2019 ABSTRACT BOOK

Summer Undergraduate Research Program

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2019 Summer Undergraduate Research Program

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Identifying Kidney Injury in DKD Mice via Immunofluorescence

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HIV positive patients have an increased risk of developing diabetes and diabetic kidney disease (DKD). Kidney disease is the 4th leading disease contributing to death in Americans living with HIV. Kidneys filter the blood and remove toxins and waste products. Diabetes, the presence of abnormally high levels of glucose in the blood, is the leading cause of kidney failure. Forty percent of diabetic patients will develop diabetic kidney disease (DKD). Antiretroviral therapy (ART) can improve kidney function in HIV-positive patients with CKD and data from the Ross lab suggests that the HIV protease inhibitor darunavir may protect the kidneys from injury by mechanisms independent of its effects on HIV and may even protect the kidneys from DKD.

In this study, we performed immunofluorescence to examine the effect of darunavir upon expression of proteins related to kidney injury in mice with DKD (eNOS-/- with streptozotocin-induced diabetes), and wild-type (control) mice. Mouse kidney sections were incubated with primary antibodies against type 1 collagen (marker of fibrosis/scarring) and synaptopodin (protein present in normal podocytes - cells that prevent leaking of protein from blood into urine). Primary antibodies were detected with secondary antibodies labeled with Alexa-Fluor 488. There was less synaptopodin detected in podocytes from DKD mice without DRV treatment compared to DKD mice with DRV treatment. Higher amounts of type 1 collagen were found in untreated DKD mice than in DRV-treated DKD mice, indicating that DRV treatment remarkably reduces fibrosis and restores glomerular structure.

In conclusion, immunofluorescence helped detect deterioration of the glomeruli in the DKD mouse kidneys. Identifying changes in these models will improve understanding of how diabetes, independent of HIV pathogenesis, causes fibrosis and kidney injury.

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How flexible are you in your predictions? Probability processing in individuals with ASD

A key feature of Autism Spectrum Disorder (ASD) is insistence on sameness and resistance to change. Recent research suggests that this inflexibility may be due to impaired formation of expectations. Predictive processing has been shown to attenuate the MMN in conditions with a higher probability of deviancies, as they are more expected in the environmental context. The mismatch negativity (MMN) electrical brain response is elicited when there is a change in a frequently presented auditory stimulus, and is thought to reflect the pre-attentive detection of violation of a prediction. In healthy adults, predictions are stronger for more frequently occurring standard stimuli (e.g., 96% versus 74%), and thus presentation of a deviant leads to a larger MMN. We used scalp electroencephalography to investigate MMN differences between individuals with ASD and typically developing controls. Participants were presented with sequences of tones containing standard stimuli on the majority of trials (1000 Hz, 500-ms Stimulus Onset Asynchrony (SOA)), interspersed with deviant stimuli in which the pitch (1300 Hz), or the SOA (300-ms) deviated from the standard stimulus. The two types of deviants (pitch and SOA) each occurred at one of three probability conditions: 4%, 8%, or 16%. It is hypothesized that conditions with deviants presented at higher probability will elicit lower MMN than those of lower probability in a typically developing (TD) control group. However, we predict this attenuation of the MMN to be less apparent in the ASD group, due to poorer flexibility in calculating the environmental statistics to generate predictions. Our preliminary data support that probability influences the MMN, a concept illustrated by a larger MMN response to lower probability deviants in TD pilot participants. Data will be presented from a cohort of TD and age-matched ASD participants.

Behavioral Verification of a Disynaptic Signaling Pathway from the Cerebellum to the Prefrontal Cortex

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The cerebellum (Cb), once thought to only be involved in motor function, has been implicated in various non-motor functions, such as social behavior and reward processing. Recently, anatomical evidence has furthered this assertion through the discovery of a projection from the deep cerebellar nuclei (DCN) to the ventral tegmental area (VTA), a structure associated with the control of reward responsive behavior. Also believed to be associated reward processing is the medial prefrontal cortex (mPFC), hinting at the existence of a Cb-VTA-mPFC signaling pathway, purposed with regulating reward responsive behavior. While various anatomical and *in vivo* imaging experiments have provided confirmatory evidence for the existence of the Cb->VTA->mPFC circuit, its effect on reward processing has not been determined. As a result, we have carried out a conditioned place preference (CPP) experiment designed to test the functionality of the circuit in the mouse model. In expressing a Cre-dependent Retro-flox channelrhodopsin-2 (ChR2) in the neurons of the mPFC, and expressing a Cre virus in the neurons of the DCN, we have singled out the latter leg of the supposed Cb-VTA -mPFC signaling pathway, leaving the VTA neurons optically active. After optically stimulating the VTA across two frequencies, 4hz and 20hz at 5mW of power, there was no significant difference in place preference between the ChR2 mice and the GFP control group, running counter to the idea that the Cb-mPFC signaling pathway is involved in reward responsive behavioral output. As a follow up to this unexpected result, as histological analysis was performed on the brains in question, with special attention given to the Cb, VTA, and mPFC. From this, we were able to identify an issue with the Cre viral injections, calling into question the results of the CPP experiment. In order to further verify the existence of the Cb-mPFC signaling pathway, a different viral vector approach will need to be explored.

Analyzing the Role of Frizzled 2 and Frizzled 9 in Ewing Sarcoma Cell Migration

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Ewing Sarcoma is the second most common malignant bone tumor in pediatrics after osteosarcoma. It is an aggressive sarcoma of the bone and the soft tissue with a peak incidence in adolescents and young adults. The five year overall survival (OS) is <30% for patients who present with metastatic disease compared to a 70% OS for patients with localized disease. Based on previous studies, it has been shown that non-canonical Wnt signaling has a major role in cancer. Wnt proteins are autocrine and paracrine signaling molecules that are secreted and are important for cell proliferation, migration and organogenesis during embryonic development. Wnt5a is involved in non-canonical signaling therefore it is the protein that we focused on for this experiment. Porcupine (PORCN) is an enzyme that post-translationally modifies Wnts by adding a palmitate tail. Without that tail, Wnts cannot bind to their receptors and cause downstream function. PORCN inhibitor Wnt974 is a pan-Wnt inhibitor in that it causes all Wnts to become nonfunctional. PORCN inhibition increases Wnt5a transcription in a majority of Ewing Sarcoma cell lines which indicates that Wnt5a and its functions are important to the cell. Wnt5a appears to increase Ewing Sarcoma cell migration. Frizzled proteins are the principal receptors for the Wnt family of ligands and they mediate canonical as well as non-canonical Wnt signaling. Through a PCR screen comparing Wnt5a responsive and Wnt5a non responsive Ewing Sarcoma cell lines, one major difference was that Frizzled 2 (Fzd2) and Frizzled 9 (Fzd9) were present in the responsive but absent in the non responsive cell lines.

The goal of this project is to determine which receptor(s) Wnt5a signals through in Ewing Sarcoma. We hypothesize that Fzd2 and Fzd9 are important receptors for non-canonical Wnt5a signaling and that loss of one or both of the receptors will adversely affect cell migration. To test our hypothesis, we worked with two different Ewing Sarcoma cell lines: TC71 and A4573. We used CRISPR/Cas9 genome editing to knock out Fzd2 and Fzd9. We were able to see the green fluorescent protein (GFP) which was an indicator that the cells were successfully transfected. We used puromycin to destroy the cells that did not take up the linear DNA and were able to observe some phenotypic changes such as the cells being: more circular, less adhesive and more prone to growing on top of each other as compared to the parental cell lines.With subsequent passages, however, the cells appeared to revert back to their normal phenotype which indicates inadequate genomic editing or a selection of cells that did not undergo genomic editing but still took up the linear DNA containing the puromycin resistant gene and the green fluorescence protein. Protein analysis of both unselected and selected Fzd2 and Fzd9 cell lines did not demonstrate complete knockout of either Frizzled which is consistent with the reversion to normal phenotype that we saw with subsequent passing of the cells. Migration assays with Boyden chambers did not show statistically significant decreases in cell migration with all CRISPR/Cas9 edited A4573 cell lines. Immunofluorescence of Fzd2 CRISPR/Cas9 edited A4573 cells demonstrated a decrease, but not complete loss of, Fzd2. Our data demonstrates that we can successfully transfect Ewing sarcoma cells although it may be a short-lived phenomenon due to their inherent genomic instability.

We plan to repeat these experiments at an earlier passage when the cells still demonstrate the abnormal phenotype and the GFP tag is the brightest. Future experiments may require a GFP selection via flow cytometry as well as an increase in puromycin dosage for selection. We will perform additional genomic confirmation via PCR with Frizzled specific primers. Additional experimentation will allow us to adequately assess the role of Fzd2 and Fzd9 as the receptors for Wnt5a in Ewing sarcoma.

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Previous research from the group on rat tumor cells showed that macrophage production of EGF was required for tumor cells to form tunneling nanotubes (TNTs). TNTs play a crucial role in the communication between cells and have also been shown to promote tumor cells to invade. For my project we want to know the relationship between EGF, the production of TNTs and protein levels of M-sec (a protein known to play a role in TNT formation) in a human breast cancer cell line, MDA-MB-231. We have seen that EGF increases TNTs in MDA-MB-231 cells. We expect the levels of M-sec to be higher in the presence of EGF. When the drug Iressa, an EGFR inhibitor, M-Sec levels should be comparable to baseline levels of M-Sec.

Elucidating the Role of Sigma Factors in M. Tuberculosis Persistence

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Mycobacterium tuberculosis (*Mtb*) remains a global threat, accounting for ~1.6 million deaths due to tuberculosis (TB) in 2017. It is not only the emergence of strains resistant to current drug/multi-drug remedies that impose a great challenge in finding new methods of treatment, but in particular, the presence of a minute subpopulation of cells knowns as the persisters. Persisters are not genetically resistant to drugs, but resist sterilization by TB antibiotics. It is thus important to investigate the molecular mechanisms by which *Mtb* steers persister formation. Sigma factors are interchangeable subunits of RNAP in bacterial cells that assist in the transcription of genes involved in survival, virulence and stress responses.

Mtb has 13 sigma factors, one of which is essential and 12 that are non-essential. The generation of sigma factor knockouts in biosafety level-2 (BSL-2) safe *Mtb* background strains can aid our understanding of persister formation when no BSL-3 facility for virulent strains is available. Our group has previously shown that deletion of sigma factor E (*sigE*) in virulent H37Rv *Mtb* increases susceptibility of the Δ*sigE* mutant strain to frontline drugs. In particular, the Δ*sigE* mutant strain sterilized upon treatment with isoniazid (INH), which was in contrast to the wild-type (WT) H37Rv that did not sterilize. In this study, we tested the hypothesis that a BSL-2 safe *Mtb* strain with a Δ*sigE* deletion will sterilize irrespective of the biosafety status of the mutant. Following INH treatment *in vitro*, we assessed the INH-sterilization phenotype of mc^{2}6230 (H37Rv Δ*RD1* Δ*panCD*) and mc^{2}8574 (H37Rv Δ*RD1* Δ*panCD* Δ*sigE*) over 28 days by measurements of $O.D._{600\ nm}$ and death curve plating. Our results showed that while there was a ~3-log reduction in growth following INH treatment relative to the untreated condition for all strains, the BSL-2 Δ*sigE* mutant strain did not sterilize. The distinct lack of sterilization recorded for the Δ*sigE* mutant in the BSL-2 background, may be as a result of a high starting inoculum or potential side-effects of the *RD1* deletion or pantothenate auxotrophy.

