The effect of Rab11b on actin dynamics and the adhesome

Nicole Weaver

Breast cancer is the most common cancer among American women, and accounts for 14% of cancer related deaths, making it the second most deadly form of cancer for women. Specifically, brain metastases are responsible for 30% of breast cancer related mortalities, largely due to the lack of therapeutics capable of crossing the blood-brain barrier. Therefore, it is essential to investigate the process of metastasis to better understand the mechanistic properties of brain metastases.

To metastasize, cancer cells must survive and adapt to new environmental stresses. To dissect the dynamic nature of brain metastatic progression, we performed transcriptome analysis and identified 108 highly up-regulated genes. By using a Drosophila melanogaster model, we identified Rab11b, a protein recycler, as an important mediator of brain metastatic outgrowth. Microenvironmental interaction relies on the composition of the cell surface, which allows a cell to respond to mechanical and biochemical cues. Mechanistically, shRab11b significantly alters the cell surface proteome, suggesting metastatic microenvironment specific cargo recycling. We show that both cellular adhesion and actin cytoskeleton proteins are dramatically altered in shRab11b cells. We hypothesize that Rab11b controls mechanical microenvironmental adaptation by disrupting focal adhesions and actin dynamics.

Here, we sought to characterize Rab11b-mediated control of the mechanical interaction between cells and the extracellular matrix (ECM) by examining the composition and control of the cellular adhesome. We live imaged focal adhesions and actin by utilizing fluorescent plasmids and performing a nocodazole washout. To identify differences in composition of the adhesome, we isolated integrin-associated complexes from control and shRab11b cells plated on collagen I or transferrin. Finally, to characterize the Rab11b interactome, we generated a construct expressing Rab11b tagged to a promiscuous biotin ligase. Taken together, this work begins to explore the mechanistic underpinnings of Rab11b-mediated control of mechanical adaptation to the metastatic microenvironment.

Cajal's battering ram employs invadosomes to enter the spinal cord

Evan L. Nichols(1,2) and Cody J. Smith(1,2)

In development and regeneration axons must coordinate with glia and other cells to properly assemble nerves. Unfortunately, how distinct cell types precisely arrange into a nerve are understudied. Recently, it was identified that the first (pioneer) axon of the dorsal root ganglia (DRG) utilizes Cajal's battering ram and cellular invasive components to cross into the spinal cord at the dorsal root entry zone (DREZ). While the role of actin in the battering ram has been described, we sought to identify additional cellular components in the battering ram by combining transgenesis and time-lapse imaging in zebrafish. We demonstrate that the battering ram also

consists of an accumulation of cytosolic vesicles at the tip of the growth cone. Inhibition of vesicle release via tetanus toxin expression specifically in DRG neurons alters pioneer axon trajectory beyond the typical DREZ location. Our data also identify the importance of matrix metalloproteinases (MMPs) in pioneer axon DREZ entry, consistent with the battering ram as an invadosome complex. By inhibiting the individual components of the battering ram, we also demonstrate that actin rearrangement into invadopodia is required for vesicle accumulation. Together, these data support a model of precise nerve assembly where axons employ invadosomes – in the form of invadopodia, cytosolic vesicles, and MMPs – to traverse a cellular and molecular boundary at the spinal cord edge. This model has importance in both development and regeneration, where axons fail to re-enter the spinal cord.

Understanding the relationship between RIPK1-mediated mitophagy and immune checkpoint inhibitor resistance

