

UMGCC/Nathan Schnaper Intern Program in Cancer Research 2014 Symposium Abstracts



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2014 Intern Presentation Schedule

Time	Intern/ Presentation Title	Mentor
8:30-8:50	Brandon Khor, RNase-L Suppresses c-fos in Mitogen Stimulated Mouse Embryonic Fibroblasts	Bret Hassel
8:55-9:15	Catherine Landis, Knockdown of Hsp70 expression and Cancer	Gerald Wilson
9:20-9:40	Angela DiNardo, Regulation of the Death Associated Protein Kinase 1 (DAPK1) expression in chronic lymphocytic leukemia	Dhan Kalvakolanu
9:45-10:05	Nick George, The Effect of a Combined Cisplatin and Sulforaphane Chemotherapy on Epidermal Squamous Cell Carcinoma Cell Survival	Rich Eckert
10:10-10:30	Courtney Culpepper, Generation of anti-CD19 CARs for NKT Cells	Tonya Webb
10:35-10:55	Alicia Greene, STAT5 Activation and ATM Expression in T-cell Lines	Arnob Banerjee
11:00-11:20	Rimsha Afzal, MYC and PARP1 Play a Role in Genetic Instability by Influencing Recruitment of Proteins Involved in Error-Prone Repair	Feyruz Rassool
11:25-11:45	Brendan King, Inhibition of PI3K pathway in obscurin-knockdown breast epithelial cells reduces mammosphere formation	Katia Kontrogianni
11:50-12:30	LUNCH BREAK	
12:30-12:50	Ashley Odai-Ofatey, The Role of RANK-L Inhibition in the Management of <i>BRCA1</i> Associated Tumorigenesis	Laundette Jones
12:55-1:15	Heather Kennedy, Re-expression of ubiquitin-conjugating enzyme E2E2 in human melanoma cells	Tom Hornyak
1:20-1:40	Haelee Pettingill, Enhancing Nanomedicines for Cancer Therapy	Anthony Kim Graeme Woodworth
1:45-2:05	Taylor Babin, Induction of Egr2 in human macrophages by IL-4	Achsah Keegan
2:10-2:30	Nathan Robinson, Targeting Novel Signaling Pathways to Treat Melanoma	Paul Shapiro
2:35-2:55	Dahlia Kronfli, The Anti-Cancer Effect of Second Generation Biquinones in Acute Myelogenous Leukemia	Rena Lapidus
3:00-3:20	Christina Worgo, The identification of novel therapeutics that downregulate PD-L1 and increase the expression of MHC class I on melanoma cells	Eduardo Davila
3:25-3:45	Asra Khan, Evaluation of the Impact of candidate Immunosensitizing Therapeutics on Melanoma Phenotypes and T cell Proliferation	Eduardo Davila
3:50-4:10	Tessa Seale, Examining the effect of candidate Leukemia suppressive microRNAs on cell growth	Wen Chih Cheng

Brandon Khor, St. Olaf University
Mentors: Dr. Bret Hassel and Dr. Sarah Laun

RNase-L Suppresses c-fos in Mitogen Stimulated Mouse Embryonic Fibroblasts

Mitogens induce genes that promote cell proliferation necessary for physiologic functions such as wound healing. Following tissue repair, mitogenic response must be attenuated to prevent uncontrolled proliferation, a hallmark of cancer. Identifying the mechanisms that constrain cell proliferation may reveal new ways to inhibit malignant growth. c-fos, an immediate-early gene induced within minutes of mitogen exposure, encodes a proto-oncogene that stimulates cell cycle progression. Inhibition of protein synthesis (e.g. cycloheximide, CHX) prior to mitogen exposure dramatically increases the induction of immediate-early transcripts. This effect, known as superinduction, is thought to involve the CHX-mediated elimination of an unstable inhibitor of immediate-early gene transcription. A mitogen-induced transcription inhibitor represents a potential antiproliferative cancer therapy target, but the molecular basis of superinduction has remained elusive. RNase-L is an endoribonuclease that mediates diverse antiproliferative activities. Mitogen treatment of RNase-L-deficient cells resulted in an enhanced induction of c-fos, mimicking superinduction. Therefore, we hypothesized that CHX targets RNase-L which inhibits a transcription factor linked to c-fos induction. To test this hypothesis, wild type and RNase-L-knockout (KO) mouse embryonic fibroblasts (MEFs) were mitogen stimulated with or without CHX, and c-fos mRNA was measured. Contrasting our prediction that CHX superinduction operates in an RNase-L dependent manner, c-fos was induced to even higher levels following CHX/mitogen treatment of RNase-L KO MEFs. This data indicates that RNase-L is not the CHX-responsive target in c-fos superinduction; rather, it suggests that CHX and RNase-L deficiency increase c-fos induction via distinct mechanisms. Thus, CHX/mitogen stimulation in the absence of RNase-L results in an excessive superinduction resulting from both conditions. Results from this work identify RNase-L as an important inhibitor of mitogen-induced gene expression that may inhibit tumor proliferation. Current studies focus on determining how CHX augments RNase-L-mediated inhibition of c-fos induction.