To continue investigating the molecular interactions of *Mtb* sigma factor E and its potential role in steering the persistence phenotype, it will be necessary to generate double *sigE*-sigma factor knockouts or *sigE*-transcription factor mutants. To achieve this, we have removed the selection cassette from the Δ*sigE* mutant by specialized transduction and sucrose selection and verified the unmarked clones for hygromycin sensitivity. We recorded no differences in the growth or sensitivity of both the marked and the unmarked Δ*sigE* mutant strains to INH.

In summary, the findings of our study were that: (1) we verified the Δ*sigE* mutant strain did not sterilize after INH treatment in the BSL-2 safe strain background; and (2) generating successful unmarked Δ*sigE* knockouts without the hygromycin resistance cassette, and testing for the INH-sterilization phenotype, showed no differences among marked and unmarked strains.

Intrinsic Effects of Pertussis Toxin on Plasma Cell Fate in Bone Marrow

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Summer Undergraduate Research Program

Plasma cells (PCs) are long-lived, terminally differentiated B-cells that migrate to the bone marrow (BM) from the lymph node, where they secrete antibodies. PCs respond to chemotactic signals for retention in the BM. Some of these chemokine signals are transmitted by G-alpha$_i$ ($G\alpha_i$) protein coupled transmembrane receptors. In this study, we determined what the effect of blocking $G\alpha_i$ signaling, using pertussis toxin (PTX), would have on PCs retention in the BM. To test this, we used a *Rosa26-PTX* inducible allele, in which PTX protein expression could be induced upon tamoxifen-inducible cre-mediated recombination at the *Rosa26* locus. Past work in the lab showed that in mice that ubiquitously expressed PTX in all cells, the number of PCs increased in the BM of mice compared to controls, suggesting that $G\alpha_i$ signaling was promoting BM exit. However, since PTX affects many signaling pathways and many cell types, we sought to determine if $G\alpha_i$ signaling was required in PCs in a cell intrinsic manner. To do so, we generated mixed BM chimeric mice where half the hematopoietic cells were PTX expressing (KO) and half were that were not (WT). We observed that T cells which produce PTX are more numerous in BM and spleen (SP) than WT or Host cells after being treated with tamoxifen (TMX). A significant loss of WT and Host cells was seen in TMX treated samples. We also saw B cells which produce PTX seem to remain in BM and SP more successfully than WT or Host cells after TMX treatment. This difference was not significant; however, the population did increase in number post treatment. Here too was a similar loss of WT and Host cells. Plasma cells that produced PTX do not seem to vary in number in the SP or BM but make up more of the total population in mice treated with TMX. We conclude that the action of intrinsically produced PTX did not alter plasma cell retention or tissue tropism.

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Modeling the role of SOCS1 in azacitidine therapy using MDS/AML cell lines

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Disease and azacitidine (AZA)-related thrombocytopenia (inefficient platelet numbers) is a serious clinical problem in the management of myeloid malignancies, such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). MDS and AML are clonal disorders caused when there is disruption in the production of functional mature blood cells induced by mutations or epigenetic aberrations in the hematopoietic stem cells (HSC). Both disorders show a marked increase in unfunctional myeloblasts in the bone marrow which crowd out normal blood cells and disrupt differentiation leading to multilineage cytopenia. Eltrombopag (EP) is a thrombopoietin receptor (TPO-R) activating drug used to stimulate the production of platelets in a process known as megakaryopoiesis; it is widely used for the treatment of thrombocytopenia in non-leukemic patients. Previous work in the Will lab has revealed that EP stimulates megakaryopoiesis in patients with MDS/AML without increasing leukemic cell growth. Based on these and further encouraging preclinical observations, several clinical studies were launched. Unfortunately, a large placebo-controlled phase III clinical trial evaluating whether EP can counteract AZA therapy-related exacerbation of thrombocytopenia in MDS and AML patients was unsuccessful as patients receiving both AZA and EP had no improvement in platelet levels and showed a trend towards increased disease progression compared to AZA monotherapy recipients. Our lab is interested in understanding the underlying mechanism of the failed EP/AZA combination therapy to develop potential mitigation strategies. We have recently uncovered that AZA interferes with EP-mediated stimulation of megakaryopoiesis by inducing Interferon type I (IFN-I) signaling in primary cells from healthy donor and MDS/AML cells. This innate immune pathway activation also triggered a rapid induction of Suppressor of Cytokine Signaling-1(SOCS1) which is activated upon stimulation of serval cytokine receptors, including TPO-R and IFN-I receptors (IFNAR). SOCS1 is an important inhibitor of interferon and cytokine receptor signaling pathways, preventing their constitutive activation in normal physiological settings. We hypothesized that super-physiological SOCS1 activation would prematurely shut down TPO-R signaling and hamper EP-mediated stimulation of megakaryopoiesis in the presence of AZA-medicated IFN-I induction.

The experimental objectives of this project were to evaluate how MDS/AML-derived cell lines (MDS-L and MOLM-14) respond to AZA treatment, specifically assessing the consequences of SOCS1 activation and IFN-I signaling induction. We employed ELISA and intracellular FACS assays to quantify the amount of IFN-α, IFN-β and SOCS1 production. We also evaluated the functional effects of AZA therapy on the short-term and long-term proliferation of MDS/AML cell lines using liquid cultures. Our data showed that there was an absence of IFN-α protein induction in the MOLM-14 and MDS-L cell lines. MOLM-14 and MDS-L cell lines used for IFN-β FACS analyses showed a decrease in IFN-β expression levels between the AZA treated and untreated samples. In contrast, our IFN-β FACS analyses showed that a primary AML patient-derived sample induced IFN-β expression up to 40% upon AZA treatment, in line with previous observations. We next assessed the expression of Type I Interferon receptors (IFNAR1/2) by FACS analysis of MOLM-14 and MDS-L cells. The vast majority of both cell lines expressed only IFNAR2 at high levels, while there was a small subpopulation in each cell line that showed expression of both receptors. This suggests that only a small subpopulation coexpressing IFNAR1/2 would activate IFN type I signaling. Our SOCS1 FACS analyses further showed that MDS-L cells increase SOCS1 expression upon EP/AZA treatment as compared to EP treated cells; MOLM-14 cells showed constitutive activation of SOCS1 and no further change.

Our data show that AZA treated MDS/AML cell lines responded similarly as compared to primary patient-derived AML cells, which we found to heterogeneously respond to AZA. As previously observed, some MDS/AML clones activate Type 1 Interferon signaling, while some clones don't. Together these results show the limitations of using MOLM-14 and MDS-L cell lines for ex-vivo modeling of the response to AZA therapy using these cell lines. In the future, we intend to do Western blot analyses to determine baseline levels of SOCS1 protein in both healthy and AML/MDS patient-derived samples. We also plan to evaluate changes to SOCS1 protein expression in these cells after AZA and EP therapy.

Changes in Gene Expression in the Livers of Prenatal Vitamin D Deficient Offspring

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Abstract

Vitamin D, a fat-soluble micronutrient, is a major regulator of bone homeostasis. Vitamin D plays vital roles in the development of the immune and cardiovascular systems, and the reduction of inflammation in tissues. Vitamin D deficiency is a condition that is widely observed in both pregnant women and infants in the United States. It is well known that maternal vitamin D deficiency leads to adverse outcomes in both the mother and offspring. These adverse health effects for the offspring include poor bone development, an increased susceptibility to asthma, hypertension and cardiovascular disease. In order to prevent diseases related to prenatal vitamin D deficiency, we need to understand the physiological role vitamin D plays in the developing fetus and to identify this risk factor during pregnancy.

To demonstrate the effects of prenatal vitamin D deficiency on offspring, we performed a dietary manipulation on C57BL/6J female mice. We observed numerous phenotypic alterations in offspring born to vitamin D deficient (VDD) female mice compared to offspring of vitamin D sufficient (VDS) females. These phenotypic alterations include a decrease in the number of immune cells, an increase in lung muscle growth, body growth defects, an enlarged heart size and an abnormal liver morphology. One outstanding alteration we found was the accumulation of lipids and fibrosis in the livers of the VDD offspring. We therefore tested the changes in gene expression in the livers of VDS and VDD offspring using RNA sequencing techniques. We performed a series of quality control checks on our RNA-Seq data. After quality control checks, we began to identify the differentially expressed genes (DEGs) between the 2 groups. 192 DEGs were identified at a p-adjusted value of 0.01 and a $\log_2$ fold change of 2. Some of the most differentially expressed genes included *Mfap4*, *Col4A4*, *Hsd17b13*, *Cyp2a4*, and *Esm1*, which are all associated with non-alcoholic liver disease (NAFLD). Based on the Gene Ontology analysis of these DEGs, the collagen-containing extracellular matrix pathway was the most enriched. We further analyzed the DEGs using gene set enrichment analysis (GSEA). We found that many liver-associated pathways were enriched, including the IL-6, TNF-α and hypoxia pathways. These findings demonstrate that prenatal vitamin D deficiency has an effect on the development of liver disease in the offspring. In the future, we will perform further experiments to confirm these findings by conducting qRT-PCR on these candidate DEGs that are upregulated and downregulated between the VDS and VDD groups.

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The Role of the Neck Linker in Kinesin1 Motor Domain Coordination

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Summer Undergraduate Research Program

Abstract

Kinesin1 is a motor protein which binds and transports cellular cargo along microtubule tracks. Its two identical motor domains bind to the microtubule and hydrolyze ATP to generate energy for mechanical work. The neck linker of Kinesin1 is a region connecting the motor domains with the dimerization domain that is believed to have a role coordinating the ATPase activities of the two motor heads for processive movement and directing the motion of the kinesin in a single direction. The neck linker transitions from an undocked to docked configuration after the binding of ATP but it is unclear whether and how the position of the neck linker (docked or undocked) may affect the nucleotide binding pocket structure and the microtubule-stimulated ATPase activity of the motor. To test the hypothesis that there is a reciprocal relationship between ATPase activity and neck linker configuration, (i.e. ATP binding affects neck linker position and neck linker position modulates ATPase activity) we made Kinesin1 constructs with distinct neck-linker lengths to be proved by ATPase assays and high resolution cryogenic electron microscopy (cryoEM). Each construct was made by taking a plasmid containing the sequence for a kinesin motor domain, neck linker, and part of the coiled coil domain and making deletion mutations using site-directed mutagenesis kits. Competent bacterial cells were transformed with the mutated plasmids and were grown overnight. The cells were lysed and the plasmids were extracted. To ensure the mutations were made, the plasmids had to be sequenced.

