The 2017 Summer Research Symposium of the Nathan Schnaper Intern Program in Translational Cancer Research

26 July 2017
Southern Management Corporation Campus Center

UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER
NATHAN SCHNAPER INTERN PROGRAM

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NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

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For more information, go to http://www.umm.edu/NSIP

2017 NSIP Research Symposium

Southern Management Corp. Campus Center, Room 351
Wednesday, July 26, 2017
8:00 am to 5:15 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	**Najah Soudan** *Assessing Immune Function in Lymphoma*	Tonya Webb
9:00 to 9:20 am	**Rachel Lent** *MERS-CoV proteins effecting yeast growth and replication*	Matthew Frieman
9:20 to 9:40 am	**Justin Montague** *Targeting diffuse large B-cell lymphomas using MyD88 specific small molecule inhibitors*	Gregory Snyder
9:40 to 10:00 am	**Smaraki Dash** *The Effect of a novel binaphthoquinone on acute myeloid leukemia*	Rena Lapidus
10:00 to 10:20 am	**Reine Leutze** *RNase-L-dependent gene regulation during the epithelial to mesenchymal transition*	Bret Hassel
10:20 to 10:40 am	**Break**	
10:40 to 11:00 am	**Bernadine Monari** *Regulation of Autophagy and Role of Extracellular Vesicles in a Picornavirus Model*	William Jackson
11:00 to 11:20 am	**Adam Schneider** *Quantifying the Effects of HIV Matrix Protein p17 on Inducing Angiogenesis and Lymphangiogenesis in AIDS-related Cancers*	Alfredo Garzino-Demo
11:20 to 11:40 am	**Caroline Schlee** *Defining the SIX1 Interactome in Leukemia Cells*	Tami Kingsbury

11:40 am to 12:00 pm	**Beita Badiei** *The Effect of the PH Domain of Obscurin on the Migratory, Invasive, and Tumorigenic Potential of Breast Cancer Cells*	Aikaterina Kontrogianni
12:00 to 12:20 pm	**Benjamin Merenbloom** *Developing a high throughput assay to screen for drugs that can inhibit hnRNP A18/RNA interaction*	France Carrier
12:20 to 1:20 pm	**Lunch with Drs. Lauren Schnaper and David Carlton**	
1:20 to 1:40 pm	**Alexandria Riedel** *The Role of Rad9 Phosphorylation on DNA Binding and DNA Repair*	A-Lien Lu-Chang
1:40 to 2:00 pm	**Emily Slaby** *Enhancing Cell-based Cancer Immunotherapies by Plasma Membrane-inserting TLR2 Ligands*	Gregory Szeto
2:00 to 2:20 pm	**Minh-Quan Pham** Toll-Like Receptor 2/1 Stimulation in pmel T Cells Increases Expression of Oxidative Stress Related Genes	Eduardo Davila
2:20 to 2:40 pm	**Kayla Reagan** *ATM affects diffuse B-cell lymphoma (DLBCL) growth by regulating SIRT1 expression*	Gartenhaus and Bhalla
2:40 to 3:00 pm	**Nicholas Montes** *Proteins in Melanoma Cells that Contribute to Resistance against Tyrosine Kinase Inhibitors that Target the MEK/ERK pathway*	Paul Shapiro
3:00 to 3:20 pm	**Anna Chippi** *Using a T-cell's JEDI to Enhance Tumor Immunity*	Nevil Singh
3:20 to 3:40 pm	**Break**	
3:40 to 4:00 pm	**Amy Yan** *Biochemical relationships between Hsp70's RNA- and protein-binding functions*	Gerald Wilson
4:00 to 4:20 pm	**Sara Barlow** *Comparative analysis of targeted nanoparticles for drug delivery across the blood-brain barrier utilizing mass spectrometry*	Kim, Winkles, and Woodworth

4:20 to 4:40 pm	**Samuel Savidge** *The Role of SETBP1 in Ph$^+$ Acute Lymphoblastic Leukemia*	Danilo Perotti and Rossana Trotta
4:40 to 5:00 pm	**Jennifer Drechsler** *Selective loss of T cell function during lymphomagenesis*	Tonya Webb
5:00 to 5:15 pm	**Bret A. Hassel, PhD** *Closing Remarks*	

Abstracts

(in alphabetical order)

Beita Badiei
University of Maryland, College Park
Mentor: Dr. Aikaterini Kontrogianni-Konstantopoulos

The Effect of the PH Domain of Obscurin on the Migratory, Invasive, and Tumorigenic Potential of Breast Cancer Cells

