The 2016 Summer Research Symposium of the Nathan Schnaper Intern Program in Translational Cancer Research

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MARLENE AND STEWART GREENEBAUM CANCER CENTER

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For more information, go to **http://www.umgcc.org/research/summer_internships.htm**

2016 NSIP Research Symposium

Thursday, July 28, 2016
8:00 am to 5:20 pm

	Speaker	Mentor
8:00 to 8:30 am	**Breakfast**	
8:30 to 8:40 am	**Bret A. Hassel, PhD** *Director's Welcome*	
8:40 to 9:00 am	**Alexandra Vlk** *The C-Terminal Domain of IRAK-3 is Necessary but Not Sufficient for Negative Regulation of Toll-Like Receptor Signaling and Induction of Apoptosis*	Eduardo Davila
9:00 to 9:20 am	**Adria Lam** *The Transcription Factor Eomesodermin Regulates Expression of B-cell lymphoma 6 in $CD8^+$ T cells*	Arnob Banerjee
9:20 to 9:40 am	**Ciara Faupel** *Therapeutic Targeting of Activated B-Cell Diffuse Large B-cell Lymphomas using Toll-like Receptor Inhibitors*	Greg Snyder
9:40 to 10:00 am	**Leah Henry** *A Novel Method for Determining the Mechanism of Action of B cell Stimulation*	Amit Golding
10:00 to 10:20 am	**Carlos Echeverria** *Evaluating the Effect of Chemopotentiation by Low-Dose Fractionated Radiotherapy on Metastatic Properties of Gastric Cancer Cells*	France Carrier
10:20 to 10:40 am	**Break**	
10:40 to 11:00 am	**Rebecca Heilman** *Functional Analysis of Novel Oncodomain Mutations in the Tumor Suppressor RNase-L*	Bret Hassel
11:00 to 11:20 am	**Christine Magana** *JEDI: Awakening the T-cell Force Against Tumors*	Nevil Singh
11:20 to 11:40 am	**Jasper Mok** *Structural and Biochemical Analysis of Thymine DNA Glycosylase, a Base Excision Repair Tool for DNA Demethylation*	Alex Drohat

11:40 am to 12:00 pm	**Haley Hauser** *Identification of Protein Domains Stabilizing AUF1 Complexes with RNA*	Gerald Wilson
12:00 to 12:20 pm	**Nathalie Chen** *Transferrin-Targeted Nanoparticles for Systemic Delivery Across the Blood Brain Barrier*	Kim, Winkles, and Woodworth
12:20 to 1:20 pm	**Lunch**	
1:20 to 1:40 pm	**Olamide Adebowale** *Targeting RUNX2 transcription factor to inhibit breast cancer proliferation*	Antonino Passaniti
1:40 to 2:00 pm	**Samuel Savidge** *Loss of the obscurin-RhoGEF and its Effect on RhoA, RhoC, and Ras in Breast Epithelial Cells*	Katia Kontrogianni
2:00 to 2:20 pm	**Jennifer Drechsler** *Targeting CARP-1 in breast cancer to decrease tumor growth and enhance immunogenicity*	Tonya Webb
2:20 to 2:40 pm	**Caroline Schlee** *The Role of ZSCAN4 in Cancer Stem Cell Phenotype and Survival*	Michal Zalzman
2:40 to 3:00 pm	**Hayley Scott** *Inhibition of Coronavirus entry by TNFα converting enzyme*	Matthew Frieman
3:00 to 3:20 pm	**Smaraki Dash** *Effect of novel binaphthoquinones alone and in combination with chemotherapy on acute myeloid leukemia*	Rena Lapidus
3:20 to 3:40 pm	**Break**	
3:40 to 4:00 pm	**Shannon Kirby** *The Impact of miR513a-1 and miR518c on Leukemia Cell Differentiation and Proliferation*	Tami Kingsbury
4:00 to 4:20 pm	**Arielle Hamburg** *ATM regulates expression of FOXO3a and mitochondrial function in response to DNA damage in diffuse large B-cell lymphoma*	Ronald Gartenhaus

4:20 to 4:40 pm	**Allysen Schreiber** *Cultured supernatant from damaged cancer cells enhances the M2-like phenotype of macrophages*	Achsah Keegan
4:40 to 5:00 pm	**Helene Kerins** *S100B inhibition of IL-6 production in melanoma cells is modulated by its interaction with calcium, zinc, and its redox state*	David Weber
5:00 to 5:20 pm	**Sydney Anchor** *Mechanisms of Resistance to Inhibitors of the ERK1/2 Signaling Pathway in Melanoma*	Paul Shapiro

Abstracts

(in order of presentation schedule)

Alexandra Vlk
Towson University
Mentor: Dr. Eduardo Davila

The C-Terminal Domain of IRAK-3 is Necessary but Not Sufficient for Negative Regulation of Toll-Like Receptor Signaling and Induction of Apoptosis

