

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



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2015 Intern Presentation Schedule

Time	Intern/ Presentation Title	Mentor
9:00-9:20	Leah Henry, A Potential Role for RNase-L in the Epithelial-to-Mesenchymal Transition	Bret Hassel
9:25-9:45	Stephanie Cerritos, TTP decreases Tumorigenicity and alters Metabolic Requirements of MDA-MB-231 Triple Negative Breast Cancer Cells	Gerald Wilson
9:50-10:10	Dahlia Kronfli, The Anti-Cancer Effect of Biquinones in Acute Myelogenous Leukemia	Rena Lapidus
10:15-10:35	Luisa Silva, Identification and testing of novel transcription factor inhibitors for breast cancer therapy that interact with the RUNX2 protein and alter DNA binding	Tony Passaniti
10:40-11:00	Sydney Anchor, Mechanisms of Resistance to ERK1/2 Inhibitors in Melanoma	Paul Shapiro
11:05-11:25	Jacqueline Bement, Development of a Multicolor Reporter System to Evaluate the Efficacy of Combination Therapies	Nevil Singh
11:30-11:50	David Myers, Interleukin-1 Receptor-Associated Kinase M Overexpression Plays a Puzzling Role in Melanoma Cell Apoptosis	Eduardo Davila
11:55-12:15	Alicia Greene, Assessing the role of the common gamma chain K315E mutation in peripheral T-cell lymphoma.	Arnob Banerjee
12:20-1:25	LUNCH BREAK	
1:30-1:50	Melanie Berger, Combined Cisplatin and Sulphoraphane Treatment as a Therapy for Epidermal Squamous Cell Carcinoma	Rich Eckert
1:55-2:15	Caroline Schlee, Establishment of Cell lines for the Study of Cancer Immortality	Michal Zalzman
2:20-2:40	Tessa Seale, Elucidating and verifying the interaction between obscurin and PI3K pathway in breast epithelial cells	Katia Kontrogianni
2:45-3:05	Tyler Rutherford, Poly (ADP-Ribose) Polymerase trapping studies in acute myeloid leukemia using DNA methyl transferase inhibitors and PARP inhibitors	Feyruz Rassool
3:10-3:30	Yuyi Zhu, Role of miR-300 in the Regulation of Leukemia Cell Proliferation and Survival	Drs. Perotti, Trotta and Silvestri
3:35-3:55	Shannon Kirby, The Impact of Mutated Ribosomal Proteins on Translation Fidelity	Tami Kingsbury
4:00-4:20	Adip Bhargav, Optimization of Nanoparticle Formulations for Systemic Circulation and Targeting	Drs. Kim, Woodworth and Winkles

**Leah Henry, The Catholic University of America
Drs. Heather Ezelle and Bret Hassel**

A Potential Role for RNase-L in the Epithelial-to-Mesenchymal Transition

The epithelial to mesenchymal transition (EMT) is an important step in carcinogenesis and serves physiologic functions in embryogenesis and tissue repair. Discovering the mechanisms of EMT could lead to therapies that induce EMT to promote tissue repair or inhibit EMT for cancer treatment. EMT results in a dedifferentiated cell with unlimited proliferative and migratory capacity due to alterations in the cytoskeleton and extracellular matrix. RNase-L is an endoribonuclease that functions as a constraint on proliferation to promote cellular differentiation through the cleavage of target mRNAs. Furthermore, RNase-L contributes to cellular barrier function as a component of the actin cytoskeleton. RNase-L thus mediates activities that are opposite to those that occur during EMT; therefore, we hypothesized that RNase-L may inhibit EMT as a mechanism of its tumor suppressor activity. Consistent with this idea, embryonic fibroblasts from RNase-L knockout mice (KO MEFs) exhibited a mesenchymal morphology as compared to wild type (WT) MEFs. We next compared the responses of WT and KO MEFs to agents that modulate actin polymerization and mimic cytoskeletal alterations that occur during EMT. Treatment with the actin depolymerizing drug Cytochalasin-D resulted in cellular detachment and aggregation in KO but not WT MEFs and resembled morphological changes associated with EMT. To determine if the mesenchymal-like phenotype observed in KO MEFs corresponded with expression of EMT markers, we analyzed E-cadherin and N-cadherin which are down- and up-regulated respectively in EMT. Remarkably, basal expression of E-cadherin was reduced and N-cadherin was increased in KO as compared to WT MEFs; this finding suggested that RNase-L-deficiency may predispose cells to EMT whereas a functional RNase-L may inhibit EMT. Studies are in progress to investigate the regulation of additional EMT markers by RNase-L and how cytoskeletal alterations impact this regulation. Together, our studies suggest a model in which RNase-L tumor suppressor and differentiation-promoting activities are mediated, in part, through the regulation of EMT-associated genes.