Repurposing the HIV Drug Tenofovir for treatment of patients with Ultra-High-Risk Neuroblastoma

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Neuroblastoma is a malignant neuroendocrine tumor of the sympathetic nervous system, where 800 new cases occur annually in the United States. Neuroblastoma prognosis is determined by the International Neuroblastoma Risk Group Staging System. 50% of children with high-risk neuroblastoma still succumb to the disease, despite intensive therapy that includes myeloablative chemotherapy with hematopoietic stem cell rescue. This 50 % of patients with high risk neuroblastomas, who do not respond to therapy, can be considered patients with ultra-high-risk neuroblastoma (UHR-neuroblastoma). Recent studies have shown that children with increased telomerase activity have a poor prognosis and that telomerase activity is increased in patients with UHR-NB. Telomerase is an enzyme in cells that is composed of a reverse transcriptase where the synthesis of telomeric DNA repeats and this enzyme is known to be active in cancer cells. Therefore, it is likely that telomerase constitutes a viable therapeutic target in neuroblastoma. Nucleoside analogs have shown to inhibit telomerase and preferentially be toxic to cells expressing high telomerase activity. We hypothesize that tenofovir disoproxil fumarate (tenofovir) demonstrates synergy with conventional chemotherapies through increased DNA damage and apoptosis and an inhibitory effect on telomerase.

An MTT assay was used on the neuroblastoma cell line IMR5 and was treated with 0-300uM of tenofovir to demonstrate a dose dependent decrease of cell viability. The IC50 of the neuroblastoma cell lines IMR5, EBC1, SKNSH, CHLA-15, and NB1643 were calculated using this method. A TRAP (telomeric repeat amplification protocol) assay (Sigma) was performed to identify the telomerase activity in the various cell lines. IMR5 was treated with tenofovir and doxorubicin at various concentrations over a 48-hour period and we used an MTT assay to identify decreases in cell viability. Synergy was identified by analyzing cell viability data with Compusyn, a statistical software. A western blot for C-PARP (a marker of apoptosis) and yH2AX (a marker of DNA damage) was used to quantify the effect of tenofovir on doxorubicin induced apoptosis and DNA damage. An MTT assay was performed to see if IMR5 became sensitized to doxorubicin or SN-38 (the active metabolite of irinotecan) after a pretreatment of 1 or 10uM tenofovir over a one-week period and decreased the IC50.

The MTT assays demonstrated that Tenofovir decreased cell viability in a dose dependent manner in NB cells and the IC50 for IMR5 was at 135 uM (Figure 1A). The IC50 of Tenofovir in neuroblastoma cell lines IMR5, EBC1, and SKNSH demonstrated that cells with high telomerase activity are less sensitive to Tenofovir (Figure 1B). CHLA-15 and NB-1643, cell lines with low activity are more sensitized to Tenofovir at lower doses (Figure 1B). Tenofovir increases the cytotoxic effect of doxorubicin in IMR5 demonstrated by the MTT Assay and Compusyn Analysis (Figure 2A). Synergy was identified in IMR5 across 50%, 75%, and 90% Fraction Affected (Figure 2B). The percent Fraction Affected represent the percentage at which the cells die from the synergistic affect. The Western Blot, at 48 hours of treatment, showed that 10uM of Tenofovir increased doxorubicin induced apoptosis (C-PARP) and DNA Damage (y-H2AX) (Figure 3A). Pre-treatment with 1 or 10uM of Tenofovir for 1 week also sensitized NB cells to doxorubicin or SN-38, and the IC50 of doxorubicin and SN-38 decreased significantly after the one week of tenofovir pre-treatment (Figure 3B ,3C, and 3D).

Tenofovir demonstrated synergy with doxorubicin in the neuroblastoma cell line IMR5 and showed a sensitization to doxorubicin with a one-week pre-treatment of Tenofovir. The finding that neuroblastoma cell lines which have high telomerase activity are less sensitive to Tenofovir, suggests that telomerase may be a therapeutic target of tenofovir. Since Tenofovir has demonstrated synergy at clinical dosages and is approved by the FDA as an HIV antiviral drug, it can be applied rapidly to clinical trials for patients with UHR-neuroblastoma who have a poor prognosis. Future experiments will focus on an expanded panel of neuroblastoma cell lines to demonstrate synergy and a telomere length experiment will identify whether tenofovir inhibits telomerase leading to a reduction in telomere length.

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Regulation of Small GTPases by PI3Kβ

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GTPases are key regulators of multiple cellular signaling pathways. GTPase enzymes are active when bound to GTP, and inactive when they hydrolyze GTP to GDP. Disruptions in the levels of GTPase activity are implicated in the development and metastasis of cancer. The GTPases Rab5, Rac1, and Cdc42 are known to bind the class I phosphoinositide 3-kinase PI3Kβ. I studied the effect of PI3Kβ on GTPase activity in the cell using two distinct assay assays for Rac1 and Rab5 activation. For Rac1, cell lysates are incubated with a GST fusion protein that specifically binds to active GTP-Rac1. For Rab5, I used a conformationally sensitive antibody that specifically binds to active GTP-Rab5. I first established the validity of the activation assays using MDA-MB-231 lysates in which GTPases were loaded with GDP or GTP. Both assays preferentially detected GTP-loaded GTPases. I next tested conditions that have been reported to activate Rab5: serum starvation and inhibition of the Vps34 PI3K. However, I saw a loss of Rab5 activity under both conditions. I also tested for EGF activation of Rac1, but was unable to detect activation at 1 or 3 min. Finally, I compared Rac1 and Rab5 activity in tumor cells expressing wild-type PI3Kβ or a loss-of-function mutant (KKDD). I was unable to detect differences in the activity of either Rac1 or Rab5. Future experiments will validate activators of Rab5 and Rac1 and test the role of PI3Kβ in this activation. Data gathered from these experiments will contribute to our understanding of PI3Kβ signaling in tumor cells.

Visualizing The Dynamic Action of Chromatin Remodeling Factors in Live Cells

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More than 50% of cancers are caused by mutations or deletions occurring in the TP53 gene.[1] This gene encodes p53, a tumor suppressor transcription factor with many downstream effectors that can attenuate cell proliferation. In response to cellular stress, p53 protein levels rise and interact with TIP60 (a chromatin remodeling protein) to help induce transcription of target genes that cause cell cycle arrest and DNA repair. TIP60 is recruited to gene targets by p53 to acetylate histones H2A and H4, resulting in an open chromatin structure (euchromatin) and transcription of the gene target. In contrast, EHMT1 is a negative regulator of p53 target genes. EHMT1 is recruited by p53 and methylates H3K9, resulting in a tightly packed chromatin structure (heterochromatin) and repression of transcription.[2] Although the interactions between p53, TIP60, EHMT1 and their gene targets have previously been studied via biochemical analysis, the dynamics and behavior of these chromatin remodeling factors within a live cell have yet to be investigated.

The goal of this project is to establish a system to study the spatiotemporal dynamics of TIP60 and EHMT1 in the presence and absence of a stress signal using live cell **S**ingle **M**olecule **I**maging (**SMI**). To achieve this goal, stable U2OS cell lines expressing halo tagged TIP60 and halo tagged EHMT1 were created. Live cell SMI of both factors in the absence of stress revealed dynamic probing and stable genomic binding in vivo. Further analysis indicated that EHMT1 localizes densely within the nucleus at specific sites whereas TIP60 is not as densely localized and spread more evenly throughout the nucleus.

To initiate cellular stress, an MDM2-antagonist called Nutlin was used. MDM2 is a ubiquitin ligase involved in proteosomal degradation of p53. Therefore, treatment of Halo-TIP60 U2OS cell line with Nutlin elevated p53 protein levels and caused a stress response in vivo. Unexpectedly, Nutlin also stabilized expression of the Halo-TIP60 protein as visualized by fluorescent imaging. In the presence of Nutlin, Halo-TIP60 had a denser localization pattern compared to unstressed conditions. Overall, TIP60 binding to target genes is shown to change in response to cellular stress.

The p21 gene, a downstream effector of the p53 protein, causes DNA repair and cell cycle arrest. In the future, this imaging system can be used to study the effects of cellular stress on the dynamic interactions between TIP60 and the p21 gene. Tracking these two factors in live cells can be used to determine how the dynamic localization of the TIP60 at the p21 gene changes in response to cellular stress. In addition, this test can also be extended to EHMT1 since the localization patterns of EHMT1-unstressed and TIP60-stressed cells are shared. It would also be interesting to see how Nutlin may cause TIP60 to associate with factors in regions occupied by EHMT1 in an unstressed state.

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High-Resolution Mapping of Binding Sites of the p53 and RNA Polymerase II Complex to the *hdm*2 Promoter

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RNA Polymerase II (Pol II) binds to the transcription start site and catalyzes a coordinated assembly of transcription factors known as the pre-initiation complex (PIC) needed for the initiation of gene expression.[1] One of the most essential transcription factors required for activation of PIC formation is the p53 tumor suppressor protein.[1] p53 regulates tumor suppression and responds to a variety of potentially harmful stimuli in the cell. To maintain genomic stability, p53 activates the expression of various cellular pathways leading to DNA repair, cell cycle arrest, and apoptosis.[1] Disruptions to the initiation and activity of regulatory response systems is of particular concern as mutations in p53 are prevalent in many cancer patients.[2] In tumors, mutations in the TP53 gene localize to the DNA-binding domain of p53 thereby interfering with sequence-specific recognition and binding necessary for transcription. p53 is central in suppressing tumorigenesis because of its role in transcriptional control and regulation of the cell cycle.

p53 is thought to be an important regulator of Pol II and was demonstrated to be integral in directing structural conformation and the elongation activity of Pol II.[1] An enhanced understanding of interactions between proteins and the p53-mediated PIC complex will help further knowledge of tumor suppression in cancer research and lead to successful methods for targeting mutant p53. Inhibiting the p53 negative regulator *hdm*2 represents a viable solution for restoring the function of wildtype tumor suppression. TP53 mutations cause abnormal structural conformations to the DNA binding domain of p53 inhibiting the ability of DNA to bind. Knowing precisely where the Pol II and p53 complex binds to the *hdm2* promoter is essential for developing an effective treatment which limits the effects of mutant p53 protein, mutations in the TP53 gene, and reactivates regulatory transcription tumor suppression pathways. Additionally, a developed strategy to elucidate the binding sites of Pol II and p53 to the *hdm2* promoter can serve as a translational model for determining bound positions of other gene regulatory proteins, such as p21. Previously, the structural binding of p53 to Pol II in constructing the complex was established as occurring "over the DNA-binding cleft proximal to the upstream DNA entry site through distinct contacts with the Rpb1 and Rpb2 subunits.[1] However, the specific locations on the promoter that the p53 and Pol II complex contact was unknown. Contact points on the promoter to Pol II were known to occur near the transcription start site, but the exact positions had not been found. Therefore, the goal of the research project was to determine the exact sites of the *hdm2* promotor that contact the p53 and Pol II complex through development of an *in vitro* formaldehyde-cross-linking/exonuclease mapping assay (xlink-exo).