Ryan Middleton, Mark Hawk, Sharif Rahmy, Xin Lu, and Zachary T. Schafer

Receptor-interacting protein kinase 1 (RIPK1) has recently been shown to control a novel cell death pathway, which is antagonized in an immune checkpoint inhibitor (ICI) resistant cell line, named C3L. This pathway normally leads to cell death by way of reactive oxygen species (ROS); thus when it is inhibited, ROS levels are lower. Our approach then was to attempt to increase ROS again by removing glutathione, an important antioxidant, from these cells. Without glutathione, restricted by CB-839, an inhibitor of glutaminase (GLS), anchorage independent growth in soft agar and viability as measured by AlamarBlue decreased significantly. 50 nM CB-839 was sufficient to prevent C3L growth in soft agar, but not growth of the ICI-sensitive parental line from which it was created, 6239. This showed specificity to the line known to mitigate ROS by antagonizing the RIPK1 pathway, supporting our hypothesis that C3L cells would be especially sensitive to this inhibition. AlamarBlue data, which compared growth in attachment to growth in detachment, was not able to concretely show specificity to either of those conditions. At 50 nM however, it did decrease growth more in the detached condition. This suggests that more AlamarBlue experiments need to be run, possibly increasing the concentration slightly, in order to find the concentration at which detached cells will be affected, but attached cells will not.

SGK-1: A Novel Regulator of ATP Production in Extracellular Matrix (ECM) Detachment in Inflammatory Breast Cancer

Taylor White

Inflammatory breast cancer (IBC) is a very rare, rapid, and deadly disease accounting for 1 to 5 percent of all breast cancers in the United States. In all cancers, like IBC, cancer cells metastasize by detaching from surrounding tissue called the extracellular matrix (ECM) and evading an ECM-

detachment induced programmed cell death known as anoikis. Along with overcoming anoikis to promote ECM-detachment survival, cancer cells must also suppress ECM-detachment induced metabolic defects to survive. Oncogenic signaling is one way cancer cells can revoke these metabolic defects. For this investigation, we were interested in the oncogene ErbB2 in IBC, which is overexpressed in 30% of breast cancers. ErbB2 is part of a prominent signaling pathway, including the signaling protein kinases of PI(3)K, Akt, and SGK-1, known to be associated with regulating metabolism in cancer cells. The focus of this research was to investigate if SGK-1 was relevant for ATP production and survival in ErbB2 driven IBC cells. We hypothesized that SGK-1 was both sufficient and required to promote ATP generation and anchorage independent growth during ECM-detachment. Using genetic approaches to manipulate KPL4 (ErbB2 positive) IBC cells to elevate SGK-1 and Akt activity in IBC cells, our results demonstrated that SGK-1 was sufficient for ATP production and anchorage independent growth in ECM-detachment. Compared to Akt, SGK-1 was in fact more effective at generating energy and forming colonies in detachment. Additionally using pharmacological inhibition and shRNA, we found that SGK-1 was required for ATP production and anchorage independent growth in detachment. The overall significance of this project was to identify a potential regulator downstream of ErbB2 that aids is facilitating energy production and anchorage independent growth in IBC cells. With future studies investigating how SGK-1 is regulating ATP production via glucose metabolism, we could perhaps highlight a potential therapeutic target for treating cancer cell metabolism in patients.

The Role of Host Cytosolic RNA Sensing Pathways in a Mycobacterium avium Infection

Emily F. Eix, Nicholas Kiene, Alexandra Tatarian, Jeffrey S. Schorey and Yong Cheng

Non-tuberculosis mycobacteria (NTM) are known to cause pulmonary infections, primarily in immune-compromised individuals or those with lung diseases. Mycobacterium avium is one of the biggest contributors to NTM infections, and while M. avium infection is less frequent than M. tuberculosis infection, its consequences are still quite significant and difficult to treat. Much is still unknown about the mechanism of interaction between M. avium and the host. Our in vitro work indicates that M. avium secretes RNA to activate the host cytosolic RIG-I/MAVS/TBK1/ IRF3 RNA sensing pathway, ultimately leading to the production of interferon-beta (IFN-b), and implicates MAVS in inhibiting M. avium replication. We further examined this mechanism in vivo by studying the role of MAVS in the RIG-I/MAVS RNA sensing pathway in murine models. Using both WT and $Mavs^{-/-}$ mice infected with M. avium, we measured bacterial growth by plating lung and spleen homogenates on 7H10 agar. Additionally, we analyzed the lung histopathology in the infected mice via H&E staining, and found that $Mavs^{-/-}$ mice exhibit greater immune cell infiltration in the lungs compared to WT mice. Finally, we used ELISA to measure IFN-b production in the serum of WT and $Mavs^{-/-}$ mice infected by M.avium, and found that IFN-b production is lower in $Mavs^{-/-}$ mice. Taken together, our results elucidate the role of MAVS in inhibiting the replication of M. avium and demonstrate the importance of the RIG-I/MAVS pathway in inducing IFN-b expression in the context of an M. avium infection.

