Developing a Technique to Label and Isolate Zebrafish Müller Glia Cells for FAC-Sorting

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The zebrafish (*Danio rerio*) is a useful model organism for studying retinal regeneration because fish and mammals have similar retinal cell types. While the damaged mammalian retina cannot regenerate, the zebrafish retina can regenerate any lost retinal neurons (Vihtelic & Hyde, 2000). In the damaged zebrafish retina, the Müller glia cells proliferate to produce neuronal progenitor cells that, that continue to proliferate and replace the cells lost during retinal damage (Bernardos *et al*., 2007; Kassen *et al*., 2007). Retinal regeneration also occurs in chicks, however only shortly after hatching and not all types of retinal neurons are regenerated (Fischer & Reh, 2000). To develop strategies to overcome vision loss in humans, it is critical to determine the similarities and differences in gene expression between Müller glia cells in the regenerating zebrafish and chick, as well as the non-regenerating mouse retina. To standardize Müller glia isolation procedures for the different species via FAC-sorting, I tested whether Diamidino Yellow Diaceturate (DiY), which was previously shown to be specifically incorporated into chick Müller glia, is also taken up exclusively by zebrafish Müller glia. DiY was injected intravitreally into the zebrafish eye and retinas were isolated and live cell imaged. The data suggests that DiY was taken up by Müller glia cells, but it may also be incorporated into the ganglion and amacrine cells. In preparation for FAC-sorting, retinas were dissociated using papain and the survival of Müller glia in different resuspension buffers was assessed. The data suggests that cell suspensions containing single Müller glia that survive for at least 6 hours can be obtained.

NF-κB1 is necessary for Müller glial proliferation in the light-damaged zebrafish retina

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Zebrafish (*Danio rerio*) is an excellent model for studying retinal regeneration because of its inherent ability to regenerate any types of lost retinal neurons upon injury. In response to retinal cell loss, zebrafish Müller glial cells dedifferentiate and re-enter the cell cycle to produce neuronal progenitor cells that continue to proliferate and differentiate into the lost neurons to replenish the damage. Previous studies found that TNFα is a critical positive regulator in zebrafish retina regeneration. The apoptotic photoreceptors release TNFα, which is necessary in Müller glia proliferation in the light-damaged retinas (Nelson et al, 2013). Moreover, recombinant soluble TNFα is sufficient to induce the Müller glia to re-enter the cell cycle without retinal damage (Conner et al., 2014). However, the signaling cascades that are activated downstream of TNFα are still largely unknown. One strong candidate is NF-κB1, which is a transcription factor known to play important roles in zebrafish embryonic neuronal development (Correa et al, 2004). Additionally, NF-κB1 is positively regulated by TNFα in various cell types (Wallach et al, 1999). Thus, this study focuses on characterizing the role of NF-κB1 in zebrafish retinal regeneration. Preliminary data showed that expression of the *nfκb1* gene increased in response to light damage at 16 and 36 hours of light treatment (hL), which is the time point when TNFα expression is upregulated in apoptotic photoreceptors and Müller glia, and then decreased

to baseline levels at later stages of progenitor cell proliferation and differentiation. Therefore, NF-κB1 might play a role in the activation of Müller glia. Additionally, SN50, an NF-κB1 inhibitor which blocks the nuclear translocation of the NF-κB1 p50 subunit, significantly reduced the number of proliferating Müller glia relative to sham injected eyes in light-damaged retinas. Together, these data suggest that NF-κB1 is necessary for the initiation of Müller glia proliferation in the regenerating zebrafish retina. Future studies will investigate whether TNFα signaling regulates the expression of NF-κB1.

