, proliferation, and metastasis by promoting metabolic activity within mitochondria. We now show that mRNA expression of MCU is elevated in a subset of pancreatic cancer patients, and that high MCU protein expression correlates with poor survival outcomes. In addition, knockout of MCU in transformed murine fibroblasts prevents mitochondrial Ca<sup>2+</sup> uptake, reducing metabolic activity, proliferation, and invasive behavior, as well as xenograft growth in an immunocompromised murine model. Furthermore, in a genetic murine model of pancreatic ductal adenocarcinoma (PDAC), the *KPCY* model, MCU knockout (KO) reduced tumor formation and metastasis in preliminary studies. A cell line developed from a *KPCY-Mcu<sup>KO</sup>* animal fails to take up Ca<sup>2+</sup> in the mitochondria in a manner which may be rescued by transfection with MCU. Re-expression of MCU in these cells is associated with a morphological change to a more fibroblastic morphology indicative of epithelial to mesenchymal transition (EMT). Results indicate that MCU-mediated influx of mitochondrial Ca<sup>2+</sup> contributes to the cancerous state and may present a therapeutic target for cancer treatment.

## Platform Presentations

## **Establishing the larval zebrafish pectoral fin as a model for targeted axon regeneration**

**Lauren Walker** and Michael Granato

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The vertebrate peripheral nervous system has significant capacity for axon regeneration. While work in many systems has addressed neuron-intrinsic factors that promote axon growth, little is known about how the regenerating growth cone interacts with cues in the environment to reinnervate target tissues with specificity. Targeting of regrowing axons to correct tissues is critical for functional recovery after nerve injury; indeed, incorrect muscle fiber reinnervation results in inappropriate movements. The larval zebrafish pectoral fin is an ideal model system to identify novel environmental cues required for target reinnervation due to its complex anatomy. The pectoral fin is innervated by four motor nerves containing dozens of axons that branch to stereotypically-innervate specific regions of the fin in two distinct muscle layers. Using a laser to transect the nerves that innervate the pectoral fin we can monitor regeneration in real time. We observe robust and specific regeneration of pectoral fin axons back to their original domains within two days, indicating that there must be regional growth and guidance cues within the fin to guide axon growth. Easy removal of the pectoral fin allows for unbiased identification of local, injury-dependent cues in vivo in a vertebrate that are not feasible in other model systems. Here, we discuss an RNAseq approach to identify factors in the regenerating pectoral fin with expression changes after axon injury that may be required for axon growth and guidance. Using this approach as a gateway to understand the underlying molecular-genetic mechanisms that promote sustained and directed growth of regenerating axons will generate a strong foundation for therapeutic applications aimed to promote functional PNS recovery.

**Joint profiling of chromatin accessibility and CAR-T integration site analysis at the single-cell level**

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Chimeric antigen receptor (CAR)-T immunotherapy has yielded impressive results in several blood cancers, establishing itself as a powerful means to redirect and enhance the natural properties of T lymphocyte subsets. However, this therapeutic approach is often limited by the extent of CAR-T cell expansion *in vivo*. A major outstanding question is whether CAR-T integration at certain genomic regions can rewire the regulatory landscape of individual T cells, enhancing the proliferative competence of CAR-T cells *in vivo*, or if a simply passive CAR-T integration in cells with intrinsic proliferative advantage can lead to clonal expansion and successful tumor killing. To determine how frequently these two scenarios occur in patients, it is critical to define the epigenetic identity of an individual CAR-T cell and simultaneously chart where CAR-T vector integrates into the genome at the single-cell resolution throughout CAR-T therapy. Here, we report the development of a novel technique called EpiVIA for the joint profiling of the chromatin accessibility and lentiviral integration site analysis at population and single-cell levels. We validate our workflow in clonal cells with previously defined integration sites and further demonstrate the measurement of lentiviral integration sites and chromatin accessibility at the single-cell resolution in CAR-T cells.

## **In vivo imaging of mutant cell growth dynamics in adult skin carcinogenesis**

**Paola Kuri**, Maylin Nishiguchi, Megan Brewster, Thomas Leung, and Pantelis Rempoulas

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The adult skin maintains homeostatic balance by relying on the highly regulated proliferative activity of resident stem cells that replenish cells lost through terminal differentiation. Spontaneous gene mutations caused by UV exposure can cause stem cells to deviate from homeostasis and develop into skin tumors. From clinical and experimental data it is known that suppression of Notch signaling is a common feature of skin carcinogenesis. However, Notch mutations are also highly prevalent in normal skin. How the same mutation can have differential effects within a cell population is a key unanswered question in the cancer field.