Catherine Landis, Towson University
Mentor: Dr. Gerald Wilson

Knockdown of Hsp70 expression and Cancer

Hsp70 is a stress inducible protein that is constitutively upregulated in many cancer cell lines. It is associated with poorer prognosis and chemoresistance. Hsp70 is thought to interact with the apoptotic machinery to prevent senescence and death. The mechanisms behind chemoresistance are still unclear. Hsp70 has been identified to bind with high affinity and specificity to U-rich mRNA. It associates with and stabilizes specific mRNAs in cells including some pro-tumorigenic factors. The RNA binding function appears to be independent of its protein chaperone function. It is suspected that constitutive activation of the mRNA stabilizing function may contribute to enhanced production of selected pro-tumorigenic factors. We have been attempting to insert a U6promoter-shHsp70 fragment into the pLL3.7 vector backbone in order to form a lenti-virus to infect appropriate cancer cell lines and knock down Hsp70. Using molecular cloning techniques we have attained a single positive ligation which has been sent for sequencing to confirm the correct shHsp70 sequence is present. Through western blots we will identify cancer cell lines with up-regulated Hsp70 levels and use these as our models. HI-60 cells will be used as a negative control and HeLa cells will be used as a positive control to identify candidate cell lines for Hsp70 knockdown studies. We are interested in the effect of the Hsp70 knock-down on gene regulation and chemoresistance. Our hypothesis is that by reducing Hsp70 protein levels chemoresistance will be diminished, and the potency of chemotherapeutic agents restored.

Angela DiNardo, Towson University

Mentors: Dr. Padmaja Gade and Dr. Dhan Kalvakolanu

Regulation of the Death Associated Protein Kinase 1 (DAPK1) expression in chronic lymphocytic leukemia

Interferons play a critical role in the inhibition of tumor growth by initiating downstream signaling pathways that promote innate and specific immunity. The Death-associated protein kinase 1 (DAPK1) is an anti-metastatic protein that controls cell cycle, apoptosis, and macroautophagy. Previously, our lab has identified the transcription factor CAAT/Enhancer binding protein (C/EBP- β) as a key regulator of the IFN- γ induced expression of DAPK1. The ER stress-induced Activating transcription factor 6 (ATF6) interacts with C/EBP- β to promote the expression of DAPK1. Several studies have confirmed a loss of DAPK1 expression in tumor cells, including those in chronic lymphoid leukemia (CLL). CLL is characterized by slow progression. Although one previous study suggested that loss of *DAPK1* expression in CLL cells is probably due to a mutation in the upstream enhancer, this appears not to be a universal mechanism. Therefore, understanding the mechanisms that control DAPK1 expression may aid in the development of targeted therapeutics that prevent CLL growth. Recently, our lab has identified Zipper-interacting protein kinase (ZIPK/DAPK3) as a putative interacting partner of ATF6 which promotes *Dapk1* expression in response to IFN- γ . We hypothesize that ZIPK plays a critical role in the activation of DAPK1-dependent autophagy through promoter-binding. To test this, we depleted ZIPK in the cells to study its impact on DAPK1-dependent autophagy. IFN- γ failed to induce autophagy in the ZIPK depleted cells when compared to the controls. In agreement with these observations, primary CLL exhibited dysfunctions in the IFN-activated collaboration between C/EBP- β , ATF6 and ZIPK. Consequently, DAPK1 expression was inhibited and resulted in a loss of growth control in CLL. Together, these studies identified novel regulatory mechanisms that control DAPK1 and tumor suppression.