Obscurins, encoded by the human *OBSCN* gene, are giant cytoskeletal proteins initially identified to play structural and regulatory roles in striated muscles. Further research has shown that mutations in the gene are implicated in severe diseases, including skeletal and cardiac myopathies. More recently, obscurins have been shown to play a role in the progression of breast cancer through interaction with the PI3K/AKT pathway, which is upregulated in nearly 30% of breast cancers affecting major cellular processes. Mechanistically, the Pleckstin Homology (PH) domain of obscurin interacts with the Src Homology 3 (SH3) domain of the p85 regulatory subunit of PI3K. We therefore hypothesize that loss of obscurins from breast cancer cells results in lack of regulation of the p85 subunit, which leads to aberrant activation of the catalytic p110 subunit, and thus upregulation of downstream cascades involved in growth, migration and invasion. My goal is to examine whether ectopic expression of the PH domain of obscurins in breast cancer cells lacking obscurins will suppress their tumorigenic, migratory, and invasive potential by restoring the PI3K/AKT activity to baseline. This will be accomplished through transient transfection of a plasmid that carries the PH domain into obscurin-knockdown MCF10A cells, and two breast cancer cell lines, MCF7 and MDA-MB-231, which lack endogenous obscurins. Cells expressing the PH domain and the appropriate controls will be analyzed for their ability to grow under low attachment conditions and form mammospheres, migrate and invade. The results of this work will shed light onto the therapeutic potential of the obscurin PH peptide for breast tumors and patients characterized by depletion of giant obscurins.

Sara Barlow
Grand Valley State University
Mentor: Drs. Jeffrey Winkles, Anthony Kim, and Graeme Woodworth

Comparative Analysis of Non-Targeted and Targeted Nanoparticles for Drug Delivery Across the Blood-Brain Barrier Utilizing Mass Spectrometry

Treatment of the most common and fatal primary adult brain cancer, glioblastoma, is extremely challenging due to the blood-brain barrier (BBB). The BBB consists of a continuous layer of endothelial cells (ECs) linked together by tight junctions, pericytes, and astrocytic foot processes. In order to freely cross the BBB, therapeutic agents need to be lipid soluble, minimally charged, and of small size (MW <400 Da). Since most small molecule drugs and biologics (e.g., antibodies) do not possess these structural properties, one strategy for BBB transit is to deliver therapeutics using targeting nanoparticles that bind to cell surface receptors expressed on brain ECs. Nanoparticle (NP) formulations with targeting moieties have been employed by others to deliver therapeutics through the BBB with limited success. We are focusing on three EC receptors that might be effective for brain drug delivery: Fn14, LRP-1, and transferrin receptor (TfR). The targeting agents we are using for these three proteins are ITEM4 (an Fn14 antibody), RAP (an LRP-1 ligand), and holo-transferrin (a TfR ligand). These targeting agents were conjugated to PGA-PEG NPs loaded with the platinum-based compound oxaliplatin. Oxaliplatin allows for the use of a sensitive assay for measuring platinum levels in the brain; namely, inductively coupled plasma mass spectrometry (ICP-MS). First, we confirmed conjugation of the three targeting agents to the nanoparticles by surface plasmon resonance assays. We then generated an oxaliplatin standard curve by intracranially injecting oxaliplatin into mice followed by animal euthanization, brain isolation and homogenization, and digestion in nitric acid. The samples were then centrifuged, the supernatant was serially diluted, and platinum was measured with ICP-MS. In our most recent experiment, either PBS, free oxaliplatin, nontargeted oxaliplatin NPs or the three targeted oxaliplatin NP types were injected systemically into nude mice. Mice were euthanized 24 hrs later, brains were isolated, and samples were prepared and analyzed in the same manner as the standards. Platinum quantitation in the brain by ICP-MS is currently in progress.

Anna Chippi
Stevenson University
Mentor: Dr. Nevil Singh

Using a T-cell's JEDI to Enhance Tumor Immunity

The immune system faces difficulties in curing cancer because of inhibitory signals tumor cells send to immune cells. These signals, known as checkpoints, are generated by ligands from tumors that bind receptors on T-cells. A promising approach in immunotherapy is to target specific checkpoints, such as PD1 and CTLA-4 by preventing their ligands from engaging receptors on T-cells. Like PD1 and CTLA-4, JEDI is a protein expressed on the surface of T-cells and is made up of an extracellular (EC) and intracellular (IC) domain. We expect that when the EC portion of JEDI binds to a ligand on a tumor cell, the IC signals a T-cell to stop responding to kill cancer. Based on this, we hypothesize that using a soluble version of the EC domain of JEDI alone can block the JEDI ligands on tumors from binding JEDI on T-cell's surface. This would bypass checkpoint activity due to JEDI and improve T-cell responses to tumors. My project aims to purify JEDI-EC through transfecting human embryonic kidney cells (HEK 293X) with a plasmid construct encoding for JEDI-EC. After purification, the protein will be run on SDS-PAGE and verified for expression via Western Blotting. Once expression and purification are validated, JEDI-EC will be tested for its ability to improve T-cell activation and can hopefully serve as an anti-tumor immunotherapy.