Ligands bind to toll-like receptors (TLRs) on the cell surface of macrophages and monocytes. The ligand-receptor complex recruits the adaptor protein MyD88 and the interleukin receptor associated kinase (IRAK) family proteins. The activated complex binds TNF receptor associated factor 6 (TRAF-6) which activates transcription factors that promote the expression of inflammatory and pro-apoptotic proteins. IRAK-3 (IRAK-M) acts as a negative regulator of TLR signaling. IRAK-M has three conserved domains: the death domain (DD), the kinase domain (KD), and the C-terminal domain (CTD). To investigate whether the CTD is necessary for induction of apoptosis by IRAK-M, plasmids were designed and introduced into melanoma cell lines by electroporation. The vectors included an empty control plasmid, the full-length IRAK-M plasmid (DD+KD+CTD), the CTD-deleted plasmid (DD+KD), and the CTD-only plasmid (CTD). The effect on the downstream signaling molecules, including TRAF-6, Bax, and caspase-3, was detected by Western blotting. Over-expression of full-length IRAK-M reduced the TRAF-6 level, the CTD-deleted IRAK-M did not change TRAF-6 expression, and the CTD alone also reduced the TRAF-6 level but not as effectively as the full-length. We measured apoptosis caused by transient over-expression of IRAK-M variants using flow cytometry (FACS). In C32 cells, presence of the full-length IRAK-M or the CTD alone resulted in increased apoptosis compared to the empty vector. These data suggest the CTD of IRAK-M is necessary but not sufficient for the degradation of TRAF-6 and the pro-apoptotic effect. The CTD may require interaction with the DD for its full function. We expect that by understanding the mechanism of IRAK-M in TLR signaling, we will identify a possible biomarker and therapeutic target in the treatment of human melanoma.

Adria Lam
Rensselaer Polytechnic Institute
Mentor: Dr. Arnob Banerjee

The Transcription Factor Eomesodermin Regulates Expression of B-cell lymphoma 6 in $CD8^+$ T cells

The transcription factors Eomesodermin (Eomes) and B-cell lymphoma 6 (Bcl6) are important regulators of effector and memory CD8+ T cell differentiation. Increasing levels of both transcription factors is associated with central-memory $CD8^+$ T cell development. Studies suggest that Bcl6 and Eomes have analogous roles in $CD8^+$ T cell memory differentiation, however it is unknown how Eomes and Bcl6 are regulated. Here we show that Eomes regulates the expression of Bcl6 in $CD8^+$ T cells. Quantitative polymerase chain reaction (qPCR) showed a greater than 80% decrease in Bcl6 mRNA expression in Eomes knockout $CD8^+$ T cells. Likewise, there was a 36% decrease in Bcl6 protein expression in the Eomes knockout $CD8^+$ T cells, as analyzed by Western blot. These results imply that Eomes regulates the expression of Bcl6 in $CD8^+$ T cells, suggesting that augmented expression of Eomes could lead to increased levels of Bcl6 and promote enhanced $CD8^+$ T cell memory differentiation. A better understanding of the generation and maintenance of these cells will allow the development of therapies aiming to enhance $CD8^+$ T cell memory responses in patients with infectious disease and cancer.

Ciara Faupel
Stevenson University
Dr. Greg Snyder

Therapeutic Targeting of Activated B-Cell Diffuse Large B-cell Lymphomas using Toll-like Receptor Inhibitors

Activated B-cell Diffuse Large B-cell Lymphoma (ABC-DLBCL) is the most common subtype of Non-Hodgkin's lymphoma. One third of all ABC-DLBCL contains a mutation in the adaptor protein MYD88. This mutation changes a leucine to a proline at position 265 within the Toll-like/IL-1Receptor (TIR) domain of MYD88 which results in chronic activation of NF-ĸB and subsequent proliferation of cancer cells. Small molecule inhibitors (SMIs) T5996207 and T6167923 are thought to bind directly to the TIR domain of MYD88 to protect against death in *Staphylococcal* enterotoxin B induced toxic shock. We hypothesize these small molecule inhibitors T5996207 and T6167923 could also inhibit NF-ĸB activation caused by the L265P mutation in B cell lymphomas. To test this hypothesis, Ontario Cancer Institute (OCI) human patient derived B cell lymphoma cell lines OCI-LY19 and OCI-LY3, which are wildtype and homozygous for the MYD88 L265P mutation, respectively, were used. The cells were cultured in 24-well plates and treated in the presence and absence of the MYD88 SMIs for 24 hours. DMSO and ML120-B were used as negative and positive controls. The expected results are that there won't be a change in NF-ĸB levels in the nucleus in DMSO and in ML120-B and the experimental small molecule inhibitors there will be a decrease in NF-ĸB levels in the nucleus. Future studies include testing additional TIR small molecule inhibitors in other B cell lymphoma cell lines and animal models of cancer.

