Presenter	Info	Lab PI	Category	Title	Funding Source and Year	Abstract
Chen, Changliang, PhD	Postdoctoral Fellow	Pradeep, Sunila, PhD	Basic	Furin-mediated ovarian cancer progression and metastasis via IGF1R/Stat3 signaling axis	The Ovarian Cancer Research Fund Alliance, research funds from Women's Health Research Program in the Department of Obstetrics and Gynecology (OBGYN), and start-up funds from the Department of Obstetrics and Gynecology at the Medical College of Wisconsin.	Introduction: Ovarian cancer is a devastating disease in women and the current strategies for therapeutic management are not very successful. Our data demonstrate that the changes in the matured form of proteins like Insulin Like Growth Factor 1 Receptor (IGF1R) and Mesothelin compare to the precursor form express high in cancer tissues and cell lines. Furin is a cellular endoprotease, which helps the maturation of several important precursor form of protein substrates through its proteolytic actions. However, the role of Furin as pro-cancer proteolytic enzyme is not well studied. In this study, we identified that Furin-mediated maturation of IGF1R protein is critical for the progression and metastasis of ovarian cancer through STAT3 signaling. Objective: Furin is an understudied proteolytic enzyme in ovarian and other gynecological malignancies. In this study, we will elucidate the mechanism of action how Furin promotes ovarian cancer progression and metastasis as well as determine the effects of inhibiting Furin to disrupt the progression of ovarian cancer. Methods: Expression of Furin and its target genes were detected in cancer cell lines and cancer tissues by western blotting, immunohistochemistry and immunofluorescence staining. To determine the role of Furin on tumor cell migration, we used target-specific siRNAs to knockdown Furin in ovarian cancer cell lines and then performed cell migration assay, matrigel invasion assay, Cancer cells were grown in extracellular matrix for 3D culture. To determine the role of Furin in preclinical models, we have knocked down Furin in ovarian cancer cells and function, ovarian cancer orthotopic implantation animal model was used, followed by bioluminescence imaging to detect tumor growth and metastasis. Results: In the high-grade ovarian cancer patients, Furin is highly expressed in tumor omentum compared with normal omentum. Meanwhile, Insulin Like Growth Factor 1 Receptor (IGF1R) expression was also upregulated in the patient tumor omentum. When Furin was silenced in different ovarian cancer cell lines, cell migration, cell invasion, spheroids size and numbers were significantly reduced. It is interesting that we also found that Furin knockout downregulated the level of IGF1R and p-stat3(Tyr705). In vivo animal data showed that when Furin was knocked down, tumor weight, tumor nodules and tumor ascites were significantly reduced. We also checked Furin, IGF1R and p-stat3 expressions in animal tissues using immunohistochemistry. All these protein expressions were suppressed in Furin knockdown group. Conclusions: Our findings yielded additional insights into newly proposed roles for Furin in ovarian cancer progression and metastasis through the maturation of IGF1R and subsequent activation of STAT3 in cancer cells. Our study demonstrated that Furin can be a new therapeutic target for ovarian cancer. Our data suggests that inhibiting the levels of Furin could modulate the tumor microenvironment and inhibit the progression and metastasis of ovarian cancer. We are expecting that our studies will improve the understanding on the role of Furin on the maturation of key precursor proteins and their contributions on ovarian cancer metastasis. Significance: Our study suggested that Furin can be a new therapeutic target for ovarian cancer. We are expecting that our studies will have an impact on other cancers with a high level of Furin expression. Funding source: Our lab is supported by the grants from The Ovarian Cancer Research Fund Alliance, research funds from Women's Health Research Program in the Department of Obstetrics and Gynecology (OBGYN), and start-up funds from the Department of Obstetrics and Gynecology at the Medical College of Wisconsin.