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

The Effect of a novel binaphthoquinone on acute myeloid leukemia

Acute myeloid leukemia (AML) is a mutationally heterogeneous cancer with poor 5-year survival rates (~ 30%). In the past 40 years, only one new drug for this cancer has been approved by the FDA. The disease is 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 chemical entity known as MCD-66, which is part of a larger class of drugs called binapthoquinones (BiQ). BiQs have also been shown to induce reactive oxygen species and DNA damage, leading to cell death. In this study, we tested the effect of MCD-66 on two human AML cell lines, MOLM-14 and MV4-11. IC50 values were calculated by treating both cell lines with a dose response of MCD-66 for 72 hours. This was followed by the addition of a MTT-like agent (mitochondrial dye) which measures whether cells are metabolically active. IC50 values for MOLM-14 and MV4-11 were 0.18 ± .05 μM and 0.20 ± .03 μM respectively. Other clinically used quinone analogs were tested in a similar fashion to determine the specificity of MCD-66. We also tested true cytoxicity of MCD-66 by counting live cells post-treatment using the Trypan Blue exclusion assay. We observed a sharp decrease in living cells around the previously found IC50 value. This shows that not only does MCD-66 slow down overall proliferation, but it may be cytotoxic and not just cytostatic. We also confirmed MCD-66's ability to induce DNA damage using gamma H2Ax experiments. Lastly, naphthoquinones have been shown in the literature to inhibit the enzyme Indoleamine 2,3-dioxygenase (IDO1). This enzyme is involved in evasion of the cancer cells from the immune system. We tested the ability of the MCD-66 and the other quinone analogs to inhibit IDO1 activity. Future work includes determining if this agent can prevent AML's ability to evade the immune system in vitro and in vivo.

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

Selective loss of T cell function during lymphomagenesis

Natural killer T (NKT) cells play an important role in initiating anti-tumor immunity. However, NKT cells are significantly reduced in lymphoma patients. In order to investigate the mechanisms by which NKT cells are impaired in lymphoma, we sought to develop and characterize a mouse model that recapitulates human disease. In our double transgenic (DTG) mouse model both IL-14, a B cell growth factor, and c-Myc, an oncogene, are overexpressed and drive B cell lymphoma development. We hypothesize that during tumorigenesis, lymphomas produce immunosuppressive factors, which lead to a reduction in the number and function of NKT cells in tumor-bearing mice, similar to human disease. To test this hypothesis, we harvested the thymus, spleen, lymph nodes, bone marrow, and liver from wild type control and DTG mice and performed phenotypic and functional analyses. The lymphocyte populations were characterized using flow cytometry. Compared to wild type mice, we observed a significant decrease in thymic T cells in DTG mice, and a concomitant decrease in NKT cells in the liver. In our *ex vivo* stimulation experiments we found a reduction in the level of T cell activation in tumor-bearing mice, compared to controls. These data suggest that our DTG mouse model has an impaired immune response, similar to our clinical data. Finally, PLZF, a NKT cell-specific transcription factor, was overexpressed within the thymus of the DTG mice compared to their wild type counterparts. In the future, we will perform immunohistochemistry studies to further characterize the development of lymphoma within the mouse model. Collectively, these studies will delineate the role of NKT cells in lymphoma development and progression and have the potential to lead to the development of novel immunotherapeutic strategies for the treatment of this devastating disease.

Rachel Lent
Towson University
Mentor: Dr. Matthew Frieman

Identifying genetic interactions with MERS-CoV using a yeast based screening model

Since emerging in 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) has resulted in over 1800 infected individuals with roughly 35% mortality. The MERS-CoV protein 4a is an accessory protein that dampens the host antiviral response by binding double-stranded RNA and antagonizing interferon signaling and activation. 4a activity can be detected through a yeast-based screening assay due to its ability to induce a slow-growth phenotype in *Saccharomyces cerevisiae.* A population of *S. cerevisiae* that contains single deletions of every non-essential gene, known as the yeast knockout library (YKO), is used as a platform to identify virus-host interactions of 4a. This was achieved by expressing 4a in YKO and selecting for clones that restore normal growth while in the presence of 4a. Proteins and cellular pathways that potentially interact with 4a were identified by sequencing these clones to identify the mutated genes that allow for restored growth. Multiple screens revealed yeast mutated in the gene for SIR2, which potentially translated into mammalian cells via the homolog SIR1. SIR2 was confirmed to impede 4a function by expressing 4a in a clean knockout strain of SIR2 that restored normal growth. Elucidating the mechanisms of 4a function is important for understanding how MERS 4a evades host immune responses and informing future therapeutic interventions.