To address this issue, I use live intravital microscopy of mammalian skin in two model systems that aim to recapitulate the canonical process of carcinogenesis from the single-cell origin. Using mouse genetic models, Notch can be deregulated in single differentially labeled clones. To expand this analysis to human skin, we have established a workflow to carry out long term live imaging of human skin xenografts at high resolution. In both systems, the long-term fate of the clones at the single-cell level can be followed over time to trace their growth dynamics and determine the conditions that favor or limit clonal expansion in vivo by identifying molecular mechanisms underlying this process. Preliminary experiments showed Notch suppression skews stem cell fate towards self-renewal in some, but not all clones, and chronic UV exposure leads to both clonal loss and expansion. Ultimately, the quantitative data generated in this study will yield a mechanistic understanding of the heterogeneity in mutant stem cell behavior in one of the earliest stages in tumorigenesis.

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### **Succinate prodrug rescues mitochondrial function in human-derived cells following acute exposure to APAP**

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Acetaminophen (APAP, paracetamol) is one of the most common over-the-counter drugs used worldwide and considered safe at therapeutic levels. Overdose, however, accounts for up to 70 % of acute liver failure cases in the western world. It is known that the formation of its toxic metabolite N-acetyl-p-benzoquinone imine, the induction of oxidative stress and mitochondrial dysfunction are central to the development of APAP-induced liver injury. And yet, despite the knowledge on the toxicity of APAP, there still is only one clinically approved treatment option, N-acetylcysteine, which has limited effectiveness in late-presenting patients. Therefore, alternative treatment strategies are warranted. In this study, we investigated mitochondria as target for the development of novel treatment strategies for APAP-induced liver injury.

The effect of APAP on mitochondrial function was evaluated *in vitro* in human-derived cells using detailed respirometric analysis. Subsequently, the ability of the cell-permeable succinate prodrug NV241 to rescue the inhibition of APAP-induced mitochondrial respiration was assessed. Characterization of the mitochondrial toxicity of APAP *in vitro* in human-derived cells revealed that complex I-linked but not complex II-linked (succinate-dependent) mitochondrial respiration was inhibited by APAP. Treatment with the cell-permeable succinate prodrug NV241 normalized APAP-induced impaired mitochondrial respiration. In conclusion, bypass of APAP-induced complex I dysfunction using cell-permeable succinate prodrugs, such as NV241, presents as a potential treatment strategy for APAP-induced mitochondrial dysfunction and, potentially, related hepatotoxicity.

## **Ultra-sensitive platinum nanoparticle based digital assay toward point-of-care diagnostics**

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Quantitating ultra-low concentrations of protein biomarkers is critical for early disease diagnosis and treatment. However, most current point-of-care (POC) assays are limited in sensitivity to meet this clinical need. Herein, we introduce an ultra-sensitive and facile microbubbling digital assay readout method toward POC quantitation of protein biomarkers requiring only bright-field

smartphone imaging. Picolitre-sized microwells together with platinum nanoparticle labels enable the discrete “visualization” of protein molecules via immobilized-microbubbling with smartphone. We also use computer vision and machine learning to develop an automated image analysis smartphone application to facilitate accurate and robust counting. Using this method, post-prostatectomy surveillance of prostate specific antigen (PSA) can be achieved with a detection limit of 2.1 fM (0.060 pg/mL), and early pregnancy detection using  $\beta$ hCG with a detection limit of 0.034 mIU/mL (2.84 pg/mL) . The results are further validated using clinical serum samples against clinical and research assays. This work provides the proof-of-principle of the microbubbling digital readout as an ultra-sensitive technology with minimal requirement for power and accessories, facilitating future POC applications.