Nick George, Loyola University

Mentors: Dr. Richard Eckert and Dr. Candace Kerr

The Effect of a Combined Cisplatin and Sulforaphane Chemotherapy on Epidermal Squamous Cell Carcinoma Cell Survival

One in five Americans will develop skin cancer during their lifetime. Epidermal squamous cell carcinoma (ESCC) is the second most common skin cancer in the United States and the incidence of this disease is on the rise with a 200% increase over the past three decades. Fully 10% of these patients develop recurrent and metastatic disease which requires systemic therapy. The most common systemic therapy utilizes cisplatin, a platinum-based chemical that binds DNA and induces apoptosis. Although cisplatin is a best-practice treatment it does not efficiently control recurrent skin cancer. It may be that this is because cisplatin does not target epidermal cancer stem cells. Epidermal cancer stem (ECS) cells are a small subset of tumor cells with enhanced self-renewal, migratory and tumorigenic capabilities. We have shown that ECS cells are able to produce large and aggressive tumors as compared to non-stem epidermal cancer cells. Our preliminary findings suggest that isothiocyanates, including sulforaphane (SFN), which are derived from cruciferous vegetables can target ECS cells. SFN has been shown to enhance the effects of cisplatin in other cancers. Therefore, we hypothesize that combined treatment with cisplatin and SFN will be more effective in treating SCC than either treatment alone. To analyze this we performed dose-dependent proliferation assays using SCC-13 cells in the presence of these agents to assess the impact of each agent and combined treatment on SCC-13 cell proliferation and survival. In all trials the combined treatment was 50% more efficient at suppressing cell growth as compared to the individual treatment. We also analyzed the invasive ability of SCC-13 cells and found that SFN treatment and possibly a combined treatment, reduces cell invasion. Results from this study suggest that a cisplatin/SFN combination therapy may provide a novel and effective treatment for ESCC.

Courtney Culpepper, Morgan State University
Mentors: Priyanka Subrahmanyam and Dr. Tonya Webb

Generation of anti-CD19 CARs for NKT Cells

Hematological malignancies, like lymphomas, remain incurable because of the high risk of relapse. Most lymphomas are B cell in origin, and are primarily divided into Hodgkin's and non-Hodgkin's lymphomas (NHL). NHL is the second fastest rising cancer in incidence and death rates in the United States, therefore the development of novel treatment strategies is essential. It has been well established that the immune system possesses strong tumor surveillance mechanisms and anti-tumor responses. To enhance anti-tumor immune responses, adoptive T cell therapy (ACT) using T cells modified to express chimeric antigen receptors (CARs) have been developed and used to targeted B cell malignancies. CARs are fusion proteins consisting of antigen recognition moieties and T cell activation domains. Since most B cells express CD19, adoptive transfer of anti-CD19 CAR T cells is now being tested in clinical trials. Treatments utilizing engineered T cells have the potential to offer long-term protection, through memory, but it is unclear which T cell subset should be used. Our lab focuses on modulating natural killer T (NKT) cells for cancer immunotherapy. NKT cell-based therapy offers the possibility of inducing an initial cytotoxic tumor response, and also activating NK cells and the adaptive immune system to produce tumor-directed cytotoxic T cells (CTL) with long-lived memory. Thus, NKT cells can mediate direct anti-tumor effects and also activate other cell types. We hypothesize that transducing NKT cells with an anti-CD19 CAR will enhance their cytotoxic functions and will be more efficient than transducing bulk T cells. In order to test this hypothesis we have prepared an anti-CD19 CAR by performing transformation studies, restriction digests, sequencing analyses and generating retrovirus. We will test our construct by transducing both T cells and NKT cells isolated from peripheral blood mononuclear cells and setting up co-cultures and cytotoxicity assays with mantle cell lymphoma cell lines. The information gained in these studies will serve to help understand and design NKT cell based immunotherapeutic approaches for the treatment of cancer.