Reine Leutze
Coppin State University
Mentor: Dr. Bret Hassel

The role of RNase L in the Epithelial to Mesenchymal Transition

RNase L is an endonuclease that functions in the immune response to viruses and bacteria and acts as a tumor suppressor by limiting cell proliferation and inducing apoptosis. The epithelial to mesenchymal transition (EMT) is a series of biochemical changes by which a differentiated epithelial cell takes on less differentiated, mesenchymal stem cell properties. Specifically, cells acquire enhanced migratory capacity, invasiveness, and resistance to programmed cell death. EMT plays physiologically important roles in embryogenesis and wound healing but the aberrant induction of EMT in differentiated cells promotes tumor metastasis and cancer progression. RNase L inhibits cell migration in a wound healing model, therefore we hypothesized that it may also inhibit migration in the context of EMT and tumor metastasis as a mechanism of its tumor suppressor activities. Furthermore, as RNaseL exerts its biologic functions by cleaving single-stranded RNA, we postulated that RNaseL will regulate specific RNAs to, in turn, inhibit cell migration and EMT. To identify mRNA targets of RNaseL in EMT, we induced EMT with Transforming Growth Factor-β in A549 lung carcinoma cells in which the RNaseL gene was present or knocked out (KO). RNaseL-dependent regulation of mRNAs encoding established EMT mediators E-cadherin, N-cadherin, and claudin-1 were then analyzed by RTPCR. E-cadherin and N-cadherin mRNAs were differentially regulated in the presence and absence of RNaseL providing a potential mechanism by which RNaseL inhibits EMT. Future studies will determine how the RNaseL-dependent regulation of specific mRNAs contributes to EMT and tumor progression. A knowledge of the specific RNAs that are targets of RNase L will provide strategies to modulate its activity and enhance anti-tumor functions in a therapeutic setting.

Benjamin Merenbloom
University of Maryland, College Park
Mentor: Dr. France Carrier

Developing a high throughput assay to screen for drugs that can inhibit hnRNP A18/RNA interaction

Heterogenous ribonucleoprotein A18 (hnRNP A18) is an RNA-binding protein primarily found in the nucleus under normal conditions. However, under cellular stress, such as UV radiation or hypoxia, which is found in most solid tumors, hnRNP A18 translocates to the cytosol. Following this translocation, the protein exhibits post-transcriptional activity on mRNA targets to stabilize them and increase translation of the targets' respective protein. hnRNP A18 mediates these effects by binding to a specific 51-nucleotide consensus motif in the 3' UTR of target mRNA transcripts. Targets include mRNA transcripts that code for the translation of proteins that help repair DNA damage (RPA and ATR) and inhibit apoptosis (TRX). hnRNP A18 has thus been associated with the proliferation of tumor cells and overall tumor growth. Because of this contribution to tumor growth, we seek to develop a high throughput (HTP) assay which can be used to screen FDA-approved anticancer drugs that could potentially inhibit the interaction between hnRNP A18 and its target RNA transcripts. As a first step to develop this assay, an A18-GST vector was induced in *E.coli* with IPTG to produce recombinant A18-GST protein. HOOK GST kit was used to extract and purify A18-GST proteins. Western blot and Coomassie stain were run to confirm A18-GST protein expression. Next, we will use a 3' Fluorescein-tagged hnRNP A18 consensus sequence to optimize HTP assay to demonstrate inhibition of hnRNP A18:RNA binding. Potential drugs will be screened from FDA-approved anticancer drug library. Provided that inhibition of binding between A18-GST and its RNA targets is demonstrated in this cell-free system, drugs will be examined further for binding inhibition activity in a physiological setting. An *in vitro* study will be performed to test potential drug activity in cell-based assays, such as colony survival and proliferation assays, followed by an *in vivo* study to test the pharmacokinetics and pharmacodynamics of the potential drugs in animal models.

Bernadine K Monari
Immaculata University
Mentor: Dr. William T. Jackson

Regulation of Autophagy and Role of Extracellular Vesicles in a Picornavirus Model

Picornaviruses, small viruses made up of single-stranded positive-sense RNA, cause a wide range of viruses, from foot-and-mouth disease to poliomyelitis to the common cold. Picornaviruses were previously thought to be nonenveloped viruses, but new data has shown that certain picornaviruses can be released in extracellular vesicles. The following study focuses on the exosomes of cells infected with poliovirus, rhinovirus 1A, and enterovirus, whether these exosomes are infectious, and what could be present in the exosomes that promotes infection. It was hypothesized that the PV and EV-D68 exosomes would be infectious, while HRV-1A exosomes would not be infectious. The potential infectivity of the exosomes could be a result of viral particles or small RNA's that trigger autophagy. Exosomes were harvested from K562's, then assayed for infectivity. Once infectivity was confirmed, proteins were collected from HeLa cells that were treated with guanidine chloride or a vehicle, and Western Blots were used to test for p62 and LC3, proteins known to be involved in autophagic process. The results of infectivity and automate assays, and current Western Blot analysis, hint at how these viruses regulate autophagy in infected cells.

Justin Montague
Loyola University Maryland
Mentor: Dr. Greg A. Snyder

Targeting Diffuse Large B-Cell Lymphoma Using MYD88-Specific Small Molecule Inhibitors