Determining the role of the streptolysin S-associated gene (*sag)* cluster in MRSA pathogenesis

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a bacterium that is resistant to multiple antibiotics including penicillin, amoxicillin, and methicillin, making it a potentially lethal infectious agent and a serious global threat to health. Recently, it has been discovered that a select strain of *S. aureus*, JKD6159, contains the Streptolysin S (SLS)-associated genes or *sag* gene cluster. This gene cluster had been previously identified in another bacterium called *Streptococcus pyogenes* It is responsible for producing a major virulence factor known as Streptolysin S in *S. pyogenes*. Its role in *S. aureus* is unknown and the purpose of this investigation is to gain a better understanding of the role of this potential virulence factor in the overall pathogenesis of *S. aureus*. Based on its analogous role in *S. pyogenes,* we hypothesize that it functions as a toxin that produces cytotoxic effects directly on host cell. We will determine the potential cytotoxic property of this novel SLS-like toxin by an *in vitro* synthetase reaction using components of the toxin that were purified. The final toxic product will be evaluated for cytotoxicity using a tissue-culture based cellular assays. We hypothesize that an alternative role of the SLS-like toxin in *S. aureus* is to inhibit the growth of other bacteria, helping *S. aureus* to compete in its environment. Growth curves were performed using condition media from a WT and sag B KO on *Staphylococcus epidermidis* and preliminary data shows that when added 25% condition media there is a growth hit at a 5 hour time point.

The Wrath of Ras; Ras Overexpression Leads to Evasion of Anoikis During Extracellular Matrix Detachment

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Cancer is a growing health concern; each year approximately 7 million deaths around the world are cancer-related. Moreover, 90% of cancer related deaths are a result of metastasis, the spread of cancerous cells from their point origin to other areas of the body. For cells to metastasize they must detach from the extracellular matrix (ECM). Normally, ECM-detached cells undergo anoikis, a type of programmed cell death; however, cancer cells evade anoikis and

survive. Ras is an oncogene that is hyper-activated in over 30% of human cancers, thus there is strong incentive to better understand its functions in tumorigenesis. For this reason, our investigation focused on studying the mechanisms by which Ras mediates anoikis evasion. Previous data generated by our lab suggested that oncogenic Ras does have the capacity to allow cells to overcome anoikis. Using mammary epithelial cells overexpressing oncogenic Ras, MCF10A:HRasG12V, we examined the molecular mechanism utilized by Ras to prevent anoikis. We performed co-precipitation assays using the CARD domain of Apaf-1 and western blots to determine if Ras was interfering with the interaction between Apaf-1 and caspase 9, which can dictate the efficacy of anoikis. Results suggest that Ras overexpression in detached conditions is inhibiting the interaction between Apaf-1 and caspase 9, thus contributing to survival. Following this we inhibited the MAPK and PI3K pathways to test if HRas was promoting anoikis evasion through these pathways that are commonly associated with Ras signaling. Inhibition of these pathways was completed in detached cells for 24 hours, which proved to be insufficient time for upregulation of apoptosomal components, giving inconclusive results. However, future studies will continue to examine if Ras is inhibiting the Apaf-1 and caspase 9 interaction through the MAPK and PI(3)K pathways, so these experiments will be altered and revisited in the future. These data are important, as understanding how cancer cells evade anoikis could ultimately lead to significant advances in the development of novel therapeutics designed to specifically eliminate cancer cells.

The effect of diet on the eukaryotic microbiome of long-tailed macaques in anthropogenic environments

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In Singapore and Bali, Indonesia, an increased risk of zoonotic transmission of eukaryotic parasites exists due to heightened human-animal contact. Long-tailed macaques (Macaca fascicularis) are known reservoirs of dangerous parasites, such as Blastocystis, Plasmodium, and Entamoeba. Food provisioning, an example of human-animal contact, causes a shift in macaque diet, which we hypothesize could affect the composition of their gut eukaryotic microbiome. We used next-generation sequencing (NGS) of the 18S rRNA gene to simultaneously detect the diversity of plants as a proxy for diet and the composition of the eukaryotic community in long-tailed macaque feces. NGS proved successful at detecting the diversity of plants in macaque feces, identifying 45 operational taxonomic units (OTUs) with one of the primers used in 18S amplification, and 38 OTUs with the second. Comparisons of plant diversity in guts of macaques from Bali and Singapore showed no correlation, indicating detectable differences in diet. Diet was shown to be significantly correlated to the composition of the eukaryotic gut microbiome. This suggests that the macaque diet has a significant effect on the whole eukaryotic microbiome.