**Stephanie Cerritos, Loyola University of Maryland
Christina Ross and Dr. Gerald Wilson**

TTP decreases Tumorigenicity and alters Metabolic Requirements of MDA-MB-231 Triple Negative Breast Cancer Cells

Triple negative breast cancer (TNBC) currently lacks effective treatments because these cancers do not express any of the biomarkers susceptible to targeted therapies: progesterone, estrogen, and HER2 receptors. Tristetraprolin (TTP), a zinc finger RNA-binding protein, suppresses selected tumorigenic properties in a variety of cancer cell types including the aggressive TNBC line MDA-MB-231. However, one feature observed in MDA-MB-231 cells that express TTP is potent suppression of the oncoprotein MYC, a master regulator of several oncogenic pathways. Gene array analyses similarly identified altered expression of known MYC-regulated genes when TTP was present. Using qRT-PCR, we verified that TTP decreased expression of six MYC-induced genes while increasing expression of three MYC-suppressed genes. The ability of TTP to decrease MYC levels and reverse features of MYC-directed gene expression suggested that some tumor suppressive properties of TTP could be mediated by downregulating MYC. Recently, MYC was shown to alter the sensitivity of some cell models to limiting glucose concentrations. To test whether TTP-directed suppression of MYC altered MDA-MB-231 cell requirements for this carbon source, we measured the growth of these cell models in low glucose medium. The parental MDA-MB-231 (Tet-Off) cells died within 4 days in low glucose medium, however, TTP-expressing MDA cells were minimally affected. Suppressing MYC independently of TTP using an shRNA construct similarly allowed MDA-MB-231 cells to survive in the low glucose environment, phenocopying restoration of TTP. Current work is aimed at identifying signaling intermediates that link TTP to MYC suppression and the metabolic consequences of the apparent “glucose addiction” observed in MDA-MB-231 cells that lack TTP. Since TTP expression normally decreases as tumors progress, it is hoped that concomitant development of extreme cellular glucose requirements might provide an opportunity for new targeted therapies that exploit this property.

Dahlia Kronfli, University of Maryland
Dr. Rena Lapidus

The Anti-Cancer Effect of 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 a novel dimeric naphthoquinone (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 have developed a novel second generation BiQ compound known as 217 and have tested its anti-leukemic effect 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. MOLM-14, THP-1, primary leukemia and normal bone marrow cells were treated with a dose response of 217 for 48 or 72 hours to generate IC50 values. This compound generated consistent IC50 values either less than 1 uM or in the single digit uM range for cancer cells. The effect of 217 was tested in the cell lines to also determine if it induces apoptosis (Western blotting for caspase 3 cleavage and Mcl-1 reduction) and whether it generates ROS.

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

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

Luisa Silva, University of Pennsylvania
Dr. Tony Passaniti

Identification and testing of novel transcription factor inhibitors for breast cancer therapy that interact with the RUNX2 protein and alter DNA binding