Alicia Greene, Stevenson University
Mentor: Dr. Arnob Banerjee

STAT5 Activation and ATM Expression in T-cell Lines

Peripheral T-cell lymphoma (PTCL) is a heterogeneous non-Hodgkin lymphoma that develops from mature T-lymphocytes. Current treatments have mostly poor outcomes with an overall survival (OS) of <40%, therefore novel therapies are needed. To identify somatic mutations in PTCL that may yield potential therapeutic targets, we conducted whole-exome sequencing of 12 PTCL patient samples including 8 different PTCL subtypes. Mutations were identified in *ATM* in 4 of the 12 samples and in components of the common gamma chain (γ c) signaling pathway (*JAK3*, *IL2RG*, *STAT5B*) in 3 of those 4 samples. Two of these mutations, identified in *JAK3* and *STAT5B*, have been previously recognized as activating mutations. The combination of activating γ c signaling mutations in patient samples with the *ATM* mutation suggests a cooperative role in the development of PTCL. In order to identify a model T-cell line for *in-vitro* testing of a novel combination therapy to inhibit *STAT5B* activation and target *ATM* deficient cells, we performed Western blotting for *STAT5* activation and *ATM* expression on a panel of human T-cell lines. We hypothesize that HuT 102 cells have constitutive *STAT5* signaling and *ATM* deficiency, and are therefore a suitable model. Our results show that there is constitutive *STAT5* activation in HuT 102 and HuT 78, mature Sezary syndrome lines, but no apparent *STAT5* activation in Jurkat, a T-cell leukemia line. All three cell lines, HuT 102, HuT 78, and Jurkat, expressed *STAT5*, which was equivalent for the stimulated and unstimulated cells of each cell line. Preliminary results for *ATM* expression showed that there was slightly less *ATM* in HuT 102 cells compared to the HuT 78 cells. From our results we conclude that HuT 102 cells have the constitutive *STAT5* activation and decreased *ATM* that is necessary for future *in-vitro* testing of a novel combination therapy for the treatment of PTCL.

Rimsha Afzal, Meredith College
Mentor: Dr. Feyruz Rassool

MYC and PARP1 Play a Role in Genetic Instability by Influencing Recruitment of Proteins Involved in Error-Prone Repair

Triple Negative Breast Cancers (TNBC) are one of the most aggressive cancer sub-types, where tumor progression is rapid and there are few available therapies. Acquisition of genetic alterations that result from errors generated through repair of DNA damage, particularly double strand breaks (DSBs), is a potential cause of tumorigenesis. Understanding how these repair errors occur can help identify therapies to halt or reverse genetic instability. DSBs are predominantly repaired through two pathways: Homologous Recombination (HR) and classical Non-Homologous End-Joining (C-NHEJ). The Rassool Lab recently reported that compared to non-tumorigenic cells, TNBCs upregulate an alternative and highly error-prone form of DSB repair, (*aka* ALT-NHEJ), by increasing the expression and activity of known ALT-NHEJ components, poly-ADP ribose polymerase (PARP1) and DNA ligase III (LIG3). Recent studies in the lab suggest that the oncogenic transcription factor MYC promotes ALT-NHEJ repair via transcriptional regulation of PARP1 and LIG3. However, little is known about the role of MYC or ALT NHEJ factors, such as PARP1 in recruitment of DSB repair proteins to the site of DSB damage in TNBCs that may influence error-prone repair. My project was to determine in TNBCs (SUM149Pt), the extent to which MYC or PARP1 influences recruitment of DSB repair proteins, to sites of DNA damage. Thus, we examined levels DSB repair proteins in chromatin extracts by Western blotting analysis following X-ray (IR) (2Gy) –induced DSBs and in these same cells in which MYC- or PARP1 was inhibited. In cells exposed to IR, there is a rapid increase in the expression of PARP-1 and LIG3, as well as C-NHEJ protein: Ku80. However, MYC and PARP inhibition results in a delayed recruitment of these proteins to DNA-damaged chromatin. This suggests that inhibition of MYC or PARP1 may delay or reduce error-prone repair and thus genomic instability in TNBCs and other MYC-driven tumors.