Regulation of the StARD9 Motor Domain by Phosphorylation

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Niemann-Pick Type C disease (NPC) is a neurodegenerative lysosomal storage disorder due to mutations in NPC1 and/or NPC2 proteins. StARD9 is a novel monomeric kinesin found in membranes containing wild-type, but not mutant NPC1 protein. StARD9 is potentially involved in lysosomal transport and contains a kinesin motor domain that is likely to bind microtubules. This investigation aims to characterize the microtubule binding activity of the StARD9 motor domain in both the phosphorylated and dephosphorylated states. Because the two phosphorylation sites at amino acid positions 170 (serine) and 172 (threonine) are not highly conserved across human kinesin families, but are conserved across different species' StARD9 sequences, they are the focus of this investigation. In order to characterize the wild-type, phosphorylated, and dephosphorylated states of the StARD9 motor domain, we created GFP-tagged constructs of the first 1-684 amino acids. We created three different versions of the construct with regards to the phosphorylation sites: serine-170/threonine-172 (wild-type), aspartic acid-170/aspartic acid-172 (phosphorylated), and alanine-170/alanine-172 (dephosphorylated). These constructs contain mutations at the phosphorylation sites that mimic states of constant (de)phosphorylation, which will be referred to as either "phosphomimetic" or "de-phosphomimetic." Both the phosphomimetic and de-phosphomimetic StARD9 motor domain constructs displayed diffuse protein localization and non-specific binding throughout the cell. Since these constructs were created by mutation rather than the natural process of (de)phosphorylation by kinase(s)/phosphatase(s), the microtubule binding activity of the StARD9 motor domain may have faced interference arising from complications in protein folding, especially in the change from the wild-type to acidic amino acids. Another explanation for why both motor domain constructs deviated from the normal, wild-type localization to be similarly diffuse is that the phosphorylation states of the two sites may need to be different and not both phosphorylated or both dephosphorylated. Future directions for research may include determination of kinases and phosphatases involved in regulating StARD9, and the development of drugs specific to these kinases and phosphatases.

Determining downstream regulators of wallenda-induced cell degeneration and axon sprouting in *Drosophila melanogaster* photoreceptor cells

John Miller, Stephanie Freed, and Joseph O'tousa

The Jun N-terminal kinase (JNK) pathway is controlled by the Jun kinase kinase kinase *wallenda* (*wnd)* in response to injuries of the axonal cord. To study this pathway, my lab has developed the photoreceptor cells in the retina of *Drosophila melanogaster* as our model system. Our analysis has revealed that the over-expression of *wnd* (*wndE*) will accelerate the light-induced cell degeneration and will induce axon sprouting at the synaptic terminals of the photoreceptor cells. To study these mechanisms, I conducted a candidate gene screen for probable contributors to the wnd *pathway*. To determine the role of each of the candidate genes, I used RNA interference (RNAi) to knockdown the genes expression in a *wndE* environment. After conducting the screen for cell degeneration, the knockdown of the gene *hemipterous* (*hep*) yielded a partial rescue, and the knockdown of *kayak* showed a complete rescue of the cell body. In contrast, the knockdown of *puckered* (*puc*) showed accelerated degeneration of the cell body. After conducting the screen for axon sprouting in the synaptic terminal, the level of expression of the gene *hep* revealed to

have a proportional relationship to the amount of axon sprouting occurring in the synaptic terminal region.

Investigation of novel insecticides through the manipulation of mosquito G-protein coupled receptors (GPCRs)

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According to the World Health Organization (WHO), in 2015 there were an estimated 214 million cases of Malaria and about 440,000 deaths. Malaria, yellow fever, and dengue are only a few of the many diseases transmitted frequently via mosquito vectors. In addition to high transmission rates, few advances have been made in the insecticides used to cull these prevalent vectors, and thus, resistance to the few available drugs have developed over time. Such resistance has increased the need for the research and development of novel insecticides that will help to reduce the spread of many diseases via mosquito transmission.
G- Protein Coupled Receptors, such as the octopamine and serotonin receptors, are heavily involved in research investigating the development of novel insecticides due to their large role in various essential physiological processes. Through contact assays, we have tested the impact that various agonist and antagonist compounds of these GPCRs have on mosquito mortality, sugar and blood feeding, and oviposition.