Metastatic breast cancer is a treatable but incurable disease. Metastasis is a very common feature of triple negative breast cancers, which are estrogen receptor-negative, progesterone receptor-negative, and HER2-negative. These cancers are more likely to recur than other breast cancers and have a poorer prognosis than other breast cancers due to the lack of targeted treatment. The genes that regulate metastasis are not well understood. However, it has been shown that the RUNX2 transcription factor is highly expressed in triple negative breast cancer and mediates breast cancer metastasis to bone. With computer-assisted drug design (CADD), we identified a novel RUNX2-targeted compound that inhibits DNA binding and breast cancer cell growth. To test the hypothesis that the drug might interact with the RUNX2 protein, the sensitivity of RUNX2 to trypsin cleavage was determined. We found that at low concentration of the drug, reduced RUNX2 proteolysis was observed but at higher concentrations, proteolysis was increased. These results suggest that a possible drug interaction with RUNX2 might alter its trypsin sensitivity. Since the CADD drug was designed to fit into the putative RUNX2:DNA binding pocket, our proteolysis results are consistent with a direct and/or indirect RUNX2:drug interaction that regulates levels of RUNX2 protein expression in breast cancer cells. Future experiments will consist of time course proteolysis experiments comparing solutions of DMSO alone to solutions with different concentrations of drug dissolved in DMSO. These will also explore whether DNA:drug interactions contribute to the mechanisms by which CADD drug inhibits RUNX2:DNA binding.

**Sydney Anchor, University of Mississippi
Dr. Paul Shapiro**

Mechanisms of Resistance to ERK1/2 Inhibitors 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 and 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 treatments exist. Consequently, much attention has been directed towards developing new therapeutic approaches which can selectively inhibit the ERK1/2 pathway. Common to 40-50% of melanoma cases is an activating mutation to BRAf. Vemurafenib, the first successful compound to treat melanoma, is a BRAf inhibitor with selectivity towards the mutant form of BRAf. While dramatic clinical success was initially seen, almost all patients quickly developed resistance. Similarly, later MEK1/2 inhibitors showed promising early results. However, resistance to these drugs emerged as well. 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. In this study, we investigated the mechanisms by which resistance to these inhibitors develops. Drug-resistant melanoma cell lines were developed and evaluated for changes in protein expression. Resistant cells showed increased expression of pMEK1/2, ppERK1/2, and α -tubulin as well as upregulation of MEK1 and Akt. These results indicate a reactivation of the ERK1/2 pathway and continued phosphorylation of the ERK1/2 proteins despite inhibition. While ERK1/2 inhibitors alone may not be able to overcome resistance, future treatments may involve ERK1/2 inhibitors combined with other targeted therapies. These combination therapies may be able to reduce the activation of the ERK signaling pathway while simultaneously preventing future resistance to the compounds. Further investigation into how resistance occurs is necessary to determine the most effective combinations of drugs.

**Jacquelyn Bement, Elon University
Dr. Nevil Singh**

Development of a Multicolor Reporter System to Evaluate the Efficacy of Combination Therapies

A vast number of negative regulatory pathways known as checkpoints play a role in inhibiting prolonged T cell effector functions. These mechanisms prevent extensive autoimmune responses to self-tissues after T cell activation, but also inhibit anti-tumor immunity. Therefore, blocking checkpoint molecules has become a popular clinical approach to upregulate T cell responses and allow for more effective clearance of tumor cells. Interestingly, combining multiple blockades works better than single blockades. So far, however, there is no way to choose ideal combinations from a vast array of potential checkpoints to block. In this study, a multicolor reporter system is being developed to evaluate the efficacy of combining checkpoint blockades. The goal is to evaluate the individual and combinatorial effects of different checkpoints on gene expression in activated T cells. Towards this end, we are developing multicolor reporters coupled to transcriptional response elements. Reporters will be validated by transfecting into primary T cells. Fluorescence will be quantified by fluorescent-activated cell sorting (FACS). The system allows for a simple and efficient way to measure transcription factor activity during T cell activation and, in extent, predict the fate and response of T cells when individual blockades are combined for immunotherapy.

David Myers, Washington College
Drs. Degui Geng and Eduardo Davila

Interleukin-1 Receptor-Associated Kinase M Overexpression Plays a Puzzling Role in Melanoma Cell Apoptosis