Brendan King, University of Virginia
Mentor: Dr. Katia Kontrogianni

Inhibition of PI3K pathway in obscurin-knockdown breast epithelial cells reduces mammosphere formation

Obscurins (~70-870 kDa) are giant, multidomain, cytoskeletal proteins encoded by the *OBSCN* gene located on chromosome 1q42. Early sequencing analysis of 13,023 genes in breast and colorectal cancers identified mutations within *OBSCN*, implicating its involvement in tumor formation and progression. Previous studies have shown that normal MCF10A breast epithelial cells treated with shRNA targeting giant obscurins display increased survival and proliferation and decreased apoptosis following exposure to the cytotoxic drug, etoposide. Furthermore, obscurin-knockdown MCF10A cells formed spherical mammospheres greater than 100 μm in anchorage independent conditions, while control cells treated with scramble shRNA failed to do so. Finally, loss of giant obscurins from MCF10A cells resulted in an increase in the protein expression of N-cadherin, a mesenchymal protein upregulated in cancer cells. Herein, we demonstrate that obscurin-knockdown MCF10A cells treated with the PI3K/AKT/mTOR pathway-inhibitor, BKM120, exhibit reduced mammosphere formation in anchorage independent conditions and decreased N-cadherin expression compared to control MCF10A cells. These findings suggest that loss of giant obscurins may promote growth in anchorage independent conditions by upregulating the PI3K pathway.

Ashley Odai-Afotey, Cornell University
Mentors: Dr. Laundette Jones and Nnamdi Edoboki

The Role of RANK-L Inhibition in the Management of *BRCA1* Associated Tumorigenesis

Females with a mutated Breast cancer gene-1 (*BRCA1*) are more prone to develop breast cancer in their lifetime. Tumors characteristic of this mutation often lack receptors that are targeted by current hormonal therapy, including the estrogen receptor (ER). Thus, the creation of new therapies that target proteins involved in the development of *BRCA1*-associated breast cancers is of clinical significance. Using a preclinical mouse model of human *BRCA1*-associated breast cancer, prior studies in the lab showed that the development of ER/PR negative preneoplastic lesions was inhibited in mice treated with a RANK-L inhibitor (Rank Fc). The objective of the current study was to determine whether there were differences in mammary epithelial cell expression of ER, PR, and cyclin D1, a key molecule in the proliferation pathway, in Rank Fc- compared to placebo-treated *Brca1* mutant mice. Western blot analysis and immunohistochemistry (IHC) were used to examine protein expression in mammary gland samples from mice treated with Rank Fc or Placebo, 3x/week beginning at 3 mo. and mice euthanized at 9 mo. or 15 mo. of age. Preliminary IHC data shows that PR expression is increased in RANK-Fc treated mice compared to control mice. Studies are underway to measure ER and Cyclin D1 protein levels and we predict that compared to control mice, Rank-Fc treated mice will show decreased cyclin D1 expression and increased ER expression. We anticipate that this study will provide further insight into a cellular pathway that may be exploited to protect against *BRCA1*- associated breast cancers.

Heather Kennedy, CCBC
Mentor: Dr. Tom Hornyak

Re-expression of ubiquitin-conjugating enzyme E2E2 in human melanoma cells

Cancer is currently the second leading cause of death in the world. Melanoma, a cancer of the skin, is among the many skin cancers leading to this number 2 ranking. Annually 13% of all melanoma diagnoses lead to death; but, with each new medical discovery, it is starting to fall in the ranks. One of the tumorigenic properties of melanoma cells is their uncontrolled cellular proliferation, in contrast to the cellular senescence state of benign melanocytic nevi which can be precursor lesions to melanoma. Many proteins have been found to have an altered expression level in melanoma cells compared to benign nevi. In metastatic melanoma, specifically the UCD-Mel-N human melanoma cell line, there are some proteins that are expressed at higher levels and others with no expression in comparison to normal nevi. This altered expression has been seen to have an effect on the melanoma cell's proliferation abilities. One of the proteins that is overexpressed is a homologue of *Drosophila* Enhancer of zeste, EZH2. EZH2 has been shown to 'turn off' or repress the expression of specific genes when expressed at high levels. Our lab found that Ubiquitin-conjugating enzyme E2E2 is one of these proteins; it assists in the ubiquitination of specific proteins to mark them for degradation. EZH2 expression is highly correlated with unchecked cell proliferation. We performed an experiment that studied the correlation between UBE2E2 and cellular proliferation by analyzing expression of the cell proliferation marker Ki67 which is present in all cell cycle phases except for G₀. It was hypothesized that the re-expression of the UBE2E2 protein would cause the melanoma cells to go into a non-proliferation state. UBE2E2 was ligated to the pmCherry-N1 vector and re-expressed, via transfection, into a human melanoma cell line. Re-expression was confirmed with the use of Western blot and fluorescence. When the pUBE2E2-mCherry protein was expressed, fewer cells expressed the Ki 67 marker than when the mCherry protein was expressed. It was concluded that further experiments needed to be performed in order to confirm the re-expression of UBE2E2 was responsible for the cells entering quiescence or senescence when EZH2 was depleted.