Temperature manipulation of *wallenda (wnd)* expression induces axon outgrowths in *Drosophila melanogaster* R7 photoreceptor cells

Catherine A. Bruno, Stephanie Freed, Kirk Mecklenburg, Joseph E. O'Tousa

Photoreceptor cells in *Drosophila melanogaster* are able to produce axon outgrowths beyond normal synaptic regions of the optic lobe. Our laboratory is looking at axon outgrowths induced by a Jun-N-terminal kinase (JNK) pathway controlled through the gene *wallenda* (*wnd).* Through the GAL4-UAS expression system, GAL4 protein initiates overexpression of *wnd* driving axon outgrowths in photoreceptor cells. In this study, temperature manipulation of *wnd* was conducted using the temperature sensitive transcription repressor $GAL80^{ts}$ to block production of GAL4 protein thereby creating the ability to monitor the occurrence of axon outgrowths. Two different rearing techniques were conducted to view their effects on *wnd* expression and axon outgrowths; one set of flies were reared at 25℃ until eclosion then moved to 18℃ for 3 days; whereas, the second set of flies were reared at 25℃ until the third instar larval stage then moved to 18℃ until 3 days old. These experiments showed that at any point in development lower temperatures repress *wnd* activity due to $GAL80^{ts}$, thereby inhibiting axon outgrowths. The difference in temperature at which sprouts are first seen between rearing methods is hypothesized to be due to the difference in developmental time when the temperature shift occurred. The shift allows a longer time for rhodopsin (Rh3) to drive *wnd* and turn on axon outgrowths at higher temperatures. We conclude that a temperature change at eclosion is sufficient to trigger

repression of GAL4 protein through $GAL80^{ts}$ compared to temperature change at earlier stages of development. Axon outgrowths can be temperature manipulated based on the activity of $GAL80^{ts}$ since levels of *wnd* expression is critical for inducement of axon outgrowths. The ability to control axon outgrowths will be helpful for new techniques in neuronal regeneration especially since an analogous gene to *wnd* has been found in humans, leading to the exciting possibilities of partial axon regeneration in human photoreceptor cells.

Investigation of IT-139 Downstream Activity in Pancreatic Ductal Adenocarcinoma

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Pancreatic Ductal Adenocarcinoma (PDAC) is the third leading cause of cancer related death in the country. Pancreatic cancer has such a high mortality rate due to a lack of early detection and prevalent chemoresistance in late stage disease. Inflammation is prevalent in PDAC and can cause an increase in unfolded proteins, resulting in endoplasmic reticulum (ER) stress. In response, the cell activates the Unfolded Protein Response (UPR), whose main function is to remove the accumulation of unfolded proteins. Activated survival pathways associated with the UPR are believed to play an important role in chemoresistance. The major regulator for the UPR is GRP78, which in healthy cells is bound to transmembrane proteins IRE1a, ATF6, and PERK. Upon ER stress, these transmembrane proteins are activated, leading to signaling cascades that have been associated with cellular survival. A ruthenium based drug known as IT-139 has been shown to block the induction of GRP78 and extend the overall survival of PDAC mice when used in combination with gemcitabine. However, the mechanisms of IT-139 are completely unknown. As GRP78 has been shown in other cell types to co-localize with AKT at the cellular surface upon ER stress and AKT has been associated with chemoresistance, we began investigating the possibility that IT-139 can block this colocalization and remove AKT associated chemoresistance through the use of immunofluorescence techniques. No significant effect was observed, suggesting that this pathway was not the primary mechanism of IT-139 activity. After further investigation into downstream pathways affected by IT-139 via Western Blot, it was determined that IT-139 is capable of blocking the ER stress induction of the IRE1a branch of the UPR, known to be tied to cell survival. Possible future directions would be to examine the expression of proteins further downstream in the IRE-1a pathway and perform functional tests measuring cell survival with IRE1a pathway knockdowns.