Leah Henry
The Catholic University of America
Dr. Amit Golding

A Novel Method for Determining the Mechanism of Action of B cell Stimulation

B cells are an important aspect of autoimmune diseases, as these patients usually have high titers of secreted autoantibodies. Determining how certain drugs or cells impact B cells would be useful for creating treatments for autoimmune diseases. One of the early stages of Ig regulation is the conversion from transmembrane/surface to the secreted form, which occurs via alternative splicing. Past methods of examining Ig regulation have primarily looked at the Ig in the serum of stimulated B cells but have not determined the mechanism of action. Currently, there is no convenient way to determine whether B cells are expressing the transmembrane form vs. the secreted form. The lab developed a novel quantitative PCR method for measuring the different IgM mRNA forms, including total, transmembrane specific, and surface specific message. This method uses three sets of primers designed by the lab that are specific for either total IgM mRNA or are specific for the transmembrane or secreted forms. We have tested this method using RNA from the stimulation of the Ramos B cell lymphoma line. We hypothesized that stimulation of these B cells will increase the total IgM expression as well as the ratio of secreted vs. transmembrane forms. We have found that supernatant from the stimulated Ramos cells show a significant increase in secreted IgM by ELISA. RNA from these cells using the qPCR conditions described showed a significant increase in total IgM mRNA and approximately 10-fold increase in the secreted vs. transmembrane ratio. Therefore, this novel method was successful for convenient measurement of the early stages of regulation of Ig forms, since it could detect changes in the specific mRNA levels due to stimulation. Determining the regulation of this early stage of Ig secretion would be helpful for discovering how drugs and/or regulatory cells may influence B cell activation and antibody secretion.

Carlos Echeverria
Loyola University Maryland
Mentor: Dr. France Carrier

Evaluating the Effect of Chemopotentiation by Low-Dose Fractionated Radiotherapy on Metastatic Properties of Gastric Cancer Cells

Metastasis is the leading reason for the resultant mortality of patients with cancer. In patients with metastatic cancer, systemic chemotherapy and radiation therapy are among the most commonly used methods of treatment. Metastatic and/or advanced gastrointestinal (GI) tumors remain difficult to treat despite recent advances in chemotherapy and technology. In these cases, the current standard of treatment involves combining 5-fluorouracil-based (5-FU) chemotherapy with localized radiation. Despite initial responses to treatment, the overall long-term prognosis for these patients remains poor. The use of standard doses of radiation therapy with full doses of chemotherapy in the treatment of GI carcinomas often leads to increased toxicity. Chemopotentiation by low-dose fractionated radiotherapy (LDFRT) is a novel treatment paradigm that allows for the safe use of full-dose systemic chemotherapy in combination with LDFRT. The clinical benefit of combining these modalities remains unclear due in part to a lack of understanding of the basic mechanisms underlying chemopotentiation by LDFRT. Recently, dual oxidase 2 (DUOX2), an enzyme with the sole function of regulated production of hydrogen peroxide, was identified as an important mediator of chemopotentiation by LDFRT. The goal of this project is to investigate the ability of DUOX2 to influence the migration and invasion of human gastric cancer cells in response to chemopotenation by LDFRT.

Rebecca Heilman
University of Connecticut
Mentor: Dr. Bret Hassel

Functional Analysis of Novel Oncodomain Mutations in the Tumor Suppressor RNase-L

RNase-L is an endonuclease involved in interferon-mediated innate immune and anti-proliferative responses. RNase-L functions to restrict cell proliferation through multiple mechanisms including promoting cellular senescence and inducing apoptosis. Consistent with its roles in tumor suppressor activities, mutations in the *RNASEL* gene have been linked to cancer. Most cancer-associated mutations to date, including those in *RNASEL*, have been identified through DNA sequence analysis but provide no information about their functional consequences. Another method to identify mutations has been to map the mutations using protein domain “landscapes”. This technique tracks alterations to specific protein domains, which provides functional insights into the role of the protein in oncogenesis. In the predicted oncodomain of RNase-L, this analysis revealed previously uncharacterized mutations from multiple human malignancies. We hypothesize that RNase-L mutations that contribute to oncogenesis will disrupt its tumor suppressor activities. To determine the functional consequences of a novel RNase-L oncodomain mutation, a thymine to cytosine base change at nucleotide 98 was introduced into the RNase-L cDNA by PCR. This mutation corresponds to an oncodomain mutation in skin cutaneous melanoma. Expression vectors containing wild type and mutated forms of RNase-L were transfected into HeLa cells to analyze RNase-L activity. Expression of transfected RNase-L was confirmed by Western blot. RNase-L activity was measured by two methods: directly through the appearance of rRNA cleavage products and indirectly through interferon expression. Transfection of the mutated cDNA slightly reduced rRNA cleavage activity relative to wild type RNase-L consistent with impaired enzyme function. However, the amount of IFN induction did not differ between these two transfectants, indicating more replicates are required to conclusively assess its impact. Structural modelings of the amino acid change corresponding to the oncodomain mutations suggest that novel pockets may comprise sites for RNase-L-targeted therapies.