## **The E3 Ubiquitin Ligase Itch Restricts the Magnitude of Germinal Center B Cell responses**

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More than 23 million Americans suffer from autoimmune disease, driven in large part by germinal center (GC)-derived autoantibodies. In GC B cells, somatic hypermutation of immunoglobulin genes can render autoreactivity, but strict regulation of GC B cell expansion and survival helps prevent the emergence of autoreactive plasma cells and antibody. However, these regulatory mechanisms are still ill-defined. The E3 ubiquitin ligase Itch prevents the emergence of

autoimmune disease and autoantibodies in humans and mice, and patients lacking Itch develop multi-faceted autoimmune disease; yet how Itch regulates GC B cell fate or function has not been explored. By studying spontaneous and immunization-induced GC B cell responses in Itch deficient mice, we now show that Itch directly limits B cell activity to shape antibody responses. While Itch-deficient mice displayed normal numbers of pre-immune B cell populations, they showed elevated numbers of antigen-experienced B cells (e.g. GC B cells, memory B cells, and plasma cells). Mixed bone marrow chimeras revealed that Itch acts within B cells to limit GC B cell numbers. Proteomic profiling of acutely activated B cells uncovered that Itch regulated a subset of proteins involved in cell cycle and mTORC1 activity. We found that B cells lacking Itch showed increased proliferation, glycolytic capacity, and mTORC1 activation *in vitro* and *in vivo*. Moreover, immunization-induced stimulation of these cells *in vivo* resulted in elevated GC B cells, plasma cells, and serum IgG. These results support a novel role for Itch in limiting B cell metabolism and proliferation to suppress GC B cell responses, supporting the idea that overly-exuberant GC B cell responses may contribute to autoimmunity in Itch deficiency.

## **Defining the role of pulmonary endothelial cell heterogeneity in the response to acute lung injury**

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Gas exchange between the lungs and the cardiovascular system is critical to sustain life. To facilitate this process, the lung possesses the largest vascular network in the mammalian body. Pulmonary endothelial cells (ECs) that line capillaries in the distal lung interface closely with type I alveolar epithelial cells (AT1s), enabling transfer of oxygen and carbon dioxide between them. Despite the importance of ECs, the extent and function of their heterogeneity within the lung remains incompletely understood. As distinct populations of ECs may possess cellular plasticity

that enables them to act as endothelial progenitors at homeostasis or during regeneration, this question is critical to our understanding of maintenance and repair of the gas exchange machinery. We have profiled pulmonary EC heterogeneity at single-cell resolution using scRNA-seq in the adult mouse lung at homeostasis and after acute lung injury, identifying several unique EC populations. These include a subset of ECs that express high levels of signaling molecules and preferentially localize to regions of dense alveolar damage, as well as a population of highly proliferative ECs that arises upon acute lung injury. Each population likely contributes to revascularization of the alveolar space during regeneration. Determining the unique functional role of each population within the alveolus, its preferential response to injury, and how each type of EC interacts with alveolar epithelial cells will facilitate a better understanding of how the lung vasculature is rebuilt and gas exchange is reestablished as the lung regenerates. These studies will contribute to the future development of regenerative therapies that restore gas exchange in respiratory disease or lung cancer, leading causes of death worldwide.

## **Investigation of the transcriptional signature of autism spectrum disorder**

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Autism Spectrum Disorder (ASD) is a heterogeneous disease with complex genetic and environmental causes. Persons with ASD display compromised social communication and interaction, repetitive behavior, restricted interests, and intellectual disability. ASD is currently defined by clinicians using behavioral criteria as there is no known biomarker. The heterogeneity of the causes and etiologies of ASD makes it exceptionally difficult to develop a unifying model of the molecular processes affected in these disorders and thus to develop relevant treatments. As such, current treatments focus on behavioral intervention and modification rather than correction of the underlying molecular pathology. However, emerging evidence has shown that several ASD-linked genes encode epigenetic regulators. We thus hypothesized that chromatin modifiers linked

to ASD converge on a common transcriptional output. Knockdown of these genes resulted in a wide range of differential gene expression in both directions. Remarkably, our preliminary analyses demonstrated significant overlap in gene expression changes for all targets tested. Even more surprising was that not only do the gene targets overlap, but they also change in the same direction; upregulated genes overlap with other upregulated genes and the same is true for downregulated genes. Furthermore, this was true for every overlap despite the fact that each of the targets works through a different mechanism. We also found several genes were common between all groups. Finally, the genes shared between all groups contain several that are potential drug targets. Ongoing studies will greatly expand our understanding of the molecular mechanisms of ASD thus the potential to develop new treatments