The MyD88/IRAK-1,-4 signaling pathway contributes to the expression of various inflammatory and tumor growth factors, and is often constitutively activated by unknown mechanisms in a variety of malignancies. IRAK-M is a unique IRAK family member that negatively regulates IRAK-1,-4 signaling, therefore decreasing inflammation, but is downregulated in malignant cells. The goals of this project were to understand how IRAK-M is downregulated, and to understand IRAK-M's contribution to melanoma cell survival and cell death. To understand the mechanisms by which IRAK-M is absent in melanomas, we treated cells with actinomycin D to inhibit transcription and assess IRAK-M mRNA stability. Melanomas and melanocytes treated with actinomycin D expressed lower levels of IRAK-M RNA than untreated cells, but the reducing rate of expression in melanomas was similar to that of melanocytes. This data suggests that downregulation of IRAK-M expression is not due to mRNA stability. Furthermore, we have observed that expressing IRAK-M in tumor cells induced cell death. To study the effects of IRAK-M overexpression, melanoma cell lines were transfected with an IRAK-M vector, and extracted proteins were analyzed by Western blot. The results showed that IRAK-M overexpression was associated with downregulation of two pro-apoptotic proteins (Casp9 and FADD) and two pro-survival proteins (p70s6k and mTOR) mostly in the melanoma cell line C32. Contrary to our expectation, however, the pro-survival protein Bcl-2 was upregulated in SK-MEL-24 cells expressing IRAK-M. Collectively, our data highlight the need to further study the mechanism by which IRAK-M induces apoptosis in melanomas, as well as how IRAK-M is downregulated in various malignancies.

Alicia Carole Greene, Stevenson University
Dr. Arnob Banerjee

Assessing the role of the common gamma chain K315E mutation in peripheral T-cell lymphoma.

Peripheral T-cell lymphoma (PTCL) is a group of incurable heterogeneous non-Hodgkin lymphomas that develop from mature T-lymphocytes. Improving our understanding of the oncogenic drivers of PTCL will be important for establishing novel therapeutic targets and approaches. Data from our lab supports the importance of the common gamma chain (γ_c) signaling pathway in PTCL oncogenesis since multiple mutations in components of this pathway (*JAK3*, *IL2RG*, *STAT5B*) were identified by whole-exome sequencing of 12 primary patient samples. One specific mutation in the cytoplasmic tail of the γ_c , K315E, was identified in a patient with the T-cell prolymphocytic leukemia (T-PLL) subtype of PTCL. This mutation has previously been identified in one other T-PLL patient, but its role in PTCL remains unknown. Our overall goal is to assess the role of the γ_c K315E mutation as a potential oncogenic driver. We hypothesize that the γ_c mutation will decrease cell dependence on IL-2, resulting in increased proliferation in the absence of IL-2, while concurrently increasing the level of phosphorylated STAT5. To test the hypothesis, we will use KIT225 cells, an IL-2 dependent cell line derived from a T-PLL patient. The γ_c K315E mutation will be overexpressed in the experimental group, while KIT225 cells expressing wild type γ_c and with pWCC43 empty vector will be used as controls. Preliminary results indicate that the γ_c mutation does not lead to independent cell proliferation or phosphorylation of STAT5 in the absence of IL-2. The levels of IL-2 will be reduced to study the impact of the mutation on cell proliferation or phosphorylation of STAT5 with minimum amounts of IL-2. The presence of γ_c on the cell's surface was tested using flow cytometry and revealed that the γ_c mutant cells have a statistically significant 13% increase ($p < 0.05$) in the amount of γ_c on their surface compared to γ_c wild type cells. γ_c K315E may increase γ_c expression on the cell surface but does not lead to complete cytokine independent proliferation or constitutive activation of STAT5.

Melanie Berger, William and Mary College

Dr. Rich Eckert

Combined Cisplatin and Sulforaphane Treatment as a Therapy for Epidermal Squamous Cell Carcinoma

Skin cancer is the most common type of cancer in the United States. Between 40 to 50 percent of Americans who live to age 65 will develop basal cell carcinoma or squamous cell carcinoma (SCC) at least once. Cisplatin, a systemic therapy used to treat SCC, induces apoptosis in cancer cells. However, cancer stem cells (CSC), which are responsible for growth and metastasis of tumors, are “slow cycling,” so they are able to avoid these anti-cancer agents. Dr. Eckert and colleagues have revealed that epidermal cancer stem (ESC) cells are able to produce large and aggressive tumors as compared to non-stem epidermal cancer cells. Dr. Eckert has also shown that sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables, can target ESC cells to stop or slow tumor growth and metastasis. We therefore hypothesize that combination therapy with cisplatin and SFN will more effectively treat SCC. To study the combined effects of both compounds we performed migration, proliferation, invasion, and spheroid assays with HaCaT cells. HaCaT are an immortalized keratinocyte cell. Our studies show that combined treatment of SFN and cisplatin is more effective in preventing HaCaT migration, invasion, and proliferation, suggesting combined treatment may be an effective treatment for SCC.