Evaluation of the temporal species composition and behaviors of molecularly characterized Anopheles species in Karama, Indonesia

Stephanny Lizarraga, Victoria Makuru, Julie Niedbalski, and Neil F. Lobo

Malaria is a life-threatening disease caused by *Plasmodium* parasites. It is transmitted between humans by the act of blood feeding by the female *Anopheles* mosquito. This disease was

responsible for 438,000 deaths in 2015 alone, along with 214 million reported cases. Currently, there are 430 known *Anopheles* species, but only 30-40 of them are known malaria vectors. There is a need to correctly identify *Anopheles* species and their role in malaria transmission as morphologically misidentified specimens result in incorrect conclusions on species compositions, downstream analysis on species-specific bionomics, and species contributions to disease transmission as well as intervention effectiveness. This leads to suboptimal resource allocation and ineffective intervention strategies. The goal is to compare molecular identification done in the lab with morphological identification done in the field to determine species compositions and quality of morphological identifications. Data including *Anopheles* species compositions and behaviors between wet season and dry season were also analyzed. A total of 16 species were identified of which only 8 could be identified to specific species. The most common *Anopheles* species in Karama, *An. barbirostris*, was found to have both indoor and outdoor biting behavior and was observed to be present all year round. Some novel *Anopheles* species were observed to be more prevalent than others depending on the season and year, and display different biting habits.

Future Goals: Determine year long and multi-year species composition and distribution, infectivity, and their feeding preference (anthropophily vs zoophily), biting habits (indoor vs outdoor), feeding times, and resting behavior. Use this information to fill in missing gaps on the vector contribution to malaria transmission in Karama, Indonesia.

Three-Dimensional Analysis of Microglia and Vasculature in the Brain during Neuroinflammation

Vanessa Quintero, Victoria Zellmer, Ian Guldner, and Siyuan Zhang

Microglia are a central nervous system (CNS)-specific family of monocytes. They play a key role in monitoring their microenvironments for foreign invaders or signs of injury. In this study, we defined the relationship between microglia and blood vessels. Based on preliminary studies, we hypothesized that the microglia would have an impact on the blood vessel architecture in the response to inflammation. Three dimensional analysis and subsequent deep tissue imaging was conducted to explore our hypothesis. We observed a change in microglia distribution near blood vessels and quantification demonstrated that there was an increase of contact between the microglia and vasculature in our neuroinflammation model. To further explore microglia-vasculature relationships, we examined microglia-enforced BBB integrity by altering microglia function and conducting a BBB leakiness assay. Based on preliminary observations, BBB leakiness is a possible indicator for microglia contribution to the BBB. Together, our results suggest microglia and vasculature interact during induced neuroinflammation conditions.

Investigating the Role of *MMAR_0039* in Cell Wall Stability in *Mycobacterium*

Jasmine Mayorga, Cristal Reyna, Dr. Champion

PDIM is a mycobacterial lipid required for cell stability and virulence (Cox et al., 1999). *In vitro* studies in *M. tuberculosis (M. tb)* have noted that *M. tb* frequently loses phthiocerol dimycocerosate (PDIM) during standard laboratory manipulation. Loss of PDIM results in attenuation, which is often ascribed to a different intended genetic manipulation. *Mycobacterium marinum* is an established model system for *M. tb*. We found that unlike *M.tb, M. marinum* does not lose PDIM. Interestingly, the loss of a single gene destabilized PDIM in *M. marinum.* Based on these data we hypothesized that *MMAR_0039* was required for PDIM stability in *M. marinum.* To test this we introduced *MMAR_0039* into *M. tb* and monitored PDIM loss. We were surprised to find that our strains of *M. tb* do not lose PDIM. Together our data is not sufficient to determine if *MMAR_0039* stabilizes PDIM.