Haelee Pettingill, St. Mary's College of Maryland
Mentors: Drs. Graeme Woodworth and Anthony Kim

Enhancing Nanomedicines for Cancer Therapy

Drug-containing nanoparticles (nanomedicines) are a hot topic of cancer research. This heightened interest is due to the fact that nanoparticles can be engineered to target specific tissues and release drugs over a desired time frame, potentially reducing the amount of damaging side effects. Interestingly, it has been observed that conventional non-coated particles tend to stick non-specifically to various body tissues, in particular tumor tissue. This may be due to their relatively hydrophobic and electrostatic surface properties. As a result, relatively hydrophilic, neutral charge surface coatings (e.g., polyethylene glycol (PEG)) have been used to make particles less adhesive, enhancing their ability to diffuse within and penetrate tissues. However, the optimum particle size and surface PEG density for nanoparticles to diffuse in tumor tissue has not been studied. Using confocal microscopy imaging we tracked the movement of polystyrene (PS) polymeric nanoparticles with varying sizes and surface PEG densities in Matrigel (a gel that mimics the extracellular matrix of tumor tissues). We calculated mean-squared displacement (MSD) over time using a multiple particle tracking software to compare the diffusion of the particles. Here we show 100nm, 40nm and 20nm uncoated particles do not diffuse in Matrigel. The 40nm and 20nm coated particles move freely, however the 100nm PEG coated particles are immobile in the gel. Next, we show that our particles with 0.25x and 5x PEG density diffused slower than the 0.5x, 1x, and 2x PEG particles. This suggests that the size and amount of PEG surface coating is very important in the ability of nanoparticles to move. It is likely that larger sized particles cannot fit through the pores in Matrigel. Additionally, particles with insufficient or too much PEG do not diffuse, possibly due to hydrophobic interactions or increased size. Further research must be done in order to determine if particles with a PEG density of 0.25x-0.5x diffuse similarly to the other PEG particles. This study can be useful in improving current FDA approved nanomedicines such as liposomal doxorubicin, similar to FDA approved Doxil, or when making nanoparticles in the future.

Taylor Babin, Sacred Heart University
Mentor: Dr. Achsah Keegan

Induction of Egr2 in human macrophages by IL-4

In the past, macrophages were regarded as immune cells that were capable of destroying tumor cells. However, recent literature has found that macrophages have the ability to promote tumor development due to secretion of mediators, such as growth and angiogenic factors. In fact, in most tumors, macrophages possess the M2-phenotype, which has been observed to aid in tumor growth and development. This change in macrophage phenotype has been attributed to the cytokines IL-4 and IL-13, which actively affect the development of macrophages into what is called "alternatively activated macrophages" (AAMs also called M2). In previous experiments, IL-4 was shown to induce AAM differentiation in mouse macrophages via the induction of the transcription factor Early Growth Response gene-(Egr)2. The purpose of this experiment was to analyze the kinetics of Egr2 induction by IL-4 and IL-13 in THP-1 human monocytes. Western blots were used to analyze how human monocytes, when stimulated by IL-4 and IL-13, alter expression of Egr2. After utilizing densitometry, results indicate that IL-4 stimulates maximal expression of Egr2 at six- and eight-hours. After longer times of treatment, the Egr2 levels declined. Conversely, IL-13 was much less effective at inducing Egr2 than IL-4 at all time points. Further experiments with a more effective anti-Egr2 antibody are required to elucidate the role IL-13 has on the expression of Egr2.