Chowdhury, Shreya Roy	Postdoctoral Fellow	Bishop, Erin	Basic	SNRK-mediated metabolic reprogramming plays a crucial role in the initial stages of ovarian cancer metastasis	Wisconsin Ovarian Cancer Alliance and Cancer Center Pilot Grant	Introduction: Ovarian cancer tends to metastasize to the fat-rich areas of omentum. Reports suggest that release of free fatty acids by the omental adipocytes act as an alternate source of energy to the tumor cells and aid the process of metastasis. Nevertheless, the molecular players and the exact mechanisms underlying this switch in fuel consumption has not yet been elucidated properly. Sucrose non-fermenting related kinase (SNRK) has been reported to be a crucial regulator of energy homeostasis in adipocytes and cardiomyocytes. The role of SNRK in cancer and metastasis remains elusive. Our previous data showed that SNRK is highly expressed in primary ovarian tumors but not in the late stage metastatic samples. Objective: Decipher the role of SNRK in promoting ovarian cancer metastasis Methods: Ovarian cancer cell lines A2780, SKOV3ip1, CaOV3, OV90 were used for the study. Stable cell lines were generated with SNRK knock down to study the effect of SNRK. Immunohistochemistry was used to detect the expression of SNRK in primary ovarian tumors, normal omentum and omental metastatic samples. The tumorigenic potential of SNRK was assessed by migration, invasion and proliferation assays. SNRK expression was studied by immunofluorescence imaging and western blotting. Mitochondrial membrane potential was studied by JC-1 staining and the ultrastructure was determined by transmission electron microscopy. Extracellular flux analyzer was used to study the bioenergetic profile and the status of fatty acid oxidation in the cells. Protein association studies were done by co-immunoprecipitation. Results: Our data shows that SNRK is highly expressed near the tumor-invasive fonts but could not be detected in the advanced metastatic tissue sections. Expression analysis in a panel of cell lines revealed that SNRK shows mostly a nuclear localization. SNRK was found to be important for cellular proliferation but was noted to have an inhibitory effect on the migratory potential. Extracellular flux analysis revealed that the proliferation profile was associated with a change in the bioenergetic state of the cells with SNRK knockdown. SNRK seemed to be crucial and necessary for mitochondrial respiration as compared to cellular glycolysis. Studying the mitochondrial physiology revealed that SNRK uncouples mitochondrial respiration thereby deterring ATP production. Mitochondrial ultrastructure analysis revealed that SNRK is essential to maintain mitochondria in a fused state to assist the process of fatty acid oxidation. Knocking down SNRK led to the appearance of bigger lipid droplets in the vicinity of truncated mitochondria. To study the role of extracellular fatty acids on the expression of SNRK, we treated ovarian cancer cells with palmitic acid and found that palmitic acid led to an increase in SNRK expression in a dose dependent manner. SNRK was found to associate with Salt-Inducible Kinase 2 (SIK2), which is reported to be necessary for the initial stages of ovarian cancer metastasis. Conclusions: Ovarian cancer cells display heterogeneity in their energetic behavior which accounts for their dependence on SNRK. Cells which are highly energetic (rely both on glycolysis and mitochondrial respiration) are more vulnerable to SNRK-mediated changes as compared to glycolytic cell lines. This implies that SNRK is a crucial regulator of mitochondrial respiration. SNRK uncouples mitochondrial respiration thereby restricting the ATP supply and propelling the cells to depend on alternate sources for energy production. Significance: Our data puts forward a novel role of SNRK in the initial stages of ovarian cancer metastasis. Inhibition of SNRK might help in developing new therapeutic regimens to restrict