Caroline Schlee, University of South Carolina

Dr. Michal Zalzman

Establishment of Cell lines for the Study of Cancer Immortality

Telomeres are repetitive DNA sequences located on the ends of the chromosomes that shorten every time the cell divides in order to regulate the cell lifespan. Once the telomeres reach a critically short length, the cell enters a stage called senescence, or cellular aging, in which they stop replicating and will eventually die. One of the hallmarks of cancer is its ability to enable replicative immortality, thus avoiding senescence. Cancer cells can achieve this immortality by regenerating their telomeres. This regeneration is normally attributed to the enzyme telomerase, but recently, other mechanisms have been discovered and studied. One of these mechanisms requires the protein ZSCAN4, originally found to regulate telomeres in mouse embryonic stem cells. Interestingly, ZSCAN4 has been demonstrated to work independent of telomerase. Recently, our lab has shown that ZSCAN4 is reactivated in human Head and Neck Squamous Cell Carcinomas (HNSCC), whereas it is not expressed in normal, noncancerous tissues, thus making it a productive target for cancer therapy. Additionally our lab found that ZSCAN4 binds telomeres and triggers rapid telomere extension. In this project, we successfully expanded and confirmed ZSCAN4 expression in multiple cancer cell lines including Breast, Colon and Pancreatic cancer. Additionally, we generated cell lines by introducing either ZSCAN4 knockdown vector or transduction with inducible ZSCAN4 overexpression lentiviral vector. We have successfully established breast and colon cancer cells lines which will allow us to modulate ZSCAN4 levels. Establishment of new cell lines in multiple cancer types will allow our lab to expand its knowledge of ZSCAN4 in cancer, potentially further implicating it in cancer therapy.

Tessa Seale, University of Maryland Baltimore County

Dr. Aikaterini Kontrogianni-Konstantopoulos

Elucidating and verifying the interaction between obscurin and PI3K pathway in breast epithelial cells

Obscurins are large cytoskeletal proteins with structural and regulatory roles. The *OBSCN* gene that encodes obscurins is highly mutated in multiple cancers, including breast cancer. Our studies have shown that breast cancer cells express reduced levels of obscurin proteins. Consistent with this, knockdown of the *OBSCN* gene in normal breast epithelial cells results in increased tumorigenicity and metastasis, through loss of adherens junctions, and induction of Epithelial to Mesenchymal Transition (EMT). More importantly, down-regulation of the *OBSCN* gene leads to up-regulation of the PI3K pathway that regulates cellular growth and survival as well as motility and invasion. The goal of my project was to verify the up-regulation of the PI3K pathway in obscurin-depleted cells, and biochemically elucidate the interaction between obscurins and components of the PI3K pathway. To do so, I used western blot analysis and protein lysates prepared from obscurin-depleted MCF10A breast epithelial cells that were treated with two different PI3K inhibitors, BKM120 and LY294002. My results demonstrated that both inhibitors resulted in increased amounts of beta-catenin and E-cadherin, and decreased amounts of N-cadherin. I obtained similar findings following down-regulation of Akt2, which is a major mediator of the PI3K pathway. Taken together, my results showed that inhibition of the PI3k pathway in obscurin-knockdown cells reverts their mesenchymal phenotype to their normal epithelial phenotype. Moreover, to examine the biochemical association of obscurins with components of the PI3K pathway, we performed co-immunoprecipitation assays. We found that obscurins are able to efficiently and specifically associate with the regulatory component of PI3K. We are now in the process of investigating the direct interaction of obscurins with the regulatory component of PI3K using pull-down assays. In summary, our goal is to understand the molecular mechanisms that are altered in breast cancer cells lacking obscurins in order to develop new therapeutic targets.