The binding sites of the p53 and Pol II complex to promoter DNA were ascertained through using the xlink-exo assay for digesting fluorescently labeled promoter DNA with exonuclease activity followed by running the products on a mapping gel. The novel xlink-exo mapping assay demonstrated the potential of determining binding sites with high precision. Fluorescent *hdm2* promoter was incubated with p53 and Pol II to instigate PIC formation. Formaldehyde was added to cross-link the DNA with p53 and Pol II to establish the contact points of protein to DNA. The p53 and Pol II complex bound to fluorescently labeled, crosslinked *hdm2* DNA was digested with Exonuclease III to remove any DNA not in direct contact with protein. Exonuclease III cleaves DNA directionally from the 3' to 5' end, however, after encountering the p53 and Pol II complex the enzyme released thus preserving areas desired for analysis. Fluorescent imaging was then used to view the binding regions of *hdm2* to p53 and Pol II. With this experimental approach, the mapping of binding positions of the p53 and Pol II complex was determined. The mapping assay confirmed p53 binding locations at numerous locations on the *hdm2* promoter, including specific target sites such as the p53 response elements 1 and 2 and at the TATA box. Pol II was shown to have a high frequency of targeted binding further downstream on the promoter at the transcription start site in the presence of p53. In the absence of p53, Pol II was unable to effectively bind to *hdm2*. Initial results have shown that p53 is essential for directing the binding of Pol II to the *hdm2* promoter. In the future, continued improvements will be for determining the binding of p53 and Pol II to the hdm2 promoter through optimization of xlink-exo reaction conditions and procedures. Additional xlink-exo mapping assays will also be designed for testing the exact, high-resolution binding sites of p21 and other proteins closely connected to cancer.

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Actin Composition in Dendritic Spines of Fragile X Mice

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Fragile X Syndrome (FXS) is one of the most common inheritable disorders that leads to intellectual disabilities and autism spectrum disorders (ASDs). The syndrome results from silencing of the fragile X mental retardation 1 gene (*Fmr1*). While the role of mTOR Complex 1 (mTORC1) has been investigated in FXS, less is known about mTOR Complex 2 (mTORC2). mTORC2, defined by its primary component Rictor, influences the development of the structure and actin skeleton of neurons. Cofilin, an actin depolymerization agent that determines spine structure and density, is thought to act downstream of mTORC2 and Rac1 activation. Previous work in the lab has shown decreased cofilin activity in *Fmr1* knockout (KO) (a common mouse model for FXS), which alters the ratio of filamentous actin (F-actin) and globular actin (G-actin). As actin ratios are important for final cell structure and motility, a shift in this ratio is thought to lead to the development of irregular and immature dendritic spines. A higher F-actin to G-actin ratio in *Fmr1* (KO) mice compared to wildtype suggests that FXS leads to the misregulation of mTORC2 and thus cofilin expression within dendritic spines. The objective is to rescue F-actin/G-actin ratios in *Fmr1* KO mice by the genetic reduction of Rictor. We hypothesize that the overactivation of mTOR2 and the resulting decrease in cofilin signaling are related to altered actin composition in neuronal dendritic spines. To accomplish this localization of Rictor and PDS95 from neuronal cultures was analyzed. Rictor and postsynaptic density components have a higher presence in FXS cortical dendritic spines, however proximal localization of the two did not increase significantly between wildtype and Fmr1 KO. Furthermore, F-actin and G-actin ratios were evaluated by homogenizing samples of the somatosensory cortex of wildtype, *Fmr1* KO, and *Fmr1*+Rictor conditional knockout (cKO) mice. The F-actin and G-actin fractions from the homogenate were run via Western Blotting and visualized using Licor imaging. Higher F-actin to G-actin ratios suggests and increase in cofilin phosphorylation status between wildtype and *Fmr1* KO. The preliminary data from the *Fmr1*+Rictor cKO mouse provides encouraging results that temporal knockdown of Rictor rescues F/G actin ratios in FXS. Further research should be conducted to determine how knockdown of Rictor influences the shape and density of neuronal dendritic spines.

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Categorical Processing Of Sounds in Adolescents Using Event-Related Potentials

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This study sought to gain a better understanding of how adolescents categorize the sounds in their surroundings. We hypothesized that speech would have a processing advantage in the adolescent brain, and thus we predicted that there would be faster and more accurate responses to speech sounds, compared to non-speech sounds. An oddball paradigm was used, which consisted of a sequence of frequently presented complex tones, and rarely and randomly presented novel tones (the targets). The novel sounds were taken from three categories: music, speech and environment. Subjects were tasked with classifying each novel tone using three response keys designated for each category. Electroencephalogram (EEG) was recorded during sound presentation. From the continuous EEG, the event-related brain potentials (ERPs) were extracted for each participant. We found that participants had the highest hit rate, fastest reaction time, and lowest false alarm rate to speech sounds, as compared to music and environmental sounds. Speech also elicited the greatest amplitude response for the P3a ERP component reflecting attention orienting, and the P3b ERP component reflecting target detection. These results suggest that speech was processed more robustly than music or environment.

The Effect of Conditional Deletion of Par1a and Par1b in Podocytes on Glomerular Morphology and Filtration Function

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Nephrotic syndrome is a disease of the kidneys and is characterized by the leaking of protein (albumin, the main protein in the blood) – known as albuminuria. Albuminuria results from damage to glomeruli. Our research focuses on the podocyte (glomerular epithelial cell). Podocytes are cells that cover the outside of the glomerular basement membrane. They form a system of foot processes that interdigitate to form a size-selective barrier for the glomerular filtration system. Injury of the podocytes is characteristic of glomerular disease, especially minimal change disease and focal segmental glomerulosclerosis. These diseases are common causes of nephrotic syndrome. Polarity is the organization of the proteins to specific cell domains. Apicobasal polarity proteins contribute to cell polarity by establishing cell domains. The podocyte has apical membrane domains and basal domains (where it attaches to the glomerular basement membrane). Podocyte damage leads to loss of podocyte polarity and foot process effacement. Par1a (*Mark*3) and Par1b (*Mark2*) are members of a family of apicobasal polarity proteins known as Partitioning defective or Par proteins. The podocyte expresses these polarity proteins during development and they maintain apicobasal polarization. Our hypothesis is *Par1a* (MARK3) and *Par1b* (MARK2) are required for the development and cytoskeletal structure of the podocyte foot processes. Methods used are breeding of conditional knockout mice, kidney extraction and preparation, quantifying albuminuria by urine dipsticks, and morphology staining by hematoxylin and eosin staining. The experiment resulted in increased albuminuria being detected and changes in glomerular structure appear to have been seen in the histopathic staining. These findings confirm the hypothesized effect of protein deletion, meaning Par1a and Par1b do have a role in glomerular filtration function and morphology. For further experimentation, electron microscopy should be performed to look at podocytes in more detail. Future studies on this topic would look at the effect of Par1a and 1b on recovery from podocyte damage. Acknowledgements go to Dr. Reidy, the Diversity Student Summer Research Opportunity Program, and the Summer Undergraduate Research Program for support and guidance throughout this project.

Characterization of IgG1 and IgG3 antibodies isolated from a HSV-1 seropositive subject

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Herpes Simplex Virus (HSV) is a prevalent virus that affects about half of the global population. There are two serotypes of HSV; HSV-1, which is the predominant cause of oral lesions, ocular disease and acute infectious encephalitis, and HSV-2, which worldwide is the most common cause of genital vesicles and is associated with increased risk of HIV acquisition and transmission. There is no clinically available vaccine to prevent primary or recurrent HSV-1 or HSV-2 infection.

Our lab has developed a candidate vaccine, called ΔgD-2, which is fully protective against HSV-1 and HSV-2 infection in murine models. The vaccine elicits high titer antibody (Ab) responses and passive transfer experiments demonstrate that these Abs alone are sufficient to protect against disease. Abs mediate protection by several different mechanisms including neutralization (binding to viral particles to prevent viral entry) and non-neutralizing functions that depend on the Fc component. Viral-bound Abs can bind to Fcgamma receptors (FcγR) on innate immune cells to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis, or activate complement. Our lab has shown that ΔgD-2 elicits Abs that act primarily by non-neutralizing functions. The ability of Abs to activate FcγR depends in part on their antigenic target, subtype (IgG1-IgG4). In humans, IgG1 is associated with neutralization for several pathogens, whereas IgG3 has been linked to more potent ADCC activity**. However, the specific antigenic targets, isotypes and function of Abs elicited by HSV in humans and whether they correlate with immune protection is not known**.

My summer project focused on isolating IgG1 and IgG3 from known HSV-1 seropositive subjects to evaluate the functionality of those isolated Abs. We used Protein-G coupled magnetic beads to isolate IgG from human plasma and then used negative selection to isolate IgG1 or IgG3. We used an ELISA to quantify and validate our isolation. Our next steps will be to optimize the isolation protocol, increase Abs concentration and then test for neutralization, C1q binding and ADCC. While targets of neutralizing Abs have been identified, the antigenic targets of ADCC and C1q binding are not known. Thus, future directions will involve designing studies to identify those targets.

I want to thank all of the members of the Herold lab for their support these past two months. I would like to specifically thank Dr. Prameladevi Chinnnasamy and Dr. Maria Visciano for their help with my project by providing insight into how to effectively isolate IgG1 and IgG3. I would also like to thank Dr. Betsy Herold for providing her knowledge and wisdom for my project. Lastly, I would like to thank Aakash Mahant and Dr. Richard Hunte for their guidance on this project as well.

Mapping determinants of neurotoxicity on Human immunodeficiency virus type 1 gp120 protein

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HIV associated neurocognitive disorders (HAND) affect up to 50% of HIV-infected individuals. An important goal of research on HAND is to identify the viral and cellular determinants that elicit the molecular and cellular sequelae leading up to neurocognitive disorders. In our laboratory, we have been focusing on identifying the neurovirulence determinants by taking advantage of two HIV-1 clades (clade B and clade C) that exhibit differences in their neurotoxicity. One of the differences is in the viral envelope protein, gp120, which is expressed on the surface of virion particles and infected cells. It is used by HIV-1 to initiate binding to the CD4 receptor and CCR5 or CXCR4 coreceptor on CD4 T cells and macrophages via the CD4 receptor. HIV is known to enter the central nervous system by crossing the blood-brain barrier. Once in the CNS, neurons are exposed to gp120 present on virions and cell membranes as well as shed into the extracellular medium.

gp120 has been shown to be neurotoxic and neurovirulent, disrupting dopamine reuptake and driving the production of excess glutamate, ultimately causing synaptodendritic injury. Our laboratory has shown that gp120 from HIV-1 subtype C isolates found on the Indian subcontinent has a lower level of neurotoxicity relative to gp120 from HIV-1 type C from Southern Africa and HIV-1 type B from the United States. These results are in agreement with the fact that neuroAIDS is much more robust in Southern Africa and the US when compared to India. The fact that the Indian and Southern African HIV-1 are subtype C, yet display large differences in neurovirulence provides us an opportunity to map these differences. It has already been shown in studies using recombinant, chimeric HIV-1 molecular clones that the V3 loop domain of gp120 may encode neurotoxicity.