Christine Magana
Binghamton University
Mentor: Nevil Singh

JEDI: Awakening the T-cell Force Against Tumors

Activated T-cells can fail to reject established tumors because immune checkpoint interactions inhibit T-cell activity. This mechanism of evasion occurs when ligands expressed on tumor cells bind to checkpoint receptors on T-cells. Blocking the interaction of these ligands and checkpoints can essentially improve anti-tumor immunity. Accordingly, antibodies developed to block known checkpoints, such as PD-1 and CTLA-4, are already in the clinic for tumor immunotherapy. Since just blocking PD-1 or CTLA-4 checkpoints aren't always sufficient, discovery of newer checkpoints can lead to improved therapies that significantly enhance T-cell responses and reduce tumor growth. Our lab has identified JEDI, as a potential checkpoint receptor. JEDI was highly expressed in chronically stimulated or exhausted T cells in a microarray analysis. We hypothesize that by blocking JEDI on T-cells from binding to its ligands on tumor cells it will relieve the inhibition experienced by T cells. This would prolong T-cell activation long enough to eradicate tumors. In order to construct a reagent that will block JEDI, my project was to express and purify the soluble extracellular domain (ECD) of JEDI to be used as the blocking reagent. The mouse JEDI ECD was cloned into an expression vector with Biotin and Histidine tags. After sequence confirmation, this was transfected into 293X cells. The secreted JEDI-ECD will be affinity purified and analyzed by Western blotting. Finally, we will evaluate if the purified JEDI-ECD can increase the activation of exhausted T cells in a mouse model and enhance the T-cell's ability to reject tumors. Studies with JEDI-ECD can lead to a better understanding of T-cell checkpoints by helping to identify ligands that bind to JEDI and provide a wider array of therapeutic options for tumors.

Jasper Mok
University of Maryland, College Park
Mentors: Drs. Alex Drohat and Lakshimi S Pidugu

Structural and Biochemical Analysis of Thymine DNA Glycosylase, a Base Excision Repair Tool for DNA Demethylation

It is well established that the methylation of cytosine at the 5'-C site, particularly at CpG islands, plays an integral role in the regulation of gene expression. The normal functionality of genes can be compromised if these cytosine bases are abnormally methylated and remain intact. 5-methylcytosine can undergo deamination to form thymine, or be oxidized by ten-eleven translocation (TET) enzymes to form the derivatives 5-carboxylcytosine and 5-formylcytosine. Thymine DNA glycosylase (TDG) is a base excision repair enzyme that removes thymine from G/T mismatches or uracil from G/U mismatches. Recently, it has been found that TDG also eliminates the oxidized derivatives of 5-methylcytosine. In this study, we seek to further our understanding of the specific TDG-DNA interactions during DNA demethylation by analyzing TDG through crystallography and biochemical studies. A new construct, $TDG^{108-301}$, was made by deleting disordered residues from the previously crystallized constructs. Conditions for overexpression of $TDG^{108-301}$ in *E. coli* were optimized and the protein was purified through nickel affinity, SP ion exchange, and size exclusion chromatography. The sitting drop vapor diffusion method was used to crystallize $TDG^{108-301}$ bound to a DNA substrate. We found that $TDG^{108-301}$ crystallized faster and more efficiently compared to other TDG constructs. Analysis of these crystallized structures is currently in progress. The rate constant for excision of T from a G/T mismatch by $TDG^{108-301}$ is 7.23×10^{-2} min^{-1} at room temperature (~22°C). This rate constant is over three times faster than that of $TDG^{111-308}$, which had been considered the catalytic domain. Our findings indicate that $TDG^{108-301}$ better represents the minimal catalytic domain. Future aims will focus on additional activity studies and mutagenesis. The effect of mutations on substrate binding and catalysis by $TDG^{108-301}$ will reveal amino acids that are important for activity.

Haley Hauser
Bucknell University
Dr. Gerald M. Wilson

Identification of Protein Domains Stabilizing AUF1 Complexes with RNA

AUF1 is a family of four RNA-binding proteins that associate with the AU-rich RNA elements (AREs) responsible for controlling the decay and translation of select mRNAs, including many that encode factors that regulate cell proliferation, inflammation, and immunity. More recent work has shown that AUF1 can also bind to a subset of microRNA targets including let-7b and enhance their loading into the RISC complex. While numerous studies have described features of RNA substrates that contribute to AUF1 binding, much less is known about the roles of AUF1 protein domains in RNA recognition. Although all AUF1 isoforms contain two tandemly arranged RNA Recognition Motifs (RRMs), using quantitative fluorescence anisotropy-based methods we show that these are not sufficient for RNA substrate binding, prompting the hypothesis that N- and/or C-terminal flanking protein domains must contribute to its RNA-binding activity. In this study, we prepared and purified a series of recombinant N- and C-terminally truncated AUF1 proteins to test whether these flanking domains aid in binding to canonical ARE and let-7b RNA substrates, and found that each domain plays distinct roles in stabilizing AUF1:RNA complexes. These findings help explain how AUF1 recognizes its physiological RNA targets, but also suggest a means by which different RRM-containing proteins might discriminate between highly diverse RNA populations.

Nathalie Chen
Carnegie Mellon University
Mentors: Drs. Anthony J. Kim, Jeffrey A. Winkles, Graeme F. Woodworth

Transferrin-Targeted Nanoparticles for Systemic Delivery Across the Blood Brain Barrier