Diffuse Large B-Cell Lymphoma (DLBCL) is an aggressive form of Non-Hodgkin's Lymphoma comprising of two district subtypes: Activated B-Cell like (ABC) & Germinal Center B-Cell like (GCB) DLBCL. The former subtype has a 40% cure rate, indicating a need for additional novel therapies. A recurring single amino acid somatic mutation of a Leucine to a Proline at position 265 (L265P), associated with nearly one out of every three of human ABC DLBCLs, has been identified within the central signaling adaptor molecule Myeloid differentiation factor 88 (MYD88). This mutation results in increased cell proliferation and chronic NF-kB signaling. The MYD88 domain architecture is comprised of TIR and Death signaling domains connected by an interdomain linker. We propose this oncogenic mutant, MYD88-L265P, located in the MYD88 TIR domain, induces a molecular change within MYD88 domain architecture resulting in dysregulated and chronic NF-kB signaling in B-cell lymphomas bearing this mutation. Our objective was to characterize small molecule inhibitors designed to target MYD88, which were previously shown to protect mice from staphylococcal enterotoxin B induced death by binding MYD88 to inhibit a fatal immune response. We tested these molecules for their ability to inhibit cell proliferation and signaling in lymphoma cell lines bearing the MYD88 L265P mutation. Using a MTS-cell proliferation assay, we evaluated the MYD88-specific small molecule inhibitors for their ability to limit cell proliferation in lymphoma cell lines OCI-Ly3 (L265P) and OCI-Ly19 (WT). Additionally, we are characterizing the molecular interactions of small molecule inhibitors and recombinant MYD88 protein using thermal shift, NMR and X-ray crystallography. Using a structure-based drug design approach, our long-term goal is development of improved small molecule therapeutics that inhibit lymphoma as well as acute and chronic inflammation.

Nicholas Montes
University of Maryland, College Park
Mentor: Dr. Paul Shapiro

Proteins in Melanoma Cells that Contribute to Resistance against Tyrosine Kinase Inhibitors that Target the MEK/ERK pathway

Malignant Melanoma is the most dangerous of all the skin cancers. According to the American Cancer Society an estimated 88,000 people will be diagnosed with malignant melanoma in 2017, and a projected 10,000 of those people will unfortunately succumb to the illness. What makes melanoma especially difficult to treat is the recurring rate of resistance to the drugs that are used to treat it. Many of the drugs used to treat melanoma patients target the MEK/ERK pathway which is vital for the cancer's survival. The reason that this pathway is targeted is because the proteins that initiate it (BRAF/NRAS) are generally the ones that carry mutations, and together they account for about 60%-70% of all melanomas diagnosed. There have been drugs developed that target mutant BRAF, as well as MEK. However, within a short period of time all patients invariably acquire resistance to the drugs. My lab has looked into the resistance mechanisms of these melanoma cells. Using mass spectrometry a list of upregulated and downregulated proteins present in drug resistant melanoma cells compared to a control group of non-treated melanoma cells was compiled. We cultivated four cell lines in the lab, in order to verify the mass spectrometry data. First we grew A375 melanoma cells, and from there grew three cell lines that were resistant to BRAF inhibitors, MEK inhibitors, and ERK inhibitors. There are BRAF and MEK inhibitors currently used in the clinic, and there are ERK inhibitors that are currently in clinical trials. This proves that these cells can acquire resistance to these drugs. Western blots were then conducted on these cell lines to verify the mass spectrometry data. I hypothesized that the mechanisms of resistance would be different across cells because the drugs these cells were resistant to targeted different aspects of the pathway. Identifying key proteins that allow the cells to circumvent the drugs used to treat them would allow companies to manufacture a secondary line of drugs that can help relapsed patients with acquired resistance to the other tyrosine kinase inhibitors in the clinic.

Minh-Quan Pham
Virginia Commonwealth University
Mentor: Dr. Eduardo Davila

Toll-Like Receptor 2/1 Stimulation in pmel T Cells Increases Expression of Oxidative Stress Related Genes

T cell-based immunotherapies have shown great promise in treating advanced cancers. Although these immunotherapies have achieved success against advanced disease, a highly immunosuppressive tumor microenvironment (TME) limits the efficacy of T-cells in producing a durable response. Myeloid Derived Suppressor Cells (MDSC) contribute to this effect through various mechanisms, including production of reactive species. Previous data has shown that co-activation of TLR2/1 in activated T cells increases T-cell resistance towards MDSC mediated suppression. In this study, we hypothesized that TLR-MyD88-mediated production of oxidative stress response genes may decrease MDSC-mediated suppression. Using quantitative Polymerase Chain Reaction (qPCR) we have shown that co-activation of TLR2/1 with ligand Pam3csk4, increases mRNA transcript levels of Superoxide Dismutase 1/2(SOD1and 2), Thioredoxin1 (TXN1), Peroxyredoxin 1/2(PRDX1 and PRDX2) and Glutathione S-Transferase Pi 1 (GSTP1). Additionally we expect increases in protein expression of the antioxidant response element Nrf2 which may directly control expression of these genes.