Therefore, in this study, we will examine if the V3 loop on gp120 plays a significant role in the gp120 neurotoxicity differences between Indian and Southern African HIV-1 type C. We hypothesize that the V3 loop determines neurovirulence and thus the V3 loop of Indian HIV1C will confer absence of neurovirulence on either of the other two viruses. Similarly, the V3 loop of either Southern African or US HIV-1 will confer increased neurovirulence on the Indian HIV-1C. To this end, we are beginning to build chimeric constructs of infectious, full-length HIV-1 constructs. We will utilize HIV-1IndieC1 (Indian clade C), HIV-11084i (Zambian HIV-1C). Using restriction digestion and cassette mutagenesis, we will swap V3 loops of each of these such that the resulting chimeric viruses will be based on HIV-1IndieC1 or HIV-11084i and contain gp120 with V3 loops from Indian HIV-1C, Southern African HIV-1C or US HIV-1B. The neurotoxicity of each of the chimeric proteins will be measured by a methodology established in our laboratory where, we will expose primary neuronal cells to each protein. Neurotoxicity will be quantified by measuring dendritic and axonic lengths.

The results to be obtained will allow us to confirm whether the V3 loop indeed is mainly responsible for the neurovirulence differences between the three viruses being studied. Furthermore, we can use these results to create site-directed mutagenesis to identify specific residues that are associated with greater or lower neurovirulence. Additionally, the results from this project will allow for further research and understanding into inter- and intra-clade differences in CCR5 and CXCR4 tropism.

Tet1 Non-Catalytic Functions in Regulation of Bivalent Genes in Mouse ES Cells and Development

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Embryonic stem cells (ESCs) have the capacity to differentiate into three germ layers of the body, a process governed by transcription factors, epigenetic modifiers, and signaling pathways. A feature orchestrating this plasticity in ESCs is bivalent gene promoters. Bivalently marked genes are essential for developmental processes and lineage specification, with PRC2 and Trithorax MLL2/COMPASS (KMT2B) catalyzing the deposition of the bivalent marks, H3K27me3 (repressive) and H3K4me3 (activating), respectively. The epigenetic modifier Tet1, a member of the Tet family of enzymes that catalyze the oxidation of 5-methylcytosine (5mC) to hydroxymethylcytosine (5hmC), has been shown to bind to bivalent gene loci. Interestingly, preliminary data show that loss of Tet1, without loss of its catalytic activity, results in upregulation of lineage marker expression in ESCs, with many being bivalent genes. Further, some bivalent gene targets of Tet1, such as *Foxa2*, show reduction in H3K27me3 levels as well as diminished Ezh2 recruitment upon Tet1 depletion. Hence, we hypothesize that Tet1 safeguards the embryonic pluripotent state by facilitating the maintenance of repressive histone marks, such as H3K27me3, at lineage gene loci, independent of its catalytic activity.

To characterize the noncatalytic functions of Tet1 in the epigenetic regulation of bivalent genes, we first defined the lineage genes controlled by H3K27me3 levels in WT mESCs. We chemically modulated H3K27me3 levels using the methylase inhibitor GSK-126 and the demethylase inhibitor GSK-J4 and determined gene expression changes by RT-qPCR. We then tested whether upregulation of lineage marker expression observed in $Tet1^{-/-}$ mESCs could be rescued by chemically increasing H3K27me3 levels with the demethylase inhibitor. Indeed, lineage markers, such as *Eomes* and *Sox17*, were downregulated, reaching levels equivalent to those of WT and $Tet1^{m/m}$ mESCs. This implies that Tet1 non-catalytic functions include the maintenance of H3K27me3 marks mediating repression of lineage markers in mESCs. Concurrently, we studied developmental differences *in vivo*, in WT, $Tet1^{m/m}$, and $Tet1^{-/-}$ mouse embryos during *in utero* development. As early as embryonic day (E) 8.5 and E11.5 we could detect size differences, with $Tet1^{-/-}$ embryos being significantly less developed than WT and $Tet1^{m/m}$ embryos. Future work will include mapping the whole genome H3K27me3/H3K4me3 distribution by ChIP-sequencing to determine global changes in bivalent marks upon Tet1 depletion. Additionally, further analysis of embryonic and adult $Tet1^{m/m}$ and $Tet1^{-/-}$ mice will establish the importance of Tet1 beyond its role in DNA demethylation in stem cells and during development.

Does hOATP1B1 interact with PDZK1?

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Background: A major function of the liver is clearing organic anions from the circulation. The molecular basis of this process has been the subject of ongoing studies in our lab. One important discovery was the family of organic anion transport proteins that mediate the uptake of compounds such as hormones, bile salts, and drugs. Mutations of these transporters have been associated with toxicity in patients due to the reduction of drug clearance by the liver. These transport proteins cycle between the cell surface and intracellular domains in endocytic vesicles that can traffic via microtubules. Several of these transporters have binding sites on their C-terminus that bind to the protein PDZK1. This scaffolding protein helps link the transporter to kinesins which are motors that traffic towards the plus end of microtubules located near the cell surface. PDZK1 binds to its OATP ligands only if their terminal four amino acids are present. Without these amino acids PDZK1 is unable to bind. The present study was designed to determine if hOATP1B1, a transporter in the human liver, interacts with hPDZK1 as has been predicted by computer analysis. **Methods:** Transient transfection of a plasmid encoding hPDZK1 tagged on its N-terminus with FLAG into human embryonic kidney (HEK293) cells was performed with the following protocol: 20μg of N-FLAG-hPDZK1 was mixed with 50μl of 2.5M $CaCl_2$with final volume of 500 μl with dH_2O and 500μl of 2x HBS (280 mM NaCl, 100mM HEPES. 1.5mM Na_2HPO_4 pH 7.12). This was then mixed with the DNA and $CaCl_2$. These mixtures were incubated at room temperature for ten minutes, then added to the cells and incubated overnight. The next day the growth media was changed, and incubation resumed for another twenty-four hours. The cells were then harvested and lysed with 1% chaps in PBS containg protease inhibitor. The protein concentration was measured by the Pierce BCA kit. An SDS polyacrylamide gel was loaded with 50μg of cell lysate. A standard Western blot protocol was followed and probed with anti-FLAG. In a second experiment $HEK1B1^{WT}$ and $HEK1B1^{\Delta ETHC}$ cells were studied for hOATP1B1 expression. These cells have been stably transfected with plasmids encoding full length wild type hOATP1B1 (WT) or hOATP1B1 in which the terminal four amino acids have been deleted (ΔETHC). These cells were harvested, lysed in 1% CHAPS-PBS and 50μg of cell lysate were loaded onto a 10% SDS polyacrylamide gel to determine their expression by Western Blot with anti-hOATP1B1. **Results:** Analysis by Western blot showed that N-FLAG hPDZK1 was expressed well in the transiently transfected HEK293 cells. The cell lines that had been stably transfected with $hOATP1B1^{WT}$ and $\mathrm{hOATP1B1}^{\Delta ETHC}$ had good expression of both proteins as judged by Western blot. **Conclusion:** These basic experiments provides the reagents for further pull-down experiments to examine whether hOATP1B1 binds to hPDZK1 and the effect of deletion of its C-terminal four amino acids. These studies will require transfection of hPDZK1 into hOATP1B1 expressing cells and pull-down of proteins bound to hPDZK1 with anti-FLAG linked agarose beads.These studies are now underway.

Association Between Serum Bicarbonate Concentrations as a Measurement of Metabolic Acidosis and Cognitive Function

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Abstract

The kidneys play an important role in the regulation of acid-base metabolism. Low serum bicarbonate levels have been associated with cognitive impairment, specifically executive function, in patients with chronic kidney disease (CKD).

We aimed to determine the association between serum bicarbonate levels (as a measure of metabolic acidosis), and cognitive function in an ongoing prospective cohort study of community residing older individuals with and without CKD. We used data from the Einstein Aging Study (EAS), a longitudinal cohort study of cognitive aging and dementia in community dwelling adults, 70 years of age and older. We evaluated the cross-sectional association between low serum bicarbonate level and performance on cognitive tests such as visuospatial (Block Design test), language (Boston Naming Test- BNT) as well as attention and executive function (WAIS III Trail making tests part A- TMT A).

The mean age of participants was 78 years (SD 5.4), 63% were female, 62% were non-Hispanic white. Mean serum bicarbonate level was 25 mEq/L (SD 2.58). We found that low serum bicarbonate levels were associated with better performance on Trail Making A and Block design. For TMT-A, completion time for those with normal serum bicarbonate was 55.2 sec (SD 22.2) vs 59.4 sec (SD 28.2), p= 0.02. For Block design, mean time was 24.8 sec (SD 9.3) vs 23.1 sec (SD 8.8) for participants with metabolic acidosis (p=0.005). The association with attention and executive function persisted after multiple variable adjustments ($p = 0.003$ and $p = 0.008$ respectively). There was no association between serum bicarbonate levels and the domains of memory and language.

Metabolic acidosis, as measured by low bicarbonate level, is associated with better performance on certain neuropsychological evaluations of executive function and attention.

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Structure-function study of Zika virus E glycoprotein quaternary epitopes using mammalian cell display

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Zika virus is an emerging global health concern, currently without any approved therapeutics. Broadly neutralizing monoclonal antibodies (bNAbs), isolated from the sera of convalescent patients, are of particular interest due to their high therapeutic potential; these antibodies are potent neutralizers of Zika virus and multiple stereotypes of dengue virus. bNAbs bind the homodimer interface of E-glycoproteins on the viral particle surface, and are proposed to neutralize by preventing critical conformational changes needed for viral infection.

The goal of this work is to determine which residues within the epitope are critical for mediating bnAb binding to the E-glycoprotein. To accomplish this, we selectively mutated epitope residues to Alanine using site-directed mutagenesis. Candidates were selected based on analysis of direct contacts found in the crystal structures of the bnAbs complexed to the glycoprotein. Using a mammalian cell display system, we evaluated the effect of specific mutations on bnAb binding using flow cytometry.

We have found at least two residues which completely abrogated antibody binding to the E-glycoprotein; however, further work is needed to distinguish whether this is due to differences in bNAb binding or global misfolding of the glycoprotein. A better understanding of the bNAb epitope and the critical residues driving the interaction will inform both flavivirus vaccine design and antibody engineering.