Determining the Effects of Climate Warming on the Active Layer of Permafrost Regions in Alaska

Floyd Nichols

Permafrost soils are becoming increasingly important to study, more specifically the microbial communities that make up these soils. Permafrost regions play a major role in the carbon cycling process on the Earth since most of Earth's carbon is stored in those regions. However, there is a lack of knowledge in permafrost soils and how climate change has affected these regions. In this study, our objective was to determine different driving factors in the microbial community of the seasonally-thawed layer (active layer) of permafrost regions in the Alaska tundra. Samples were collected at 17 different sites across an Alaskan transect that differed in elevation; however, this study focused on the Upper Sagwon and Lower Sagwon site.

To analyze the microbial composition of the soils, phospholipid fatty acid (PLFA) extraction was used to produce fatty acid methyl esters (FAMEs) which could be used to identify microbial organisms. PLFAs were the chosen biomarkers used to analyze the microbial communities because of its uniqueness to bacteria and fungi and because PLFAs degrade very rapidly, allowing us to view the microbial community structure of viable microbes. Fungi and bacteria are the primary focus for soil microbes because of their decomposition of organic matter to inorganic matter such as CH_4 or CO_2. The primary goals that were looked at were whether fungi:bacteria ratio changed with depth, if soil pH had a significant impact on PLFA content, and if the elevation gradient contributed to differences in C:N ratio. The data obtained was also used to characterize which microbes were the dominant microbes at each site. Our preliminary data showed that there was a significant difference in soil, total organic carbon (TOC), and total organic nitrogen (TON among both sites (p-value < 0.05, $n=13$).

Somatosensory response to noxious cold begins in early embryogenesis in Danio rerio

Jawuanna McAlister

The ability of animals to sense their external environment through somatosensory transduction is critical for their development and survival. In vertebrates, this involves dorsal root ganglia (DRG), collections of neuronal cell bodies placed along the outside of the spinal cord. Sensory neurons within the ganglia develop during distinct developmental windows and are specialized to sense specific sensory modalities such as temperature, pressure, and pain. In mammals, the ability to sense these modalities is already established by birth. However, the time at which DRG neurons are first sensitive and become functionally active is not yet known. To investigate somatosensory development during early embryogenesis, we use trangenesis, immunohistochemistry, and confocal microscopy in Danio rerio. Tg(HuC:CaMPARI) zebrafish, which photo convert from GFP emission to RFP emission upon neuronal activation, show an increase in photoconversion after noxious cold exposure at 3, 4, and 5 days post fertilization. Sensory neurons are specifically

activated upon noxious cold exposure. Additionally, starting at 3 dpf, animals exposed to noxious cold exhibited a distinct tail movement, which we term a shiver phenotype. Together, these results support the hypothesis that somatosensory response to noxious cold begins in early embryogenesis in vertebrates.

Deletion of the von Hippel-Lindau Gene in mouse Hemangioblasts

Karolina I. Pellot Ortiz[1], Fang Liu[2], and Xin Lu[2]