Nathan Robinson, Stevenson University
Mentor: Dr. Paul Shapiro

Targeting Novel Signaling Pathways to Treat Melanoma

Melanoma is a type of skin cancer that will affect 76,100 Americans and kill 9,710 in the year 2014 alone (source-American Cancer Society). While it is more difficult to treat in its later stages when it has metastasized, some targeted therapeutics can be used to treat it. However, some targets can be turned back on, leading to drug resistance, so other pathways and proteins are being targeted by newer drugs. Most melanoma cells have a BRAf protein mutation which is a promising drug target, because BRAf activates MEK1/2 and ERK1/2 which leads to cell survival and proliferation. Cells tend to develop resistance to available drugs. Sf-3-O3O has been developed by the Shapiro lab and may potentially overcome resistance. To better understand the mechanism of action in sf-3-O3O, mRNA sequencing was done to compare sf-3-O3O with a MEK inhibitor (AZD6244) in melanoma cells. It was addressed whether sf-3-O3O would behave like AZD6244 indicating that it had a similar mechanism of action through analysis of mRNA sequencing data including the use of a heat map and other comparisons. Although there were similar transcripts regulated, the data showed sf-3-O3O affected mostly different genes than AZD6244 and those affected largely were up regulated. The data revealed 112 and 60 transcripts were regulated in cells with AZD6244 and sf-3-O3O treatment, respectively. A few of the transcripts down regulated correspondingly with AZD6244 and sf-3-O3O, including c-Myc (an oncogene). The potential tumor suppressor transcript, EGR-1, was down regulated with AZD6244 and up regulated with sf-3-O3O. These data suggest that although sf-3-O3O has features like AZD6244, it also works through different mechanisms. In future experiments it will be determined if mRNA in the sf-3-O3O compound correlates directly to the protein level, the effects of the drug on other pathways, and if sf-3-O3O has clinically relevant effects in vivo.

Dahlia Kronfli, University of Maryland
Mentor: Dr. Rena Lapidus

The Anti-Cancer Effect of Second Generation Biquinones in Acute Myelogenous Leukemia

Acute myeloid leukemia (AML), a blood cancer that targets white blood cells, is the most common type of acute leukemia found in adults. This study assessed the anti-cancer properties of the second generation of a novel dimeric naphthoquinones (BiQ) in AML. BiQs are unique molecules that have been shown to induce cytotoxicity in human prostate cancer cells by inducing reactive oxygen species¹ (ROS). ROS are formed as a byproduct of the normal metabolism of oxygen and play significant roles in cell signaling and homeostasis in the citric acid cycle. Through voltammetry studies measuring ROS, it has been shown that BiQs exert their cytotoxic effects through oxidative stress and mitochondrial dysfunction². In this study, we tested two second generation BiQ compounds known as 217 and 218 in two AML cell lines, MOLM-14 and THP-1. We hypothesize that treating these AML cells with BiQs will produce a large amount of ROS and DNA damage, thus inducing apoptosis. The MOLM-14 and THP-1 cells were treated with a dose response of 217 and 218 for 72 hours to generate IC50 values. Both compounds generated consistent IC50 values either less than 1 uM or in the single digit uM range. 218, the more potent of the two, was selected for further studies. The effect of 218 was further evaluated in MOLM-14 and THP-1 cells using viability, clonogenic, and apoptotic assays to determine survival, proliferation, and ROS generation of cancer cells.

¹ Ross et al, *BJU International*, 108, 447-454. 2010.

² Emadi et al, *Bioorg Med Chem*, 19(23), 7057-7062. 2011.

Christina Worgo, Stevenson University
Mentors: Alexander Tsai and Dr. Eduardo Davila

The identification of novel therapeutics that downregulate PD-L1 and increase the expression of MHC class I on melanoma cells

While traditional chemotherapies target tumor cells nonspecifically, recent advances in melanoma therapy focus on blockade of specific immune checkpoints. Despite significant success, one remaining obstacle to this type of treatment is low response rates. These low response rates are due in part to the ability of tumor cells to co-opt several immune checkpoint pathways while also driving other immunosuppressive processes. Collectively, these mechanisms limit T cell function. Several studies have targeted immunosuppressive mechanisms in order to stimulate anti-tumor T cell activity. In particular, a significant focus has been placed on an emerging immunosuppressive ligand called programmed death-ligand 1 (PD-L1). The expression of PD-L1 on tumor cells allows tumors to evade normal tumor surveillance functions by binding to programmed death-1 (PD-1) on T cells. Recent studies have shown that the PD-1/PD-L1 pathway plays a key role in the negative regulation of T cells in the context of cancer. Further, it has become clear that PD-L1 downregulation has the potential to augment anti-tumor T cell function. Yet, PD-1/PD-L1 signaling is only one of numerous immunosuppressive players in the tumor microenvironment. Thus, in this study over 800 FDA-approved drugs and natural compounds were screened on a human melanoma cell line in order to identify drugs with the potential to downregulate PD-L1, PDL2, CD155 while upregulating MHC class I. We identified several novel compounds which favorably altered immune molecule expression on melanoma. In particular, glucocorticoids consistently decreased PD-L1 expression. In addition, we discovered several compounds which undesirably upregulated PD-L1 and CD155. Future studies will focus on further characterizing the effects of candidate therapeutics on several melanoma cell lines using an expanded panel of cell surface molecules.