Du, Meijun	Research Scientist I	Wang, Liang, PhD	Basic	Plasma cell-free DNA genome abnormalities predict response to abiraterone acetate/prednisone and prognosis in castration-resistant prostate cancer	NCI CA212097	Introduction: Abiraterone acetate/prednisone (AA/P) is used for treatment of metastatic castration-resistant prostate cancer (mCRPC), but no molecular predictive biomarkers are known. Objective: This study aimed to identify plasma cell-free DNA (cfDNA) based molecular biomarkers predictive of treatment resistance and/or prognostic of overall survival for mCRPC state. Methods: We collected serial plasma specimens from 88 mCRPC patients progressing on androgen deprivation therapy (ADT) before and after 12-weeks AA/P treatment. We performed low-pass whole genome sequencing and subsequent copy number alteration (CNA) analysis in 174 plasma cfDNAs. We used Fisher's exact test to evaluate CNAs with primary resistance. We applied Cox regression and Kaplan-Meier survival analyses to associate the CNAs with time to treatment change (TTTC) (secondary resistance) and overall survival (OS). Results: By comparing treatment response status, we identified CNAs in 4 genes (AR, OPHN1, ZFHX3, and PIK3CA) that showed significant association with AA/P primary resistance (False discovery rate (FDR≤0.05)). Survival analyses revealed significant association of CNAs at 11 gene loci with TTTC and at 12 gene loci with OS. Notably, amplifications at AR locus was associated with primary resistance to AA/P treatment (P=0.0039), shorter TTTC (P=0.0003) and OS (P=0.05). Interestingly, amplification of OPHN1 at AR downstream showed more significant association with primary resistance (P=0.0037), shorter TTTC (P=0.0002) and poor OS (P=0.004). To compensate for genetic heterogeneity, we built multi-gene models to predict risk of secondary resistance and OS. By combining CNAs from OPHN1, ZFHX3, PIK3CA and BRCA2, we developed a CNAs-based risk score that was significantly associated with TTTC (P=7.8E-04). We also developed another CNAs-based risk score by combining CNAs from ZFHX3, PIK3CA, and SPOP loci that predicted OS (P=0.002). These genomic risk scores were independent from known predictive and prognostic factors including circulating tumor cells (CTC), plasma tumor content, and clinical factors (age, baseline PSA level and volume of metastasis). Conclusions: Plasma cfDNA-based CNAs in multiple gene loci are predictive of treatment resistance to AA/P and survival in mCRPC state. Significance: Liquid biopsy is a minimal invasive technology that may supplement tissue biopsy to predict treatment response and clinical outcome. Funding source: NCI CA212097

George, Jasmine, PhD	Postdoctoral Fellow	Chaluvally-Raghavan, Pradeep, PhD	Basic	FXR1-mediated Oncogenic Adaptations via Post Transcriptional Regulation of c-MYC	Ovarian Cancer Research Fund Alliance (OCRFA), DoD Breast Cancer Research Program (W81XWH-18-1-0024), the Women's Health Research Program (WHRP) at MCW, American Cancer Society's IRG, and research funds from MCW Cancer Center.	Introduction: Breast and ovarian cancers are the most lethal malignancies in women. Our data shows that Copy Number Variations of RNA binding proteins (RBPs) changes the post-transcriptional events contributes towards tumor initiation and metastasis. However, the functional role of RBPs has not been explored carefully in cancer. We identified that an RNA binding protein FXR1 is highly amplified in breast and ovarian cancers and this genomic amplification upregulates the expression of FXR1. In this study we characterized the key targets of FXR1 and its mechanism of action in breast and ovarian cancers. Our analysis and assays identified that c-MYC is the key target of FXR1 and FXR1 upregulates c-MYC levels by altering post-transcriptional and translational mechanisms. We further demonstrated that the upregulation of c-MYC through FXR1 dysregulates cell cycle, provides metabolic and proliferative advantages, and hijack pro-apoptotic mechanisms in cancer cells. Objective: This study aims to evaluate the mechanism, how FXR1 upregulates c-MYC and other oncogenes for the growth and metastasis of cancer cells. Methods: FXR1 expression in human breast and ovarian cancer cells and tissues were determined by Western blotting, immunohistochemistry and qPCR. We have either knocked down or overexpressed FXR1 in breast and ovarian cancer cells and performed MTT proliferation assay, colony formation assay, matrigel invasion assay, wound healing assay, protein array, cell cycle analysis, and real-time quantitative PCR (qPCR) array. We used this medium