The blood brain barrier (BBB) is a complex interface that protects and separates the central nervous system from the rest of the body. By extension, the BBB plays a major role in controlling the access of therapeutic agents to the brain. This often results in reduced treatment efficacy in diseases such as brain cancer. Research is currently being conducted on methods for delivering therapeutics across the BBB including the use of focused ultrasound and conjugating therapeutics to immune cells and nanoparticles. Transferrin receptors (TfRs) are expressed on the luminal membrane of cerebral endothelial cells, and have therefore been used to deliver therapeutic agents across the BBB. It has been demonstrated that transferrin targeted nanoparticles (Tf-NPs) can reach the brain parenchyma via receptor-mediated transcytosis. My project evaluated the ability of Tf-NPs to reach the brain via systemic delivery. Forty nanometer PEG-coated fluorescent polystyrene-NPs (CNPs) were coated with low (10%) or high (50%) concentrations of transferrin (Tf). The hydrodynamic size, polydispersity index, and zeta potential of the NPs were characterized by light scattering and Laser Doppler Microelectrophoresis. Tf conjugation to the NPs was confirmed via surface plasmon resonance using a TfR antibody-coated chip. We observed a positive correlation between Tf coating density and binding affinity. Next, BALB/c mice were injected with the NP formulations via the tail vein. After eight hours, the mice were euthanized and the brains removed. Brain tissue was homogenized, centrifuged, and the supernatant was analyzed for fluorescence using a plate reader. A statistically significant increase in fluorescence was observed in the brains of mice injected with low Tf-NPs compared to mice injected with non-targeted NPs. These results indicate that Tf-NPs accumulate in the brain more than non-targeted NPs, suggesting that the TfR is a potential target that could be used for the systemic delivery of therapeutics. Future experiments will test CNP accumulation in brain tumors and use electron microscopy to directly observe if CNPs collect within endothelial cells or if they truly cross the BBB.

Olamide Adebowale
Claflin University
Mentor: Dr. Antonino Passaniti

Targeting RUNX2 transcription factor to inhibit breast cancer proliferation

Breast cancer (BC) is the second most prevalent cancer in women next to skin cancer in the United States and is a persistent public health issue. Although great strides have been made in prognosis of early stage cancers, more aggressive metastatic cancers remain resistant to treatment. Recent studies have shown that the RUNX2 transcription factor regulates the expression of oncogenes such as the matrix metalloproteinase, MMP13, through all stages of BC and increases metastasis. The more aggressive cancers express the highest levels of RUNX2. Computer Assisted Drug Design (CADD) was used to find compounds that would inhibit RUNX2 DNA binding and transcriptional activity. One of the compounds, CADD522, has been shown to inhibit RUNX2:DNA binding and to decrease the expression of RUNX2 downstream genes. The aim of this study is to find the most effective analog of the lead CADD522 compound in inhibiting proliferation and colony formation in MCF7 (ER+/PR+/HER2-) luminal cell line. Cell proliferation assays show that some of the CADD522 analogs inhibit the proliferative and clonogenic ability of MCF7 cells. These results provide important information regarding the structural components of the drugs with the most favorable therapeutic index. Further studies will be necessary to define transcriptional regulation of RUNX2 downstream genes.

Samuel Savidge
Washington University in St. Louis
Mentor: Dr. Aikaterini Kontrogianni-Konstantopoulos

Loss of the obscurin-RhoGEF and its Effect on RhoA, RhoC, and Ras in Breast Epithelial Cells

Obscurins are giant, RhoGEF-containing, cytoskeletal proteins whose loss from breast epithelial cells has been shown to promote epithelial-to-mesenchymal transition, tumorigenicity, metastasis, and confer a growth and survival advantage within the cells. The mechanisms by which these changes become possible still remain unclear. However, it was recently demonstrated that the loss of obscurin-RhoGEF decreases RhoA activity and increases microtentactle formation and the attachment of breast epithelial cells. Herein, we sought to study the effects of sh-RNA mediated obscurin downregulated MCF10A cells on RhoC, Arf1, and Ras activity. Thus, using GTPase specific activation assays, we confirmed the increased activity of RhoA in obscurin deficient cells to validate proper procedural techniques. Then we measured the activities of Ras, RhoC, and Arf1 in obscurin downregulated and scramble control cells. We observed that in obscurin deficient cells, Ras was upregulated compared to the scramble control, and assays for RhoC and Arf1 are currently underway. However, the upregulation of Ras in sh-RNA mediated obscurin downregulated cells may shine some light on the mechanisms that promote epithelial-to-mesenchymal transition, tumorigenicity, metastasis, and confer a growth advantage in obscurin deficient breast epithelial cells. Furthermore, results demonstrating varying activities of RhoC and Arf1 will only advance the knowledge surrounding the loss of obscurins and its role in breast cancer.

Jennifer Drechsler
University of Maryland, College Park
Mentor: Dr. Tonya Webb

Targeting CARP-1 in breast cancer to decrease tumor growth and enhance immunogenicity