Asra Khan, York College of Pennsylvania
Mentor: Dr. Eduardo Davila

Evaluation of the Impact of candidate Immunosensitizing Therapeutics on Melanoma Phenotypes and T cell Proliferation

Cancer immunotherapies focus on using immune responses to aid in the identification and elimination of tumors. Several clinical studies have shown that the immune system can be harnessed to target cancer, particularly in melanoma. Melanoma is the deadliest primary skin malignancy, and despite recent advancements in targeted therapies and immunotherapies, the prognosis for patients with advanced melanoma remains poor. Recent studies have suggested that constitutively active oncogenic signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, reduce melanoma immunogenicity and drive immunosuppressive mechanisms which limit anti-tumor T cell responses. To counter these tumor-dependent immunosuppressive mechanisms while increasing melanoma immunogenicity, we have identified several novel candidate therapeutics which decrease the expression of programmed death-ligand 1 (PD-L1) and CD155, while increasing the expression of MHC class I. To further characterize these therapeutics, we have examined their impact on the proliferative capacity of human peripheral blood mononuclear cells (PBMC), while also studying their ability to alter the phenotypes of several human melanoma cell lines. The effects of these therapeutics on PBMCs will be crucial to the development of effective immunotherapies which favorably alter melanoma phenotypes without significantly altering T cell proliferation and function. Although many of the candidate drugs consistently altered the expression of the immunosuppressive molecules on melanoma cell lines, some were also toxic to PBMCs. Select compounds, including, MEK, VEGFR, PI3K, RAF, and p38 MAPK inhibitors were effective in immunosensitizing melanoma cell lines without significantly impacting PBMC proliferation, and thus, warrant further investigation.

Tessa Seale, UMBC
Mentor: Dr. Wen Chih Cheng

Examining the effect of candidate Leukemia suppressive microRNAs on cell growth

MicroRNAs (miRs) are short noncoding RNAs that bind to mRNAs, inhibiting their expression. Deregulation of miRs can lead to cancer. This project focuses on leukemia-suppressive miRs associated with Acute Lymphoblastic Leukemia (ALL), the most common cancer in children. Our goal is to utilize leukemia-suppressive miRs to identify molecules/pathways important for ALL growth. To identify candidate leukemia-suppressive miRs, we combined a miR- high throughput functional screen with available miR expression data. We focused on miR candidates that had growth-inhibitory activity and reduced expression in ALL samples.

This project investigated two such candidates, miR-151a and miR-190b. We first overexpress these miRs in leukemia cells to validate that they decrease cell growth. MiR-151a was overexpressed in a T-cell ALL (T-ALL) cell line through infection with a lentivirus. Then three growth assays were applied to analyze leukemia cell growth. Data from viable cell count, alamarBlue, and GFP competition assays indicated that cells with miR-151a overexpression had less cell growth than did cells infected with an empty vector, validating that miR-151a has a leukemia-suppressive effect on T-ALL cells. MiR-190b was already validated to inhibit the growth of a B-cell ALL (B-ALL) cell line, thus, we set out to determine if miR-190b inhibits cell growth through apoptosis. We infected B-ALL cells with a lentivirus that overexpresses miR-190b and examined apoptosis using Annexin V/7AAD staining. The results showed that cells overexpressing miR-190b had ~3-fold more apoptotic cells than cells infected with an empty vector. Therefore, apoptosis induction is one of the mechanisms that miR-190b inhibits leukemia growth. Future directions for this project include (1) identifying mRNA targets and pathways regulated by miR-190b and (2) increasing the dosage for miR-151a to potentially further repress leukemia cell growth. Ultimately, we aim to identify miR-regulated targets and pathways important for leukemia growth, and potentially develop therapies based on those molecules/pathways.