throughput qPCR arrays to determine the expression of cell cycle genes, metabolism genes and the expression of tumor suppressor genes and oncogenes differentially expressed upon the alterations of FXR1. We also performed seahorse bioassay to measure the oxygen consumption and extracellular acidification rate upon FXR1 altered cancer cells. To identify the proteins which interact with FXR1, we performed co-immunoprecipitation (CoIP) analyses followed by immunoblotting. We also performed RNA immunoprecipitation (RIP) to show the interaction between FXR1 with c-MYC mRNA. Results: Our analysis identified that FXR1 is highly amplified in several types of human malignancies, particularly in lung, ovarian, cervical and breast cancers. We show that FXR1 is overexpressed in a panel of cancer cell lines and tissues, and the high expression of FXR1 promotes the growth, migration and invasion of cancer cells. In contrast, silencing of FXR1, reduced the proliferation, clonogenic potential, invasion, and wound healing abilities of cancer cells. We also found that the loss of FXR1 modulated the levels of apoptosis-related proteins, as well as promoted G-1 phase arrest. FXR1 silencing also reduced the glucose consumption, lactate secretion, mitochondrial membrane potential and the generation of reactive oxygen species (ROS). Importantly, we found that FXR1 expression associate with poor outcome of breast, ovarian and lung cancer patients. Our data revealed that all the oncogenic effects mediated by FXR1 are orchestrated through c- MYC. We also found that FXR1 level associate with c-MYC expression in the tissue microarrays (TMAs) of ovarian cancer patients. Our data shows that FXR1 stabilizes c-MYC mRNA by preventing its degradation. Specifically, we showed that FXR1 improves the half-life of c-MYC transcript. Furthermore, FXR1 binding on c-MYC mRNA facilitates the recruitment of eukaryotic translation initiation factors (eIFs) to the translation initiation site and activates c-MYC translation. Conclusions: Our results demonstrate that the RNA Binding Protein FXR1 is an unexpected driver of breast and ovarian cancers. Our study characterizes the previously unknown roles of FXR1 on the progression and metastasis of cancer cells. Herein, we identified that the oncogenic effects of FXR1 are mediated through c-MYC oncogene. Importantly, we identified that FXR1 facilitates the recruitment of translation initiation factor complex to the translation initiation site of c-MYC mRNA and enhances the translation of c-MYC protein. Significance: Our study suggests that FXR1 is a prognostic and therapeutic target for ovarian cancer. We found that FXR1 has a critical role in the regulation of c-MYC mRNA and protein. The
Gronseth, Emily	Research Assistant	Ramchandran, Ramani, PhD	Basic	Astrocyte secreted factors influence medulloblastoma tumor cell phenotypes	The MCW Department of Pediatrics and the Children's Research Institute	Introduction: Medulloblastoma (MB) is a pediatric brain tumor that forms in the cerebellum or brainstem of the brain. Various subtypes of the tumor are known to be highly metastatic and recurrent, although the mechanisms of these phenotypes are still largely unknown. Astrocytes are the most abundant cell type in the brain and MB microenvironment. Our lab has shown that factors secreted by astrocytes can increase invasive behavior of breast cancer cells metastasizing to the brain. Objective: The goal of this research was to investigate the signaling events that promote the metastasis of MB, focusing on the role of astrocytes. We hypothesized that astrocytes secrete factors which induce invasive characteristics in MB cells, leading to MB metastasis. The overall objective of these studies is to elucidate a potential therapeutic target(s) to inhibit MB metastasis. Methods: To determine the effects of astrocyte secreted factors on MB cells, we utilized media conditioned from the culturing of neonatal rat astrocytes, or astrocyte conditioned media (ACM). Invasion of Daoy and UW228 (human MB cell lines) cells was assessed in vitro using a Boyden chamber invasion assay, where ACM was used in the lower chamber. In addition, gene expression analysis was performed on Daoy cells cultured in ACM which led to further investigation of the adhesive