Breast cancer (BC) is the most common malignancy in women worldwide and is currently considered the leading cause of cancer-related mortality. While significant advances have been made over the last 30 years, novel approaches are essential for the treatment of BC. Preclinical studies and clinical trials have demonstrated that immunotherapy can effectively treat many cancers; however, immunotherapeutic strategies targeting BC should also focus on removing negative factors that inhibit the immune response in order to maximize their therapeutic effects. In fact, natural killer T (NKT) cells are important in initiating anti-tumor immunity, but these cells are significantly reduced in BC patients. The tumor-associated factors that lead to the reduction in NKT cells are unclear; however, it is known that during malignancy, BCs upregulate pro-survival proteins in order to proliferate and evade immune detection. One factor, CARP-1/CCAR-1, is overexpressed in BC and plays a critical role in regulating apoptosis. Thus, we hypothesize that inhibiting CARP-1 through the use of functional mimetics (CFMs) will induce apoptosis and restore anti-tumor immune responses. In this study, BC cell lines were treated with CFM-4&5 and then specificity, proliferation, cytotoxicity, and immunogenicity were assessed. It was found that pretreatment of MCF-7 and E0771 BC lines with 20μM CFM-4&5 resulted in 80-100% cell death within 48-72h. In order to assess immunogenicity in the absence of cytotoxicity, BC cell lines were pretreated with 10μm for 24h, washed, and cultured in fresh medium. This conditioned medium was collected and used to treat CD1d-expressing cells for 4h prior to co-culture with NKT cell hybridomas. Importantly, it was found that pretreatment with CFM-4&5 abrogated the secretion of immunosuppressive factors by both human and mouse BC cell lines and restored CD1d-mediated NKT cell activation. Collectively, these data implicate a novel role for targeting CARP-1 for the treatment of BC.

Caroline Schlee
University of South Carolina
Mentor: Dr. Michal Zalzman

The Role of ZSCAN4 in Cancer Stem Cell Phenotype and Survival

ZSCAN4 is an early stage developmental gene that was first identified in mES cells and preimplantation embryos. ZSCAN4 was shown to facilitate the nuclear reprogramming in induced pluripotent stem cell (iPSC) through activation of early embryonic genes. In mES cells, we found ZSCAN4 regulates telomere length and genomic stability, thereby maintaining long term developmental potency. Recent data from our lab indicates that ZSCAN4 is upregulated in a large array of human cancers. We further show that ZSCAN4 expression is required for long term survival of cancer cells in vitro and coincides with several cancer stem cell properties. Cancer stem cells (CSCs) represent a sub-population of cancer cells within a tumor that maintain the ability to self-renew, drive tumor growth and contribute to drug and radiation resistance. To study the role of ZSCAN4 in cancer stemness, we overexpressed ZSCAN4 and monitored several hallmarks of the cancer stem cell phenotype. We used multiple methods to assess the impact of ZSCAN4 on CSC properties including tumorsphere formation, telomere length regulation and CSC marker expression. In this project, we also defined the epigenetic changes in chromatin state in multiple types of cancer cell lines. This work will offer insight into the mechanism by which ZSCAN4 promotes CSC formation and will allow us to develop ZSCAN4 as a therapeutic target in cancer.

Hayley Scott
Loyola University Maryland
Dr. Matthew Frieman

Inhibition of Coronavirus entry by TNFα converting enzyme

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are emerging coronaviruses that are highly pathogenic and represent a large public health threat to people around the world. Neither of these viruses have approved therapeutics. Novel approaches to antiviral drug development are needed to combat these and future viral threats. The receptor for SARS-CoV is a protein called ACE2 and the receptor for MERS-CoV is DPP4. Both of these receptors are present on the plasma membrane of susceptible cells. Also present on the plasma membrane of those cells is a TNF
mentioned receptors as a homeostatic control mechanism. We hypothesize that a novel antiviral therapy could be created that hyper activates TACE/ADAM17, therefore cleaving the receptors off of the surface of cells. This will inhibit SARS-CoV and MERS-CoV by removing the receptor from the cell surface, as well as having free receptor in lungs to prevent infection. As proof of concept, we transfected 293T cells with either receptor alone, as well as each receptor in combination with TACE/ADAM17. We predict that over expression of TACE/ADAM17 will result in the release of the virus receptors into the cell media thus reducing viral infection. This work will identify whether hyper activation of TACE/ADAM17 is a potential anti-coronavirus therapy.

Smaraki Dash
University of Maryland College Park
Mentor: Dr. Rena Lapidus

Effect of novel binaphthoquinones alone and in combination with chemotherapy on acute myeloid leukemia

Acute myeloid leukemia (AML) is a cancer characterized by an accumulation of immature white blood cells in the bone marrow, which prevents the development of red blood cells, white blood cells, and platelets. This study assessed the anti-cancer properties of a novel class of drugs called binapthoquinones (BiQ) both alone and in combination with known chemotherapeutics used to treat AML. BiQs have been shown to induce reactive oxygen species and damage DNA, which leads to cell death. The known chemotherapeutics in this experiment were cytarabine and cladribine, both classified as antimetabolites. These agents are reported to incorporate into DNA, alter DNA synthesis, and damage DNA with eventual prevention of cell division. Specifically, araC is a pyrimidine antagonist while cladribine is a purine analog and may interfere with the enzyme adenosine deaminase. In this study, we used two FLT3-ITD mutant human AML cell lines, MOLM-14 and MV4-11, and treated both with a dose response of each of the novel drugs and chemotherapeutics independently for 72 hours. Following incubation, these cells were terminated by an MTT-like agent (mitochondrial dye) and IC50 values were calculated.
As AML treatment usually includes a cocktail of different drugs, combination studies are also an important component of drug testing. In particular, one binaphtoquinone called 217 was further evaluated in combination with each of the chemotherapeutics in both cell lines for potentiation effects. Interestingly, 217 potentiated araC and cladribine in MV4-11 cells while this effect was not observed in MOLM-14 cells. Following this, we are currently testing for synergy with 217 and each of the chemotherapies in MV-411 cells. In the future, we intend to look at mechanistic studies, specifically 217's effect on DNA methylation in AML cells.