Tyler Rutherford, Stevenson University

Nidal Muvarak and Dr. Feyruz Rassool

Poly (ADP-Ribose) Polymerase trapping studies in acute myeloid leukemia using DNA methyltransferase inhibitors and PARP inhibitors

Acute Myeloid Leukemia (AML) is a cancer of the myeloid line of blood cells that is caused by the uncontrolled proliferation of myeloid precursor cells, myeloblasts, in the bone marrow, disrupting its normal function. DNA methyltransferase inhibitors (DNMTi) are used to treat AML but responses are suboptimal. Previous work in the Rassool lab have shown that DNA repair is abnormal in AML cells, including increased expression of DNA repair protein PARP1. However, little is known about Poly (ADP-ribose) polymerase inhibitors (PARPi) in AML. Recently, the Rassool lab have suggested a therapeutic treatment for AML utilizing the combination of low non cytotoxic doses of DNMTi and PARPi. This is based upon the following concepts: (1) PARP inhibitors trap PARP-1 at sites of DNA damage in chromatin, leading to cytotoxic DNA double strands breaks. (2) DNMT1 and PARP interact in a multi-protein complex. (3) DNMTi covalently binds DNMT1 into DNA. (4) DNMTi in combination with PARPi leads to increased PARP trapping and increased AML cell cytotoxicity. In this study we set out to determine the optimal doses and times at which PARP trapping occur using the drugs individually and in combination. MV411 was cultured for up to 96 hours and either treated with 1nM, 2.5nM, 5nM of biomarin (BMN673) or decitabine (DAC). As expected, single treatment of low doses of BMN (1-2.5nM) trapped PARP1 optimally at 24 and 48 hours. Additionally, after single treatment of DAC, PARP trapping was witnessed optimally from 1nM-2.5nM at 48 hours. A treatment plan was developed in order to test the effectiveness of PARP trapping after 2.5nM DNMTi and 1nM-5nM PARPi in combination. The proposed treatments are expected to display a significant amount of PARP1 trapping and thus induce lethal amounts of cellular cytotoxicity in patient samples. These studies will lay the groundwork for clinical trials in AML.

Yuyi Zhu, Towson University

Drs. Giovannino Silvestri, Rossana Trotta, and Danilo Perrotti

Role of miR-300 in the Regulation of Leukemia Cell Proliferation and Survival

Chronic myelogenous leukemia (CML) is a biphasic (chronic and blastic) BCR-ABL1⁺ stem cell-derived and progenitor-driven myeloproliferative disorder. Although most of CML patients respond to ABL1 tyrosine kinase inhibitor-based therapies, TKI resistance remains a major therapeutic challenge. Several microRNAs (miRs), which are small noncoding RNAs that post-transcriptionally modulate gene expression, are dysregulated in CML. Specifically, expression of miR-300 was found strongly suppressed in a BCR-ABL1 kinase-dependent manner in myeloid progenitors from patients in the fatal blastic CML phase. We found that ectopic miR-300 expression represses proliferation and survival of CML cells likely by targeting multiple molecules of the BCR-ABL1/JAK2/SET-PP2A/ β -Catenin pathway. Among miR-300 predicted targets is SET, an endogenous inhibitor of tumor suppressor protein phosphatase-2A (PP2A). The goal of this study was to prove that SET mRNA is a direct target of miR-300. Thus, a GFP-containing lentiviral vector was used to generate Flag-tagged SET constructs containing SET cDNA lacking (*Flag-SET*) or containing a wild type (*Flag-SET-3'wt-UTR*) or a miR-300 binding site-deleted (*Flag-SET-3' Δ -UTR*) SET 3'UTR. These constructs were transduced into two BCR/ABL1⁺ cell lines. GFP⁺ cells were FACS-sorted and transduced with miR-300-containing or an empty vector. The effect of miR-300 overexpression on ectopic SET levels was assessed by anti-Flag Western Blotting. We observed that levels of ectopic Flag SET were markedly inhibited in miR-300-expressing cells transduced with *Flag-SET-3'wt-UTR* but not in those transduced with either *Flag-SET* or *Flag-SET-3' Δ -UTR*. As expected, no modulation of Flag-SET protein expression was observed in empty vector-transduced cells. Therefore, our data indicate that SET is indeed a bona fide miR-300 direct target. Future experiments will be aimed to demonstrate that β -catenin and JAK2 mRNAs are also direct targets of miR-300.