properties of cells cultured in ACM compared to control media, through cell-based assays and flow cytometry assessment of cell surface adhesion proteins. A neurosphere formation assay was also performed to assess stem-like characteristics of the MB cells. To study how the presence of ACM affects cells in vivo, a zebrafish xenotransplant model was employed to determine differences in cell movement and morphology when cultured in ACM or control media prior to injection. Results: Daoy and UW228 cells showed greater invasion and adhesion in vitro in the presence of ACM. Gene expression analysis comparing normally cultured and ACM cultured Daoy cells revealed several adhesion related genes to be upregulated, including L1CAM, NCAM2, and NFASC. TGFβ-2 is known to be a regulator of these adhesion proteins and has also been shown to be secreted by astrocytes. When Daoy cells were treated with TGFβ-2, however, both the adhesion proteins and adhesion remained unchanged. Interestingly, we did find that both TGFβ-2 and ACM upregulated apoptosis and CD133 in Daoy cells. In addition to being associated with adhesion and protrusion formation, CD133 is a known marker for MB stem-like cells. A characteristic of brain tumor stem cells is their ability to form neurospheres in culture, therefore, a neurosphere formation assay was performed. We found ACM was able to induce neurosphere formation in Daoy and UW228 cells, whereas control media could not. Similarly, time lapse confocal imaging of GFP expressing Daoy cells injected into the hindbrains of zebrafish embryos revealed changes in morphology when cells were cultured in ACM beforehand. These cells showed an increase in protrusion formation and protrusion length. However, no difference in displacement of the cells was found when comparing cells cultured in ACM versus control media. Conclusions: The results of this study indicate that astrocyte signaling mechanisms facilitated by the secretion of factors can be co-opted by MB cells to promote expression and morphology changes that may lead to more aggressive tumors, such as increased invasion and adhesion. Additionally, the role of CD133 in cell survival, or resisting apoptosis, is being evaluated. Although no difference in displacement of the Daoy cells was seen in the 24-hour time-lapse imaging experiment, the increase in protrusion formation indicates that the cells were primed to migrate. Significance: Since astrocytes are such a large component of the MB microenvironment, defining their contribution to pediatric tumor development will facilitate future targeting strategies. In this study, ACM increases invasion, adhesion, and enriches CD133 expressing MB cells. CD133 is often localized in cellular protrusions, and therefore this association and its meaning is currently being studied in the lab. In addition, the increase in CD133 and neurosphere formation indicates that ACM may be enriching cells that are have increased stem-like and self-
He, Chenxia, PhD	Assistant Professor	Bonini, Marcelo, PhD	Basic	Acetylation activates an alternative function of SOD2 as a stemness factor in breast cancer	NIH/NCI R01CA216882	Mitochondrial superoxide dismutase (SOD2) displays a dichotomous role in cancer, being a suppressor of tumor initiation while stimulating cancer progression later in the established disease. The mechanistic basis of this switch remains unknown. Our results indicate that an increase in SOD2 expression beyond a defined threshold leads to its accumulation in an acetylated state and the activation of redox stress responses including, as reported here, the activation of hypoxic signaling. Specifically, we found that increased expression of SOD2/Ac-SOD2 stabilizes hypoxia-induced factor 2α (HIF2α) in a H2O2-dependent manner. Consequently, the SOD2/HIF2α promotes core stemness gene (i.e. Oct4 and SOX2) expression increasing the cancer stem cell (CSC) subpopulation, tumorigenicity and invasiveness of breast cancer cells. Based on these findings we propose that as tumors evolve to advanced stages a mitochondrial pathway to stemness reprogramming dependent on SOD2/ROS and HIF2α is activated. It is also proposed that a SOD2high/HIF2α+ signature may identify cells in breast cancer with tumor initiating capacity and high metastatic potential.