Shannon Kirby
University of Maryland, College Park
Mentor: Dr. Tami Kingsbury

The Impact of miR513a-1 and miR518c on Leukemia Cell Differentiation and Proliferation

Erythropoiesis is the orchestrated proliferation and differentiation of hematopoietic stem-progenitor cells (HSPC) into red blood cells (RBCs). In humans, 2 million RBCs are generated per second, showcasing the role of controlled cell growth and differentiation in the maintenance of health. Defects in HSPC differentiation lead blood cancers (leukemia). MicroRNAs (miRs) are small RNA molecules that have been shown to impact differentiation through mRNA targeting. Several microRNAs are critical for progression of erythropoiesis, while other microRNAs have been linked to leukemia. Microarray expression analysis revealed increased levels of miR513a during erythropoiesis. To determine the role of miR513a, TF1 cells were transduced with control vs. miR513a expressing lentivirus. The erythroleukemia TF1 cell line is a model for human erythropoiesis. Differentiation is monitored by increased transferrin receptor (CD71) and glycophorin A (CD235) surface expression and loss of the HSPC marker CD34. TF1 cells expressing miR513a demonstrated increased numbers of erythroid (CD71+CD235+CD34-) cells compared to control cells. Western blot analysis of miR513a expressing cells revealed increased levels of hemoglobin and reduced levels of GATA2, even in the absence of erythropoietin. The "GATA switch", in which cells switch from expressing GATA2 to GATA1, is a hallmark of erythropoiesis. GATA2 is a predicted target of miR513a and our results suggest miR513a may contribute to GATA switching. In contrast to miR513a, enforced expression of miR518c reduced erythropoiesis. Our findings suggest that miR513a enhances, while miR518c inhibits erythropoiesis. In contrast to their opposing role in erythropoiesis, expression of either miR513a or miR518c reduced TF1 clonogenicity, as assessed by plating for single cell colonies. Both miR513a and miR518c reduced the number of single cell clones obtained by ~50% compared to controls, demonstrating the ability of both miRs to regulate leukemia cell growth. Future studies will determine the mechanisms by which these miRs regulate erythropoiesis and leukemia cell growth.

Arielle Hamburg
Washington University in St. Louis
Mentors: Dr. Ronald Gartenhaus and Dr. Kavita Bhalla

ATM regulates expression of FOXO3a and mitochondrial function in response to DNA damage in diffuse large B-cell lymphoma

Body: Patients with Ataxia-telangiectasia (AT), a neurological disorder that occurs due to deletion or mutation in the Ataxia-telangiectasia mutated (ATM) gene, have a propensity to develop lymphoma. Cells lacking ATM, a kinase critical to the cellular DNA damage response (DDR), have an attenuated DDR to DNA double strand breaks. Previous reports and published data from our lab indicate that ATM affects the association of HuR binding protein with target mRNAs, including FOXO3a and SIRT1. These molecules regulate vital cellular processes, including DNA repair and mitochondrial metabolism. We hypothesized that ATM exerts its effect on lymphoma development in response to DNA damage by potentially regulating FOXO3a and SIRT1. RNA expression analysis of these targets showed no difference in mRNA expression between ATM $^{+/+}$ and ATM $^{-/-}$ normal lymphocytes. Interestingly, pharmacological inhibition of ATM signaling in diffuse large B-cell lymphoma (DLBCL) cells resulted in approximately two-fold decrease in expression of FOXO3a. However, no significant difference was seen in mRNA expression of SIRT1 in DLBCL cells. We also studied the effect of ATM on FOXO3a protein expression. At the protein level we observed reduced expression of FOXO3a in ATM-null lymphocytes compared to ATM $^{+/+}$ lymphocytes. We also examined protein regulation of FOXO3a by stimulating DNA damage under hypoxic stress. In DLBCL cells where ATM was inhibited prior to hypoxic stress, expression of FOXO3a was reduced compared to untreated cells. RNA and protein expression studies indicate that ATM directly regulates

expression of FOXO3a in DLBCL lymphocytes. Next we explored if inhibition of ATM pathway affected mitochondrial respiration in DLBCL cells. We found that DLBCL cells treated with the ATM inhibitor had a diminished mitochondrial respiratory capacity compared to untreated cells. Together, our data indicates that ATM directly regulates expression of FOXO3a and mitochondrial function in DLBCL cells. Thus our findings suggest that absence of ATM activation during DNA damage may reduce FOXO3a tumor suppressor activity and mitochondrial function that in turn may contribute to development of lymphoma in AT patients.