Von Hippel-Lindau (VHL) disease is a multi-system familial tumor syndrome characterized by phenotypically similar vascular tumors in the central nervous system and viscera. The VHL tumor suppressor protein (pVHL) plays a key part in cellular oxygen sensing by targeting hypoxia-inducible factors (HIF) for ubiquitylation and proteasomal degradation. To elucidate the aetiological role of the VHL gene in hemangioblastomas, we developed a murine model of VHL-associated hemangioblastomas by conditionally inactivating VHL in a hemangioblast population using a Tie2-Cre transgenic mouse line. Conventional biallelic VHL knockout mice are embryonically lethal. Conditional knockout strains have been established via tissue-specific inactivation of VHL in the hemangioblast population. The VHL heterozygous mutation may not cause pathological change and tumorlets upon H&E staining in the cerebellum, brainstem, and spinal cord. We want to explore the role of VHL homozygous mutation in VHL-associated hemangioblastomas. First, we generate the VHL homozygous mutation in VHL heterozygous mutation fibroblast cells via cell-intrinsic gene recombination to analyze their phenotype. Further investigation into hemangioblastomas pathophysiology and the development of conditional inducible biallelic VHL mutation are needed.

MicroCT Analysis of Bone Quantity and Bone Quality un the Lizard Skull

Nicole L. Marquez-Reyes

Lizards comprise a taxonomically and morphologically diverse group of vertebrates. Although common among mammals, only some lizards employ extensive oral preparation of foods prior to swallowing and ingestion. Unlike other vertebrates such as fish and mammals, there is a paucity of evidence regarding the mechanobiology and development of cranial hard tissues in lizards (Lepidosauria). To explore if lizard cranial bones exhibit diet-related plasticity like mammals, we collected preliminary data on intra- and inter-specific variation in cortical bone thickness and biomineralization throughout the skull. To achieve this goal, high-resolution x-ray (micro CT) images of three lizard species were obtained. Bones in the feeding system were identified, and cortical bone mineralization and thickness quantified for statistical analyses ($p \leq 0.05$) of intracranial and interspecific variation in bone quantity and quality. Of the three species, Agama colonorum showed higher biomineralization, followed by Sceloporus occidentalis. Our pilot

analyses underscore the efficacy of collecting data on diet-induced variation in mineralization and thickness in lizards subjected to long-term variation in food properties during growth.

Analysis of the immune response's effect on the regeneration of photoreceptors in gosh mutant zebrafish (Danio rerio)

Steven Pesina, Maria Iribarne and David Hyde

To understand the mechanism of photoreceptor recovery in gosh mutants, we examined the role of the microglia in the retinal regeneration process.To inhibit microglia activation, 3 and 7 week-post-fertilization (wpf) gosh zebrafish were treated with the glucocorticoid agonist dexamethasone (5 µg/L and 10 µg/L) for either 4 or 7 days-post-treatment (dpt). Macrophage/microglia was visualized with a transgenic line Tg[mpeg:GFP]. Immunochemistry was performed to visualize nuclei (DAPI) and the proliferation marker PCNA, or double cone by zpr1. In the methanol treatment group, the gosh mutant has a greater number of activated microglia, with more being present and translocated to the outer nuclear layer (ONL) than the WT zebrafish. PCNA positive cells are present in Müller cell, and neuronal progenitors in the inner nuclear layer (INL), and rod precursors in the ONL. Gosh mutants showed higher numbers of PCNA positive cells compared to WT. When treated with dexamethasone, the WT and gosh fish show similar microglia features as not active. WT and gosh showed a decrease in PCNA positive cells in their INL but no change to those cells in the ONL. These results suggest that Müller cell proliferation is being inhibited by the inhibition of microglia in both gosh and WT retina. To evaluate the photoreceptor recovery, the retina was stained with the double cone marker zpr1. In the WT retina, zpr1 show a normal appearance with a long and slim cone body cell. gosh shows a discontinuous cone layer with abnormal cone, which are more round and shorter; but between the two treatment groups the WT showed no difference, along with gosh. By looking at DAPI, the number of rod cells look similar between all of the treatment groups. All together suggest that dexamethasone treatment doesn't affect the photoreceptors recovering in WT and gosh retinas.We evaluated the effect of dexamethasone in 7 wpf fish, which present a mature acquire immune system. Similar results like 3 wpf, that only has the innate immune system present, were observed.