Heimbruch, Katelyn	Research Assistant	Rao, Sid, MD, PhD	Basic	Cohesin Mutations in Core-Binding Factor AML	Midwest Athletes Against Childhood Cancer fund, Versiti Research Foundation, and NIH/NCI 1R01CA204231-01A1, NIH/NIGMS T32-GM080202 (MCW MSTP)	Introduction: Acute myeloid leukemia (AML) is an aggressive malignancy with a 5-year survival of less than 50%. Within AML, even including distinct subtypes, there is significant genetic heterogeneity. Different chromosomal translocations involving the core-binding factor complex are well-known "drivers" of leukemia development and have very similar clinical and prognostic implications. The core binding factor complex is essential for normal hematopoiesis and is composed of two subunits, AML1 (also known as RUNX1) and CBFB. Both AML1 and CBFB are involved in distinct chromosomal translocations in AML, t(8;21) and inv(16) respectively, which generate the fusion oncoproteins AML1-ETO or CBFB-MYH11. Surprisingly, these mutations alone do not induce AML in animal models, suggesting additional "hits" are required. Heterozygous mutations in one of four members of the cohesin complex (RAD21, SMC3, STAG2, and SMC1A) are commonly found in patients with AML, and frequently (up to 25%) co-occur with AML1-ETO, but never with CBFB-MYH11. This suggests a strong selective pressure for the presence or absence of cohesin mutations depending upon the driver oncogene. Specifically, we hypothesize that cohesin mutations synergize with AML1-ETO during leukemic transformation, whereas CBFB-MYH11 and cohesin display a synthetic lethal genetic interaction. Objective: In this study, we set out to determine the relationship between cohesin haploinsufficiency and core-binding factor fusion oncogenes in AML development. By learning more about the mechanism by which these driver oncogenes form distinct genetic interactions with cohesin, we may be able to develop precision medicine approaches. Methods: To explore the relationship between cohesin haploinsufficiency and core-binding factor complex mutations we have engineered murine-derived bone marrow cells that express the oncogenic fusion proteins that are either Smc3+/f or Smc3+/-. We have studied the phenotype of these cells in vitro and with next generation sequencing technologies. To gain a better mechanistic understanding we have performed ATAC-sequencing focusing on the difference between the accessibility of known transcription factor binding sites in AML1-ETO;Smc3+/f compared to AML1-ETO;SMC3+/-. Additionally, we have performed RNA-sequencing to identify transcriptome differences due to cohesin haploinsufficiency and AML1-ETO expression. Results: Our preliminary in vitro studies indicate that the loss of cohesin augments in vitro self-renewal of AML1-ETO and promotes an immature, blast-like morphology. Our ATAC-seq analysis has uncovered several motifs implicated in myeloid development (RUNX1, GATA2, ERG, PU.1), nuclear architecture (CTCF, CTCFL), and cell proliferation (AP-1, FLI1, JUN) as enriched in the Smc3+/- background. RNA-sequencing revealed downregulation of genes involved in myeloid cell differentiation, changes corresponding to Hoxa9 upregulation, and upregulation of the Rb and p53 oncogenic gene signatures in AML1-ETO;Smc3+/- compared to Smc3+/f. Interestingly, we did not identify significant transcriptional differences that correspond to the factors that bind the more accessible motifs. Conclusions: We have identified that Smc3 haploinsufficiency and AML1-ETO cooperate to promote increased self-renewal and immature cell morphology. Additionally, Smc3 haploinsufficiency and AML1-ETO result in increased chromatin accessibility and transcriptional changes. Our findings lead us to propose that alteration of cohesin's function as a regulator of chromatin accessibility allows AML1-ETO to bind new sites leading to the transcriptional changes and downstream phenotype we have observed here. Significance: Our work provides insights into how we may begin to develop more personalized, targeted therapies for leukemias, based on the combination of specific driving mutations present in an individual patient (ie cohesin targeting therapies may be used successfully for patients with t(8;21) but not inv(16)). Funding source: Midwest Athletes Against Childhood Cancer fund, Versiti Research Foundation, and NCI 1R01CA204231-01A1, NIGMS T32-GM080202 (MCW MSTP)