Shannon Kirby, University of Maryland, College Park

Dr. Tami Kingsbury

The Impact of Mutated Ribosomal Proteins on Translation Fidelity

Haploinsufficiency of ribosomal protein S14 has been linked to 5q- syndrome, while mutations in ribosomal protein S19 are found in 25% of individuals with Diamond-Blackfan syndrome. Both of these syndromes have cellular hypo-proliferative defects, leading to decreased red blood cell production. However, later in life, individuals with these syndromes are more likely to develop a hyper-proliferative disease, Acute Myeloid Leukemia. Ribosomal proteins, such as S14 and S19, have many roles within the cell, but feature most prominently in the translation of RNA to protein. Based on yeast models, it is predicted that human cells with mutant RPS14 or RPS19 will be unable to translate proteins as accurately as wild-type cells. CRISPR/Cas9 was used to mutate RPS14 or RPS19 genes in human HEK293T cells. Cell clones were selected and sequenced to confirm INDELS within RPS14 and RPS19 exons, respectively. The translation fidelity of wild type vs. mutant cells was assessed using luciferase reporter genes designed to detect stop codon read through and -1 vs. +1 frameshifting. RPS14 and RPS19 mutant cells failed to terminate translation at stop codons at a higher frequency. The bias toward stop codon read through we observe is consistent with results in yeast. Additionally, as ribosomes facilitate Nonsense Mediated Decay by recognizing premature stop codons, SYBR qPCR was performed to quantitate the levels of mRNA known to be regulated by NMD. The abundance of microRNA 10 was also measured by miR Taqman assays, to determine whether altered ribosome function stimulated miR10 expression. No consistent changes in miR10 levels were observed across the mutant cell lines. Going forward, it will be interesting to explore how read through of stop codons relates to the levels of mistranslated proteins within the cell, and how those levels could create a cell that is predisposed to cancer.

Adip G. Bhargav, University of Maryland
Drs. Anthony Kim, Graeme Woodworth and Jeff Winkles

Optimization of Nanoparticle Formulations for Systemic Circulation and Targeting

Metastatic disease stemming from primary malignancies is responsible for ~90% of cancer-related deaths. Existing treatments for metastatic disease consist of a combination of systemic (primarily chemotherapy) and local therapy (surgery and radiation). However, conventional chemotherapeutics are limited by toxicity, especially in advanced systemic metastases where the required high dosages cause major organ damage. Recently, nanoparticle formulations of chemotherapeutic drugs have been explored to minimize the toxicity of free drugs by sustained release effects and improved tumor-specific targeting. One critical limitation of nanoparticle therapeutics is clearance via the reticuloendothelial system (RES), the body's mechanism to remove blood-borne foreign particulates. Through RES clearance, nanoparticles are opsonized and eliminated before reaching their target. Here, we report our initial efforts to develop a high-throughput method to evaluate and optimize long-circulating nanoparticle formulations. Model polystyrene nanoparticles were engineered with varying amounts of surface polyethylene glycol (PEG) coating, a hydrophilic polymer shown to enhance circulation, to identify the best formulation. These nanoparticles were characterized for hydrodynamic size, polydispersity index, and zeta potential by light scattering and laser Doppler anemometry. PEG density was determined using nuclear magnetic resonance. Then, we conducted Biacore binding assays using a plasma-coated chip and correlated this finding with circulation studies in nude mice to assess circulation potential *in vitro* and *in vivo*. Increasingly PEGylated formulations correlated with longer systemic circulation *in vivo* and weaker binding on the plasma chip. Moreover, binding strength on the plasma chip was inversely correlated with circulation time *in vivo*. However, the Biacore assay was unable to resolve the difference between similarly PEGylated nanoparticles. Next, we will optimize other nanoparticle parameters including size and coating type and refine our Biacore assay. If successful, this work will set the foundation for rapid and thorough evaluation of therapeutic nanoparticle formulations, with the characteristics necessary for prolonged circulation and tumor-specific targeting.