Kayla Reagan
University of Maryland, College Park
Mentor: Drs. Kavita Bhalla and Ron Gartenhaus

ATM affects diffuse B-cell lymphoma (DLBCL) growth by regulating SIRT1 expression

Mutations in Ataxia-telangiectasia mutated (ATM) gene are found in 10-15% of DLBCL patients. Absence of activated ATM kinase and failure of DNA damage response results in predisposition to DLBCL. Our lab previously determined that HuR-associated mRNAs are regulated in an ATM-dependent manner (Mazan-Mamczarz et al., 2011). It was also suggested that HuR alters SIRT1 binding (Abdelmohsen et al, 2007). SIRT1 is associated with poor prognosis in DLBCL patients (Jang et at, 2008). We therefore investigated if ATM exerts its effect on DLBCL growth by regulating SIRT1. To determine the effect of ATM on SIRT1 expression, we inhibited ATM activity using ATM kinase inhibitor. We first compared RNA expression of SIRT1 in DLBCL cell line HLY to a normal B-cell line, GM01284. Expression of SIRT1 was induced in HLY cells by 2.5-fold compared to the normal lymphocytes. To determine changes in SIRT1 protein expression, protein lysates were obtained from GM20184, GM03332, and DLBCL cell lines HLY, SUDHL2, and SUDHL6. Absence of ATM significantly decreased SIRT1 protein expression by 48% in HLY, and 29% in SUDHL2. We further interrogated whether ATM affected SIRT1 localization by immunofluorescence using SIRT1 and Tom20 antibodies. DAPI was used for nuclear staining. Subcellular localization of SIRT1 was impacted by attenuation of ATM signaling. HLY cells with intact ATM signaling expressed SIRT1 in the nucleus and cytoplasm. SIRT1 was reduced in the nucleus of HLY cells when ATM signaling was inhibited. We determined the effect of ATM signaling on DLBCL growth by measuring apoptosis using Annexin V staining. Lack of active ATM led to significant apoptosis overall in GM cells and even greater late stage apoptosis, specifically, in HLY cells. Our findings suggest that loss of ATM activation reduces SIRT1 expression as well as its nuclear localization, which we hypothesize may contribute to lymphomagenesis. Further elucidation of regulatory nodes for the DNA damage response will facilitate the identification of novel therapeutic targets for DLBCL.

Alexandria T. Riedel
Washington College
Mentor: Dr. A-Lien Lu-Chang

The Role of Rad9 Phosphorylation on DNA Binding and DNA Repair

Cell cycle checkpoints prevent carcinogenesis and influence neoplastic cell survival due to their role in DNA damage response (DDR) pathways. When genomic DNA is damaged, DDR pathways are activated in order to repair the DNA and arrest cell cycle progression to allow time for such repair. When DNA damage is extreme, apoptosis is triggered. The heterotrimeric 9-1-1 protein complex comprised of Rad9, Rad1 and Hus1 is essential for DDR activation. 9-1-1 is a DNA damage response sensor that binds DNA and functions in base excision repair (BER) by interacting with nearly every enzyme involved in BER. Previous work from our lab and others suggested that 9-1-1 provides a platform to coordinate BER. Consistent with this model, we demonstrated that the Rad9 subunit binds DNA with the highest affinity of 9-1-1 complex proteins. Intact 9-1-1 expressed in a bacterial host, which lacks post-transcriptional modification capability, cannot bind DNA. However, expression of 9-1-1 in baculovirus–transduced insect cells, that permit post-transcriptional protein modification, was able to bind 5'-recessed DNA. The capacity of insect cell produced 9-1-1 to bind DNA corresponded with phosphorylation of 60% of the Rad9 protein, suggesting that phosphorylation is required for DNA binding. Based on these data, we hypothesize that the phosphorylation of the C-terminal polypeptide tail of subunit Rad9 causes a conformational change to enhance DNA binding. To test this hypothesis, we generated four Ser→Asp point mutants to mimic phosphorylation of the Rad9 C-terminal at residues that are known to be phosphorylated (S277D, S328D, S277D/S328D and S341D). Wild type or mutant Rad9 has been expressed in E. coli strains. The impact of phosphomimetic Rad9 mutations on DNA binding was measured by gel retardation assay. Our work has revealed a novel role of the phosphorylation of a specific Rad9 C-terminal residue in DNA binding and DNA repair. This finding provides a foundation for future studies to determine how Rad9 and the 9-1-1 complex are regulated in the context of DNA damage and oncogenesis.

Sam Savidge
Washington University in St. Louis
Mentor: Dr. Danilo Perrotti

The Role of SETBP1 in Ph^+ Acute Lymphoblastic Leukemia

Philadelphia-chromosome-positive (Ph+) B-cell Acute Lymphoblastic Leukemia (B-ALL) accounts for about one third of all adult ALL. While combined Tyrosine Kinase Inhibitor (TKI) therapy and chemotherapy has significantly improved outcome for Ph+ B-ALL patients, high relapse rates remain a major problem. Relapse of Ph+ B-ALL largely results from the inability of TKI's to eliminate Ph+ Leukemia Initiating Cells (LIC's). We recently reported that the inhibition of the tumor suppressor protein phosphatase 2A (PP2A) is essential for survival and self-renewal of Ph+ chronic myelogenous leukemia (CML) LIC's and for survival and proliferation of Ph+ CML and B-ALL CD34+ progenitors. PP2A is inactive in almost all solid and hematopoietic tumors, and its suppression correlates with poor outcome and disease progression. The inhibition of PP2A relies on aberrant expression of CIP2A, SET, and/or SETBP1. Preliminary data suggests that in Ph+ B-ALL, SETBP1 participates in the formation of an inhibitory multiprotein complex with CIP2A and SET; however, a cleaved (N-terminus) but not full length SETBP1 seems to be involved in the formation of this complex. Herein, we sought to investigate which part of SETBP1 contributes to PP2A inhibition. To do so, we generated a lentiviral construct containing the SETBP1 cDNA with a hemagglutinin (HA)-tag at the N-terminus and a 6x Histidine-tag at the C-terminus. Using this construct along with a Flag-tagged CIP2A and a Myc-tagged SET, we will be able to conduct a series of co-immunoprecipitation experiments to verify that only a cleaved SETBP1 is required for inhibition of the PP2A tumor suppressor.