Effects of cocaine on cued sucrose-reward seeking in rats

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The nucleus accumbens (NAc) region in the brain is critical for cued reward seeking[1,2]. Previous research has shown that cocaine inhibits neuronal firing in the NAc[3]. Humans binging on high doses of cocaine tend to lose interest in pursuing alternative rewards such as food and sleep[4]. However, the impact of cocaine on responding to reward-predictive cues has not been directly studied. We hypothesized that systemic injection of cocaine in rats would inhibit cued sucrose seeking.

Sixteen Long-Evans rats were trained to respond to an auditory cue by entering a receptacle to obtain a liquid sucrose reward. The rats were then divided into two groups. One group was injected with cocaine (16 mg/kg i.p.) and the other was injected with saline. After the injection, the rats were evaluated in one hour sessions in which they were presented with the auditory cue. Collected data included percentage of cues responded to with a receptacle entry and the latency of the response.

Initial analysis showed a surprising lack of difference in cue response percentage. However, cocaine caused an increase in response latency. Based on examination of video recordings, we hypothesized that the lack of difference in cue response percentage was due to the rats' increased movement, which caused them to briefly enter the sucrose reward receptacle without remaining long enough to consume the reward. Therefore, in subsequent experiments we increased the time required for the rat to leave its head in the receptacle before obtaining the liquid sucrose reward from 0.01 s to 0.5 s. After two retraining sessions with no injections, a third injection experiment was performed in which cocaine and saline groups were switched. We found that there was a 60% reduction in response rate for rats that received cocaine injections compared to saline injections in the first ten minutes of the session. This difference gradually diminished over the course of the session, almost completely disappearing by the end of 60 minutes. These findings suggest that cocaine does have a potent inhibitory effect on cued reward seeking in rats, supporting our hypothesis.

The next step is to determine which specific part of the brain is responsible for the inhibitory effect of cocaine on cued reward seeking. Four potential brain regions have been identified for further study – NAc shell, NAc core, perifornical lateral hypothalamus (PefLH), and ventral tegmental area (VTA). The rats were divided into two groups – one group had bilateral cannulas inserted into the NAc core and PefLH while the other group had cannulas inserted into the NAc shell and the ventral tegmental area (VTA). We will infuse cocaine into those localized brain regions and analyze rat performance in one hour cued sucrose-seeking sessions.

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Roles of class I histone deacetylase overexpression on gene expression levels and pathways

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The chemical modifications of histones affect the accessibility of genomic DNA to the transcription machinery. Histone modifications include adding modifiers such as methyl and phosphate groups to histones to alter the chromatin structure. Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from histones. This epigenetic (transcriptional regulatory) modification is associated with the closing of chromatin, and is linked with lower levels of nearby gene expression. HDAC inhibitors are drugs used to inhibit histone deacetylation and induce the opening of chromatin, which enhances local gene transcription. However, the specific mechanisms by which HDACs exert their effects are unknown. In this study, we wanted to better understand the specific effects of these epigenetic modifiers on gene expression levels and cellular pathways.

We performed RNA-seq on cells transfected with ponasterone A (PonA)-inducible vectors which overexpress different class I HDACs: HDAC1, HDAC3, or HDAC8. We predicted that samples expressing different HDACs will demonstrate different gene expression levels, reflecting distinct targets for each HDAC. Bioinformatic tools were used to compare HDAC protein levels and identify pathways affected by the change in HDAC expression.

We observed successful induction of HDAC1 and HDAC3 in PonA-treated samples, while induction of HDAC8 caused no change in its expression level. Both HDAC1 and HDAC3 overexpression were seen to have effects in multiple pathways. Differentially expressed genes in HDAC1-induced samples were significantly enriched in pathways involved in cell adhesion, while differentially expressed genes in HDAC3-induced samples were significant for the development of organs, including the kidneys, the epithelial tube, and the urogenital system. These data indicate that although these three HDACs are within the same class, they have distinctive roles in various biological processes. Future studies include quantifying gene expression levels via qRT-PCR, and testing how the affected pathways/genes alter phenotypes in mice.

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Uncovering the Kinetic Abilities of Methyltransferases: Important Enzymes for Gene Transcription

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In the eukaryotic genome there are approximately 3 billion base pairs of DNA. The total length of these strands of DNA are 2 meters long; they are therefore packaged in the nucleus in the form of chromatin. The fundamental repeat unit of chromatin is called a nucleosome, which contains roughly 146 base pairs of DNA coiled around 2 pairs of each of the histone proteins (H2A, H2B, H3, and H4). Aside from their affinity for DNA, histones also have the ability to undergo post translational modifications (PTMs) at their *N*-terminus tails. Amino acids on the *N*-terminus tails such as serine/threonine, lysine and arginine are modified by PTMs into phospho-serine/threonine, acetyl/methyl-lysine and methyl-arginine. PTMs cause epigenetics effects by activating or repressing all genes. Understanding the mechanisms and consequences behind the writing and erasing of PTMs can provide a pathway to curing numerous metabolic disorders.

The specific PTM we are studying is arginine methylation. This mark is deposited by the class of enzymes: protein arginine methyltransferases (PRMTs). Specifically, the experiment focuses on PRMT 5 and its kinetic ability to transfer a methyl group to Arginine, forming Rme and *S*-adenosyl-L-homocysteine (SAH). To measure the activity of this enzyme, we are using a coupled assay that was invented by the lab named: 1Step EZ-MTase. The assay relies on the functionality of the protein TM0936, a deaminase that catabolizes SAH into the *S*-inosyl-L-homocysteine product (SIH). Thus, TM0936 can reveal the catalytic properties of any methyltransferase: while SAM and SAH have identical UV absorbance spectra, SIH has a distinct UV signature, making it detectable through UV spectroscopy.

For the first part of my internship, I used various biochemical techniques to express and purify the TM0936 coupling enzyme. Additionally, I characterized the enzymatic behavior of TM0936 by measuring its K_m and k_{cat} parameters. My next step will be running the EZ-MTase assay in vitro with the histone tail peptides. The efficiency of arginine methylation will be evaluated when the neighboring serine is phosphorylated, versus when it is unmodified. The predicted result is that the arginine methyl transfer will be less favorable when serine is phosphorylated.

Engineering Vaccinia Virus as an Approach to Profile Hantavirus Cellular Entry

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Hantaviruses, members of the Order *Bunyavirales*, cause varied and highly fatal disease syndromes in people worldwide. Due to their safety profile, the study of authentic hantaviruses is restricted to a few high-biocontainment facilities. Although surrogate virus models to study hantaviruses at a biosafety level 2 are available, they are limited in their scope. Thus, the development of alternative platforms is warranted. Here, we propose the use of the poxvirus, vaccinia virus (VACV), commonly used as an expression vector for foreign genes, as a novel strategy to study hantavirus entry. VACV produces two major types of virions upon infection: intracellular mature virus (IMV) and extracellular enveloped virus (EEV). The EEV affords targeting of foreign proteins to its surface membrane by fusing such proteins to subdomains of the VACV transmembrane proteins A56R or B5R. The goal of this summer research project was to engineer recombinant VACVs expressing different hantavirus glycoproteins and displaying them on their surface membrane as a preliminary step for creating viral libraries to understand the basic properties of anti-hantavirus neutralizing antibodies. Accordingly, we generated three recombinant VACVs expressing hantavirus glycoproteins fused to either A56R or B5R. We verified the cell-surface expression of these proteins by immunofluorescence microscopy and tested their incorporation into EEVs via western blotting. Moving forward, we will generate EEV libraries bearing mutant forms of the hantavirus glycoproteins to map the epitopes of hantavirus neutralizing antibodies and the interface between these glycoproteins and their critical cellular entry receptor PCDH1.

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Developing a Phage-Packaged Point of Care Reporter Cosmid System for Detecting Urinary Tract Infections

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Urinary tract infections (UTIs) are among the most prevalent bacterial infections and are responsible for millions of doctor visits each year. UTIs affect individuals of all ages and are most commonly caused by the bacteria *Escherichia coli*. They often cause considerable discomfort and, if left untreated, may lead to more severe conditions such as pyelonephritis, permanent kidney damage, or sepsis. Antibiotics are the standard treatment, but the growing issue of resistant bacterial strains is an imminent crisis. Urinalysis procedures currently used in clinical settings can indicate whether the patient has a UTI. However, they do not provide information about the causative agent or its drug susceptibilities. The development of an inexpensive point of care test to rapidly determine the appropriate antibiotic to treat a patient's UTI would be an invaluable diagnostic tool for modern medicine.

Our lab has focused on developing luciferase reporter phages that provide a simple and easy-to-assay phenotypic test for assessing whether bacteria are alive or dead. Further, determining bacterial resistance to antibiotics can be accomplished by observing if they luminesce when treated with different drugs. The presence of phage genes can complicate this rapid readout, therefore the delivery of plasmids would be ideal. For this project, we have developed a system to package reporter cosmids into bacteriophage lambda particles. A cosmid is a bacterial plasmid that has a packaging sequence (*cos*), which allows it to be inserted into bacteriophage heads.

Under transcriptional control of the strong L5 promoter, we expressed firefly luciferase in a lambda cosmid and transformed it into the *E. coli* strain χ2764. This strain has been lysogenized by a lambda phage and contains a temperature sensitive repressor, a deletion of the excisionase enzyme gene, and a mutation in the holin protein. The first mutation allows thermal induction of the lambda phage DNA. When moved from 30˚ to 42˚C, χ2764 begins to produce lambda phage head and tail proteins. The excisionase mutation interferes with the ability of the phage genome to dissociate from the *E. coli* chromosome. This confers predominant packaging of the cosmid into phage particles and minimal packaging of lambda phage DNA. Lastly, the holin mutation prevents χ2764 cells from lysing, allowing them to be easily concentrated by centrifugation. The end result is a phage lysate primarily composed of firefly luciferase-expressing cosmids. This lysate was used to transduce *E.coli* HB101 cells, enabling them to express luciferase and emit light. To determine drug susceptibility, *E. coli* cells from a clinical sample are first treated with antibiotics, then transduced by the novel lambda phages. Only resistant cells survive, express firefly luciferase, and subsequently emit light. The samples that do not luminesce indicate sensitivity to an antibiotic, providing a treatment option for the patient.

The accomplishments of this project were (1) confirming that lambda phage systems can package luciferase reporter cosmids, (2) determining that cosmids were preferentially packaged into the phage heads over phage DNA and (3) verifying that the cosmids produce luminescence after transducing target cells.