Allysen Schreiber
Wake Forest University
Mentor: Dr. Achsah Keegan

Cultured supernatant from damaged cancer cells enhances the M2-like phenotype of macrophages

Tumor-associated macrophages (TAMs) are the most abundant immune cells in the microenvironments of primary and metastatic tumors. TAMs commonly adopt a pro-tumorigenic, M2-like phenotype that suppresses immune responses to the tumor, stimulates angiogenesis, and promotes tumor cell migration and metastasis. Signals generated by damaged tumor cells in response to anti-cancer therapies can enhance the M2 phenotype of TAMs and diminish treatment efficacy. However, the mechanism by which this occurs remains unclear. We hypothesize that direct activation of innate sensing pathways by damaged tumor cells is responsible for this observation. To test this hypothesis, we treated the murine breast cancer cell line EO771 with Doxorubicin, a common chemotherapeutic agent, and harvested the supernatant. This supernatant was then used to stimulate macrophages and the protein levels of well-established M2 markers were examined by Western blot analysis. Preliminary findings indicate that cultured supernatant from EO771 cells treated with Doxorubicin increases the M2 marker Ym1 in macrophages. These results suggest that signals released from damaged cancer cells in response to chemotherapy treatment directly enhance the M2 macrophage polarization. Ongoing work will identify the specific innate sensing pathways that are activated in this event. Findings from this study will improve our understanding of the macrophage innate sensing pathways activated in response to tumor damage and could explain the failure of some chemotherapeutic treatments and cases of tumor recurrence.

Helene Kerins
University of Richmond
Mentor: Dr. David Weber

S100B inhibition of IL-6 production in melanoma cells is modulated by its interaction with calcium, zinc, and its redox state

The small, dimeric, Ca^{2+} and Zn^{2+} binding protein, S100B, is elevated in several types of cancer including reactive gliomas, renal cell tumors, malignant T-cells, and malignant melanoma, where its expression correlates inversely with patient survival. S100B is not just a marker, it also plays a role in maintaining and promoting tumors by interacting with and regulating several intracellular proteins including p90 ribosomal S6 kinase (RSK) in a calcium dependent manner. While S100B appears to activate the MAPK kinase pathway resulting in increased amounts of the active pERK, it specifically inhibits the pERK-dependent phosphorylation of RSK Thr573. While RSK still appears to be active, S100B sequesters RSK into the cytosol preventing it from acting on nuclear targets tweaking the MAPK signaling to allow phosphorylation of the ERK specific targets while preventing phosphorylation of the nuclear RSK targets. The overexpression of S100B in

melanoma cells has now been shown to decrease activation of the IL-6 gene and shuts down IL-6 secretion by the cells, and this appears to be dependent on MAPK/RSK signaling pathway and the CREB transcription factor. The present study examines whether the intracellular S100B inhibition of IL-6 secretion in melanoma cells is modulated by calcium or zinc binding to S100B and/or by the redox modification of the critical Cys84. While the importance of these in regulating target interaction has been demonstrated in cell free systems, their intracellular relevance is still questioned. One reason for this is that S100B's affinity for calcium and zinc are thought to be much lower than the available levels of free metal ions found intracellularly. S100B and three mutants will be expressed in cells that lack endogenous S100B but express IL-6, WM1158 melanoma cells, to assess mutants effect on IL-6 secretion. Specifically, the cysteine mutant C84S to test the importance of redox regulation, calcium binding mutant E31/72A, and a zinc binding mutant H15A of S100B. All three of these cell signaling pathways have been found to be dysregulated in cancer cells and it is extraordinary that a small protein like S100B may be regulated by all three.

Sydney Anchor
University of Mississippi
Dr. Paul Shapiro

Mechanisms of Resistance to Inhibitors of the ERK1/2 Signaling Pathway in Melanoma

Extracellular-signal-regulated kinases (ERK1/2) are activated through a complex phosphorylation cascade consisting of intracellular proteins including Ras G-proteins, BRaf, and MAPK/ERK kinases (MEK1/2). Activating mutations in Ras ad BRaf have been seen in many cancers, one prime example being melanoma. Metastatic melanoma is a highly mutagenic, incredibly lethal cancer for which few effective drug therapies exist. Consequently, much attention has been directed towards developing new therapeutic approaches which can selectively inhibit the ERK1/2 pathway. While clinical success was initially seen with BRaf and MEK1/2 inhibitors, almost all patients quickly developed resistance. As a way to overcome such patterns of resistance, novel inhibitors selective to the ERK1/2 proteins are now being developed. Currently, it is not known whether patients will develop resistance to these compounds. In this study we investigated the potential for resistance to develop against BRaf, MEK1/2, and ERK1/2 inhibitors and compared mechanisms by which resistance to these inhibitors developed. Drug-resistant A375 melanoma cells were developed and evaluated for changes in protein expression. Resistant cells all showed resumed phosphorylation of MEK1/2 and ERK1/2 as well as increased expression of MEK1 and Akt, however with varying levels of magnitude. These results indicate a reactivation of the ERK1/2 pathway despite inhibition in all resistant cells with differing levels of dependence on alternative cell survival pathways. While ERK1/2 inhibitors alone may not be able to avoid the development of resistance, future treatments may involve ERK1/2 inhibitors combined with other targeted therapies. Comparison of downstream substrates between these resistant cell lines will help to determine the most effective combinations of drugs. Ideally, these combination therapies may be able to reduce the activation of the ERK signaling pathway while simultaneously preventing future resistance. Further investigation will involve the determination of proteins unique to resistant cell lines through proteomic analysis and siRNA knockdown of up-regulated proteins to determine key players in acquiring resistance.