Caroline Schlee
University of South Carolina
Mentor: Dr. Tami Kingsbury

Defining the SIX1 Interactome in Leukemia Cells

The PAX-SIX-EYA-DACH Network (PSEDN) plays an important role in the development of many types of tissues. Altered expression of PSEDN family members has been linked to the development of several types of solid cancers, as well as Hodgkin's lymphoma and mixed-lineage leukemia. A Drosophila second site non-complementation screen conducted by our collaborator recently identified PSEDN members as belonging to the GATA-FOG network. In humans, GATA-FOG function is critical for erythropoiesis. Our preliminary shRNA data suggests that SIX proteins are essential for growth of the CD34+ erythroleukemia TF1 cell line. Lentiviral mediated SIX1 overexpression stimulated erythropoiesis in the absence of exogenous erythropoietin, as measured by flow cytometry of erythroid cell surface markers and hemoglobin expression. To gain insight into SIX1 function, we are working to define the SIX1 interactome. To achieve this goal, we have conducted BioID mass spectrometry on TF1 cells expressing BirA-SIX1 fusion proteins. EYA3 and TLE3, two proteins expected to interact with SIX family members, were among the factors biotinylated in response by BirA-SIX1, suggesting the assay is working. In agreement with the fly screen, BirA-SIX1 GATA1 was also biotinylated by BirA-SIX1. Our list of candidate SIX1 interacting factors includes nucleolar proteins, transcription factors, DNA repair proteins and elongation factors. To validate the mass spectrometry defined SIX1 interactome, we are performing SIX1 co-immunoprecipitation assays, starting with EYA3, TLE3 and GATA1. We have also engineered recombinant SIX1 containing an amino-terminal Streptavidin Binding Peptide (SBP) tag, which will enable robust purification of SIX1 protein complexes in sufficient quantity for mass spectrometry identification of co-purified factors. For functional interrogation of SIX1 interacting factors, we are generating sgRNA constructs to perform targeted gene knockdown in our dCas9-KRAB TF1 cell lines. Identification of SIX1 interacting factors required for leukemia cell growth vs. SIX1-mediated erythropoiesis will provide insight into the mechanisms by which SIX1 regulates leukemia cell growth and differentiation.

Adam Schneider
Loyola University Maryland
Mentor: Dr. Alfredo Garzino-Demo

Quantifying the Effects of HIV Matrix Protein p17 on Inducing Angiogenesis and Lymphangiogenesis in AIDS-related Cancers

AIDS-related cancers account for a large portion of annual malignancies and deaths worldwide in HIV-1 infected individuals. Even with current HAART therapies available today, there are higher grades and incidence levels of AIDS-related cancers, particularly Kaposi sarcoma and Non-Hodgkin's-Lymphoma, and tumor severity and progression mechanisms have been shown to rely heavily on the formation of hypervasculature networks via induction of angiogenesis and lymphangiogenesis (A&LA), respectively. The HIV-1 matrix protein p17, a structural viral protein associated with the inner portion of the viral envelope that can be secreted from cells, induces angiogenesis at nanomolar concentrations by binding to extracellular receptors CXCR-1/2 located on endothelial cells, mimicking interleukin-8 (IL-8), the high affinity ligand of these receptors. Activation of the CXCR-1/2 signaling cascade plays a major role in the emergence of neovessels and angiogenic characteristics, such as new sprouts, fibers and increased vessel density regions. However, current data is insufficient to inform a dose-response model of the A&LA activity of p17. Therefore, in this study we aim to obtain quantitative data for angiogenic factors formed via p17-induced A&LA. To that end, we set up an *in-vitro* assay model designed for rapid quantification of A&LA. Using collagen-matrix filled microslide wells, human dermal microvascular endothelial (HMEC-1) cells were plated at 10,000 cells/well. Cell-filled wells were then treated with varying concentrations of p17 in endothelial growth media (EGM-2) – 5 ng/mL, 25 ng/mL and 125 ng/mL – just prior to 37°C incubation. At hour time intervals 2, 4 and 6, *in-vitro* assay microslides were imaged using confocal microscopy after prepped with a fluorescein complex, Calcein AM. Imaging data provides evidence for a significant statistical difference in dose-response fiber diameters, specifically between control groups and the 25 ng/mL p17 treatment group, as well as between 5 ng/mL and 25 ng/mL p17 treatment groups. Our preliminary results confirm the A&LA activity of p17, and further optimization of the assay system is needed to extend its dynamic range.