Enhanced *ex vivo* expansion of multipotent hematopoietic stem cells in albumin-free culture

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Multipotent hematopoietic stem cells (HSCs) are essential for generation and regeneration of the adult blood system. Transplantation of HSCs offers curative therapies for conditions such as leukemias and immunodeficiencies, but necessitates conditioning to deplete recipient bone marrow, due to limited HSC yield from donors. More efficient culture and expansion of HSCs *ex vivo* could eliminate this need, revolutionizing the transplant process. Here we investigate a newly developed method of culturing HSCs, involving replacement of widely used bovine serum albumin with polyvinyl alcohol, a proposed protein carrier. Mouse HSCs ($CD150^{+}CD34^{-}Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$) were sorted and cultured in either albumin-free PVA media or StemSpan, a widely used, commercially available HSC culture media. HSC expansion was assessed by FACS analysis of the hematopoietic progenitor population after 14 days of culture. We demonstrate approximately 10-fold expansion of HSCs in albumin-free PVA culture, compared to StemSpan which failed to maintain or expand the HSC population. Culture will be continued for 14 additional days, at which time the functionality of the expanded HSCs may be assessed by transplantation and evaluation of long-term chimerism in the recipient.

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Organ Size Regulation in Rp mutants in Drosophila

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Ribosomal proteins (Rp) function as components of ribosomes which are essential for the synthesis of proteins. Given how important ribosomes are for translation, organisms with homozygous mutations for Rp die, while heterozygous mutants present with abnormal disease in humans as can be seen in Diamond- Blackfan anemia and *Drosophila* with reduced size in bristles and an extended growth period (the minute phenotype). In the case of *Drosophila*, previous results in the lab suggests that Xrp1 are able to regulate this phenotype through a developmental delay that contributes to the extended growth period compensating for the missing copy of the Rp gene. The Hippo signaling pathway is a kinase cascade that plays a role in organ size control in animals by regulating the activity of a transcription co-activator yorkie (yki) that promotes organ growth. During the course of this research wing size was used as a measure for the genetic interactions of the manipulated genes. The question that we explored was whether Xrp1 regulates organ size in Rp mutants through the regulation of the hippo pathway. Our hypothesis is that there will be an increase of yki activity in Rp (ribosomal protein) mutants to maintain organ size. Crosses were set for the desired genotypes, wings from the obtained flies were dissected and measurements were taken. From the taken measurements we found evidence that RpS3 heterozygous flies display mild but significant increase in wing size and reduction in aspect ratio, a phenotype dependent on Xrp1. Moreover, additional genetic interaction data is presented with the members of the Hippo pathway. From results we were able to support our hypothesis in the case of yki but not in wts, suggesting that these measurements should be repeated with a greater number of flies.

Role of Microglia-specific Axl and Mertk during an Inflammatory Demyelinating Disease

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Abstract:

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS). During active MS, inflammatory molecules are secreted by T-cells, monocytes, and antigen presenting cells that cross the blood brain barrier, as well as by resident astrocytes and microglia. Activation of microglia, the resident innate immune cells of the CNS, can induce the production of pro-inflammatory molecules that damage oligodendrocytes, the myelin synthesizing cells of the CNS, and the myelin sheath that wraps and insulates axons.

Axl and Mertk are transmembrane receptor tyrosine kinases that belong to the Tyro3, Axl, and Mertk (TAM) family and are expressed in multiple cells. Both Axl and Mertk are expressed on the cell surface of microglia. To characterize the role of Axl and Mertk in microglia during an inflammatory demyelinating disease, mice with an inducible conditional knockout (CKO) of Axl or Mertk in microglia were generated and studied during myelin-oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE), a mouse model that shares many of the symptoms observed in the CNS during episode of active MS. We hypothesized that a deficit in Axl or Mertk in microglia will cause a more severe clinical EAE course and increase the levels of pro-inflammatory cytokines in the CNS.

The CKO of Axl was first verified by isolating microglia from naïve mice and comparing the levels of Axl protein produced by Axl-CKO treated with tamoxifen (+Tx) relative to that of the uninduced control (-Tx). The lack of Axl protein in Axl CKO +Tx microglia compared to other neuronal cells confirmed this genotype.

Axl CKO +Tx male mice displayed a significant delay in the onset of EAE symptoms relative to wild-type (WT) control, $Axl^{fl/fl}$ +Tx mice. No significant differences in clinical scores were observed between Axl CKO +Tx, Axl CKO -Tx, and WT, although the Axl CKO mice trended towards higher clinical scores during chronic EAE. Unexpectedly, the clinical scores of Axl CKO +Tx mice exhibited greater similarity to that of Axl CKO -Tx mice. The clinical scores of Mertk CKO +Tx mice did not show any significant difference compared to WT mice, although the clinical scores during the chronic phase of EAE was higher compared to WT.

To determine the contribution of Axl and Mertk to the inflammatory response observed during chronic EAE, protein homogenates were isolated from the spinal cords of mice presenting with clinical symptoms of EAE for 30 days. Analysis of the pro- and anti-inflammatory cytokines, IL-1β, IL-17, IL-6, IFN-β, TNF-α, and IFN-γ, were determined by ELISA. Cytokine levels in the spinal cords of Mertk CKO +Tx mice did not exhibit any significant differences compared to WT. We observed no significant differences in cytokine levels between Axl CKO +Tx, Axl CKO -Tx, and wild-type mice; however, we did observe an upward trend of all tested cytokine levels in Axl CKO +Tx compared to WT.

While these studies are currently being repeated with both male and female mice to obtain sufficient statistical power and confirm these results, our current data suggest that the deletion of Axl and Mertk in the microglia of male mice during EAE does not significantly impact the EAE course or severity of disease.

The role of the amygdalohippocampal area in infant-directed aggression in female mice

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Parental behavior, or behaviors that increase the evolutionary fitness of offspring, are highly conserved throughout the animal kingdom. In certain species, such as mice, this behavior is dependent on mating status. For instance, mated male mice have been seen to provide pups with parental behavior, however, virgin male mice spontaneously attack infants. This mating-induced behavioral switch produces a model to understand the neurobiological mechanisms underlying the positive and negative regulation of infant care. Urocortin 3 neurons of the perifornical area ($PeFA^{ucn3}$) have been found to be critical for the expression of infant-directed aggression. Furthermore, it has been shown that $PeFA^{ucn3}$ neurons send dense projections to the amygdalohippocampal area (AHi), a functionally novel brain area. Optogenetic stimulation of $PeFA^{ucn3}$ terminals in the AHi elicits infant-directed aggression in virgin females mice that are typically spontaneously maternal. In addition, immediate early gene studies in virgin males reveal that this region is highly active during infant directed aggression. Together, this evidence leads us to postulate that the AHi may be the functional locus for infant directed aggression. To investigate this hypothesis, we will optogenetically stimulate neurons in the AHi to assess whether this area is involved in the expression of infant-directed aggression. Furthermore we will observe if AHi neurons are excitatory or inhibitory. Based on our pilot experiments, we observe that activating the AHi is associated with decreased nesting behavior and decreased time spent with the pup in the nest. Our data supports our hypothesis that AHi activation is associated with decreased parenting.

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The breast tumor suppressor MLL3 may regulate enhancer histone marks in mammary epithelial cells

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Breast cancer is the leading cause of cancer deaths in females worldwide. There are a number of genes that have been found to contribute to tumor formation and development, some of which are known and others less well-known, such as MLL3, a methyltransferase protein. Encoded by the KMT2 gene, MLL3 and its homolog, MLL4, have been found to play a key role in regulating post-translational modifications on lysine residues of histone 3 such as H3K4 monomethylation and H3K27 acetylation. Both processes have been identified as activators of enhancer genes which promote transcription. While this gene has been established as an important tumor suppressor in cancer growth and particularly in breast cancer, it remains unknown how its pathway functions and at what stage of tumorigenesis process it affects. More specifically, we wish to determine whether various histone marks vary among different mammary epithelial cell lineages. Furthermore, we seek to determine the impact of Mll3 SET domain mutant and Mll3/Mll4 double mutants on the global levels of enhancer marks in mammary cells.

Immunofluorescence staining was used to analyze the presence of high and low-profile histone in cells within breast ducts of mouse breast gland tissue samples. ImageJ computer image analysis program was used to isolate color channels in the fluorescent images and to identify high-stained histones using the “multi-point” counting tool.

After staining for both ER positive and negative cells types, it was determined that the global H3K4me1 and H3K27ac levels were comparable between the two. Interestingly, both cell types contained distinct cells with low or high levels of enhancer marks, although more cells have high levels of H3K27ac. Double knockout mutant mice with both MLL3 and MLL4 SET domain deletions presented a greater than 50% decrease in histone content when tested for methylation yet barely changed in the case of acetylation. However, while staining of the mll3 mutant animals for both methylation and acetylation suggested that a deletion may present a drastic depletion in high-histone markers, due to heterogeneity among the animals tested, further experiments must be conducted in order to conclude whether mll3 knockout animals are depleted of histone markers when mutated. Furthermore, a ChIP-seq (chromatin immunoprecipitation sequencing) experiment would be useful in future studies in order to identify the particular binding site in which MLL3 modifies chromatin and whether that differs in ER+ and ER- cells and in double mutant genotypes.

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In vitro characterization of bradyzoite protein effector export in neurons during *Toxoplasma gondii* infection

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ABSTRACT

Toxoplasma gondii is an intracellular protozoan parasite known to affect a significant number of humans throughout the world. Of particular importance is its flexible life stages, the tachyzoite, or rapidly dividing stage, and the bradyzoite, or slowly dividing stage. The ladder is known to maintain a chronic infection within host cells that persists in the brain and muscle tissue by maintaining self-generated vacuoles called tissue cysts. Not much is known about the modes of bradyzoite control of gene expression in the host cell nucleus. Recently identified proteins at the parasitophorous vacuole membrane have been shown to be responsible for translocating nuclear effectors created by the parasite during the tachyzoite stage into the host cell nucleus. We are interested in whether bradyzoites retain the ability to export these effectors, GRA16, GRA24, GRA28, and TgIST, in neurons during infection. These effectors are known to affect host cell gene expression, with the exception of GRA28. This model has been shown to be the case in human foreskin fibroblasts (HFFs) with respect to these four known effectors through epitope tagging of their endogenous loci and by using immunofluorescence assays (IFA). These effectors accumulate in the host cell nucleus at early but not late timepoints during tachyzoite-to-bradyzoite transition (one- versus two- and three-days post-infection) and when further developed bradyzoite parasites invade new host cells. With a longer infection period, the presence of these effectors decreases. What we sought to determine is whether this model holds true in neurons, since neurons are the cell type that most commonly harbor tissue cysts in the brain. Therefore, they are a more appropriate model to study bradyzoite protein effector export. Moreover, this may explain how bradyzoites maintain a chronic infection in their hosts. We hypothesized that a similar trend of effector export may be observed as in HFFs, with continuous export of the effectors out of the parasite at early but not late stages of infection. By carrying out similar IFAs, we observed a general trend in the quantification of effector signal intensities generated from images captured after IFA consistent with the trend associated with findings in HFFs despite low signal intensity levels. When comparing each day post infection to other days, the data are not statistically significant ($p<0.05$), except for the GRA28 effector between one- and three-days post-infection. When comparing the signal intensity of each day post-infection to the signal intensity of uninfected neurons, GRA28 at day one and TgIST at days one, two, and three exhibited statistical significance ($p<0.05$). These results indicate the importance of further investigating GRA28's pattern of export, as well as repeating the experiment described in order to further elucidate patterns of all exported effector proteins, since there is evidence to suggest that this phenomenon occurs during infection of neurons. Bradyzoites may be able to maintain control of host cell gene expression through these effectors. Exploring the transcriptional response of the host cell will further inform how bradyzoites can persist within their host cell and maintain chronic infection.

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ATM Kinase Inhibition Rescues Defective Erythropoiesis in *ddx41* Mutant Zebrafish

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Myelodysplastic syndromes (MDS) are a preleukemic condition arising from mutations in hematopoietic stem and progenitor cells (HSPCs) resulting in an expansion of dysfunctional leukemic blasts and ineffective hematopoiesis. Germline mutations in *DEAD-box Helicase 41* (*DDX41*) were identified in patients with MDS with inferior overall survival, suggesting a contribution to disease pathogenesis. However, though a strong clinical correlation is found between mutations in *DDX41* and MDS, the *in vivo* role of DDX41 in hematopoiesis has not been elucidated.

Previous work in our lab has shown that *ddx41* loss-of-function homozygous mutants (*ddx41* mutants) have developmental hematopoietic defects including megaloblastoid-like anemia and increased HSPC formation at 36 hours-post-fertilization (hpf). Additionally, *ddx41* mutant cells exhibit cell cycle arrest and apoptosis, phenotypes commonly associated with MDS and mediated by DNA damage. DNA damage can trigger several signaling pathways via the activation of distinct kinases, such as Ataxia Telangiectasia Mutated (ATM) in response to double strand breaks, and ATM and RAD3-related (ATR) in response to single strand breaks or replication stress. Which of these pathways contribute to the DNA damage response in *ddx41* mutants and if DNA damage mediates the anemia or HSPC expansion seen in *ddx41* mutants has yet to be elucidated.

To explore if ATM or ATR play a role in *ddx41* mutant hematopoiesis, we inhibited the ATM or ATR pathways and assessed levels of mature erythrocytes and HSPCs. We crossed *ddx41* heterozygous zebrafish and treated the resulting embryos at 6 hfp and 24 hfp with DMSO as a vehicle control, ATM kinase inhibitor KU60019, or the ATR kinase inhibitor AZ. Embryos were then fixed and stained at 36 hpf using o-dianisidine dihydrochloride to mark mature erythrocytes. Embryos were then imaged using light microscopy and levels of erythrocytes were assessed, with intensity of staining corresponding to levels of erythrocytes. ATM kinase inhibitor treatment led to a partial but significant rescue of the anemia in *ddx41* mutants as compared to DMSO treated *ddx41* mutants. ATR kinase inhibitor treatment led to an insignificant rescue of the anemia in ddx41 mutants as compared to DMSO control treated *ddx41* mutants. Taken together, these results show that ATM partially mediates the erythropoietic defect seen in *ddx41* mutants.

Future studies seek to explore if ATM or ATR mediates the DNA damage, cell cycle arrest, and/or HSPC expansion present in *ddx41* mutants. These results highlight the important role of *ddx41* as a protector of genomic integrity and the direct role the DNA damage response plays in the erythropoietic defect seen in the absence of Ddx41. These studies provide potential therapeutic targets for treating *DDX41* mutated MDS and AML.

Vascularization in Neural Transplantation

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Neural stem cells transplantation is a promising approach to repair and regenerate aged brains and neurological diseases. Vascularization plays an important role in the survival of transplanted cells, as blood vessels provide oxygen and nutrition to the neighboring transplanted cells. There are two types of vascularization: angiogenesis, the protrusion and outgrowth of the pre-existing blood vessels, and neovascularization, the formation of new blood vessels from vascular precursor cells. It is speculated that, during neural stem cell transplantation, angiogenesis of host vessels alone is insufficient to support the survival of all transplanted neurons, so transplanting vascular precursor cells together may improve the regeneration outcome. To validate this hypothesis, we compare the contributions of the transplanted vascular precursor cells neovascularization versus the host vessels angiogenesis in multiple transplantation conditions in mice.

We transplant neural stem cells and vascular precursor cells (GFP expressing) to the stroke sites of mice brains, as well as the contralateral normal sites, at the time points of 4 days or 7 days after strokes induced. We sacrifice the mice and section the brains 2 weeks after transplantation surgeries, and we investigate the formation of the host and the transplanted blood vessels by immunofluorescent staining of the blood vessel marker (CD31) and the transplanted blood vessel marker (GFP).

From our preliminary data, the transplant neovascularization is significantly low in the “Normal Site – 7 Days” group. However, we need to increase the number in each group to validate the conclusion. Most importantly, we speculate more variables may also affect the result, such as the severity of the ischemic strokes, the number of transplant cells, the quality of the transplantation surgery, and the age and health condition of mice, etc. They should be further considered and specified in order to find the optimal transplantation method.

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Assessing the Effect of AIF1 Gene on Microglia Activity and Synapses

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Microglia are described as specialized brain macrophages that traditionally have been shown to clear debris and respond to injury. However, recent work has generated great interest in the role of neuro-glia interactions in potentially underlying neurodevelopmental disorders. Given the growing evidence of microglia in synapse formation and elimination in cortical brain structures, understanding the molecular and cellular impact of microglia dysfunction to synaptic activity is critical. The AIF1 (allograft inflammatory factor 1) gene selectively expressed in microglia codes for a protein involved in actin polymerization and potentially participates in cell motility. However, little is known about morphological and functional alterations in microglia lacking AIF1 and how this deficit can impact synapses of the central nervous system. We hypothesize that microglia AIF1 contributes to signaling cascades and neuro-glia interactions during brain development to establish normal neural circuit function.

To test our hypothesis, we used acute hippocampal slices from wild-type and AIF knockout mice to perform immunostaining, microglia and neuronal *ex vivo* structural imaging, electrophysiological recordings, and behavioral testing. Young postnatal day 15-17 male and female animals were included in our study and quantification analyses were carried out by blind experimenters. Behavioral testing was executed when animals reached adulthood at postnatal day 60. Immunostaining of specific microglia cell markers such as CD68 and P2Y12 demonstrated there was no significant alteration in microglia phagocytosis and chemotaxis. Microglia structural imaging revealed that laser ablation to induce chemotaxis was deficient in AIF KO mice. In addition, Sholl analysis showed microglia in KO animals have decreased branch complexity. Given that AIF null microglia possessed deficits in cell motility and branching, we performed electrophysiological recordings and neuronal structural imaging to identify possible alterations in synaptic function. We found that spine density, frequency of miniature ESPCs, and evoked transmission were all reduced. One metric associated with neurodevelopmental disorders such as autism is electrophysiological alterations in excitatory and inhibitory balance (E/I). Analysis of AIF null animals revealed an increase in E/I balance when compared to control mice. As irregular E/I balance is associated with autism, to strengthen our findings in synaptic alterations we decided to examine social behavior. However, AIF KO animals had no social preference as compared to control.

We have identified a synaptic phenotype and a potential behavioral deficit in microglia-deficient animals. However, to attain a more mechanistic explanation of our observations, elucidating changes in signaling cascades responsible for actin polymerization in microglia should be evaluated. Although we measured an insignificant difference between levels of P2YR12 in AIF1 WT and KO slices, we believe a more sensitive method such as Western Blot could reveal a more prominent difference. Our research highlights the importance of understanding microglial function in brain tissue to reveal mechanisms underlying neurodevelopmental disorders.

Role of Macrophage TNTs in Inducing Stemness

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Breast cancer invasion and progression can be increased by the presence of tumor associated macrophages. Macrophages have been shown to increase the elongated morphology of tumor cells, increase invasion and metastasis, the formation of tunneling nanotubes (TNTs), and chemoresistance. Although these properties exist when macrophages and tumors interact, these are also characteristic properties of cancer stem cells (CSCs). We hypothesize that traditional stem cell markers are regulated by coculture with macrophages. In addition, we propose that TNTs contribute to the ability of macrophages to enhance CSC properties. We tested this hypothesis by evaluating the expression of CD44 and CD24 on two breast cancer cell lines, MTLn3 and MDA-MB-231 (231) when cultured alone or in the presence of RAW/LR5 macrophages. We also tested the role of macrophage TNTs by comparing the use of a control macrophage cell line or line reduced in TNT production through the suppression of the protein Msec (or TNFAIP2). We found that coculture of control macrophages with MTLn3 cells led to changes in expression of CD44 and CD24 which were Msec dependent. However, expression of CD44 and CD24 by 231 cells was not altered by coculture with macrophages.

Accelerated Clearance of *Plasmodium yoelii* Infection in Anti-LAG3-Treated PDL1-KO Mice Is Independent of Parasite-Specific Antibody Titers

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Malaria is a mosquito-borne disease caused by the infection with the *Plasmodium* parasite. An estimated 200 million new cases of malaria occur worldwide each year, with approximately 90% of global malaria cases found in Sub-Saharan Africa and India (WHO 2018). Though the incidence of malaria has declined over the last decade, with an 18% reduction in cases between 2010 and 2017, the effectiveness of malaria vaccines are low. This is in part due to the lack of knowledge regarding the mechanisms of immune defense against malaria. Of the almost 200 species of *Plasmodium*, *P. falciparum* accounts for 90% of human mortality (WHO 2013), while *P. yoelii* is often used to study the immunological mechanisms of the disease in mice. CD4+ T cell inhibitory receptors, such as PD-1 (programmed cell death protein 1) and LAG-3 (lymphocyte activation gene 3), are upregulated in exhausted T cells, a type of dysfunctional T cell characteristic of chronic infection (e.g. malaria) and cancer It has been previously documented that in WT mice infected with *P. yoelii*, blockade of the PD-1 ligand PDL-1 and the inhibitory receptor LAG-3 accelerates parasite clearance by promoting germinal center responses and production of parasite-specific antibodies. Here, we use an enzyme-linked immunosorbent assay (ELISA) to compare levels of *P. yoelii*-specific antibodies in PDL1-KO *P. yoelii*-infected mice treated with LAG-3 blocking antibodies against those treated with isotype. Surprisingly, our results find that PDL1-KO mice treated with Anti LAG-3 have lower titers of MSP-specific antibody and better parasite clearance than when treated with isotype. This suggests that anti-malarial antibodies targeting MSP may not be playing a role in mediating infection clearance, and that perhaps antibodies targeting other malarial antigens are providing protection and resulting in accelerated clearance of malaria parasites. Further experiments, including a repeat transfer of *P. yoelii*-immune serum to naïve mice, are necessary to further examine the role of antibodies in mediating response to *P. yoelii* infection.