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Mentor: Dr. Gregory Szeto

Enhancing Cell-based Cancer Immunotherapies by Plasma Membrane-inserting TLR2 Ligands

Injected drugs and cell based therapies have shown efficacy preclinically and in combination. However, <50% of solid tumor patients respond to these therapies. Both have drawbacks: injected drugs become diluted in the body and nonspecific toxicity limits injecting higher concentrations; cell modification and expansion are time consuming for cell based therapies, and these cells may still suffer from tumor immunosuppression in the body. We added lipid-tailed drugs to the surface of immune cells, a process called depoting, to help combine drugs with cells to enhance drug delivery to the tumor site, as well as improve immune cell functions during immunosuppression. Toll-like receptor 2 (TLR2) is a receptor on the surface of therapeutic and host immune cells that can activate cells to help overcome immunosuppression. TLR2 ligands, Pam2CSK4 (Pam2) and Pam3CSK4 (Pam3), were depoted on immune cells to promote neighboring immune cell activation. Neighboring immune cell activation by the TLR2 pathway was measured by quantifying phosphorylation of p38 mitogen-activated protein kinase (MAPK). Splenocytes depoted with Pam2 showed a two-fold increase in activation over normalized nondepoted cell controls after 30 minutes. The functional outcome of membrane-bound ligands was studied using flow cytometry to measure B-cell activation markers – MHC II, CD69, and CD86. Depoted cells sustained activation of neighboring immune cells for greater than 3 days. T-cell proliferation was measured in the absence and presence of tumor cells to determine the ability of depoted cells to overcome immunosuppression. Depoted cells proliferated 3 more generations than cells surrounded by soluble drug. The ability of these cells to overcome suppression signals from tumor cells shows promise for enhancing therapeutic responses *in vivo*.

Najah Soudan
Spelman College
Mentor: Dr. Tonya J. Webb

Assessing Immune Function in Lymphoma

Lymphomas are a heterogeneous group of malignancies, primarily divided into Hodgkin's and non-Hodgkin's lymphomas (NHL), with B cell lymphomas representing the majority. Unlike other cancers, the incidence of NHL is steadily increasing. Natural Killer T (NKT) cells are innate-like lymphocytes that recognize glycolipid antigens in the context of CD1d- an MHC class I-like molecule. They have strong anti-tumor activity and respond by producing cytokines and mediating tumor cell lysis. However, NHL patients have a reduction in NKT cell number and function, suggesting that lymphomas may utilize mechanisms to specifically evade detection by NKT cells. In these studies, we utilized a mouse model of lymphoma to examine the impact of malignancy on the ability of B cells to stimulate NKT cell responses. We observed a significant increase in the numbers of B cells in the thymi, lymph nodes, and bone marrow of tumor-bearing mice. In addition, we found a profound reduction in the ability of splenocytes from tumor-bearing mice to activate NKT cells, indicating that malignant transformation may alter the ability of B cells to process and present antigen to NKT cells. Taken together, these data suggest that tumors are not restricted to the lymphoid organs in our mouse model of lymphoma. Our co-culture studies suggest that lymphomas develop mechanisms to evade detection by the NKT cells. We plan to extend our studies in order to identify the mechanisms by which cancerous B cells block NKT cell activation, and determine whether this mechanism of immune evasion is restricted to CD1d-mediated antigen presentation.

Zisui (Amy) Yan
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Mentor: Dr. Gerald Wilson

Biochemical relationships between Hsp70's RNA- and protein-binding functions

Hsp70 is a well-known protein chaperone that aids in protein folding and prevents aggregation. This involves repeated binding and release of protein cargoes coupled to an ATPase cycle. However, recent data also show that Hsp70 can bind and stabilize mRNAs by binding AU-rich elements (AREs) located within 3'-untranslated regions. In this project, we tested the hypothesis that RNA binding by Hsp70 might be coupled to its chaperone cycle, similar to the activities of RNA helicase enzymes. We produced and purified recombinant His-tagged proteins spanning the Hsp70 ATP-binding (1-385) and peptide-binding (386-613) domains. Binding between these proteins and fluorescein-labeled RNA, nucleotide, or peptide ligands was measured using fluorescence anisotropy-based assays. We found that: (i) Hsp70 binds RNA substrates via contacts with both the ATP- and peptide-binding domains, (ii) the Hsp70 ATP-binding domain bound adenosine-based nucleotides at low-micromolar affinities, (iii) RNA binding by the ATP-binding domain is independent of associated nucleotides, and (iv) RNA affinity by the Hsp70 peptide-binding domain is not affected by bound peptide ligands. Together, these data support a model whereby Hsp70 binding to RNA substrates is independent of its protein chaperone functions. The potential for Hsp70 to thus bind both RNA and peptide substrates simultaneously may explain some recently-discovered roles for this protein, including nascent peptide folding by tethering to polysomes and RNA sorting in stress granules.