Jondle, Christopher, PhD	Postdoctoral Fellow	Tarakanova, Vera, PhD	Basic	Title: Gammaherpesvirus usurps host IL-17 signaling to support chronic infection	NIH/NCI R01CA203923; NIH/NCI R01CA183593	Introduction: Gammaherpesviruses are oncogenic viruses that are associated with multiple cancers, including B cell lymphomas. These viruses have a natural tropism for B cells and drive a robust germinal center (GC) response, needed to establish a latent reservoir in memory B cells. The robust GC response driven by gammaherpesvirus infection increases the risk for B cell transformation. Unsurprisingly, many gammaherpesvirus cancers are derived from GC or post GC B cells. The mechanism used by gammaherpesviruses to induce the GC response is poorly understood. IL-17 is a functionally diverse cytokine that has been found in almost every cancer type. Interestingly, herpesvirus saimiri (HVS), a simian gammaherpesvirus, encodes a viral IL-17, which functions like human IL-17A, suggesting an intriguing possibility that IL-17A is important in gammaherpesvirus infection. Other gammaherpesviruses do not encode a vIL-17 and therefore would need to induce host IL-17A. Unfortunately, the role of IL-17 during gammaherpesvirus infection and pathogenesis is unknown. Objective: Utilize IL-17RA-/- mice to determine the significance of IL-17A&F signaling during gammaherpesvirus infection. Methods: Human gammaherpesviruses are very species specific, which make in vivo studies difficult. To overcome this obstacle, murine gammaherpesvirus 68 (MHV68), a related gammaherpesvirus is used for in vivo animal studies. Utilizing the power of mouse genetics in combination with MHV68 infection, we can determine the impact specific host factors have on the establishment of MHV68 chronic infection. Viral and cellular landscapes following infection of IRF-1-/- and IL-17RA-/- mice were determined 16 days post infection with MHV68 and compared to infected BL6 mice. Results: MHV68 infection increases IL-17A expression. Loss of IL-17RA signaling during MHV68 infection led to significant attenuation in establishment of viral latency as well as viral reactivation. Correspondingly, loss of IL-17RA signaling decreased the germinal center response seen during MHV68 infection. Lack of IL-17RA signaling resulted in a significant reduction in both viral specific and irrelevant antibody titers following MHV68 infection. Interestingly, these results were not recapitulated during LCMV infection, suggesting that IL-17 signaling is selectively required to support the germinal center response during gammaherpesvirus infection. Further, neutralization of IL-17A during infection led to a decrease in viral latency and reactivation as well as attenuation of the germinal center response. Taken together these results establish a proviral role for IL-17RA signaling during gammaherpesvirus infection. Conclusions: IL-17RA signaling supports the germinal center response and establishment of chronic infection during gammaherpesvirus infection. Significance: This study shows for the first time a proviral role of IL-17RA signaling in an oncogenic virus infection.
Maddirela, Dilip, PhD	Postdoctoral Fellow	White, Sarah, MD, MS, FSIR	Basic	Inhibition of HIF-1⍰ Sensitizes Primary and Metastatic Liver Cancer Cells		Introduction: Transarterial chemoembolization (TACE) is the standard of care for unresectable primary liver tumors. However, robust data demonstrating the superiority of chemoembolization over bland embolization is lacking. Recent studies have shown that embolization induces transient hypoxia which further drives proliferation of hypoxia-adapted cancer cells and leads to resistance to drugs. Objective: The purpose of this study is to demonstrate that HIF-1⍰ inhibition sensitizes cancer cells to doxorubicin and therefore can improve the outcomes of embolization. Methods: Hepatocellular carcinoma (MCA-RH777), colorectal liver metastasis (CC-531) and immortalized liver (Clone 9) cell lines were grown in DMEM medium at 37°C in a humidified atmosphere with 5% CO2 in normoxic and <1% O2 hypoxic conditions. For survival assays, the cells were exposed to doxorubicin (drug used for TACE), R59949 (HIF-1⍰ inhibitor) or a combination of the two under normoxic or hypoxic conditions. After 24h and 48 h of incubation, images were acquired and cell viability was determined by trypan blue staining. For wound healing assay, cells were seeded into 12-well plates to achieve confluency. The following day, a scratch wound was made, and the cells were cultured in medium and subjected to the above described treatments. Images of the cells migrating into the wound were captured prior to and after 16h. Results: The combination treatment of doxorubicin and R59949 showed greater than 12.5% cell death under hypoxic conditions compared to normoxic conditions in the cell lines tested. Although cell death was noted with either treatment alone under hypoxic conditions, the combination was shown to be markedly effective (>30%). Similarly, combination therapy indicated greater inhibition of migration (>20%) across the wound edges under hypoxic conditions compared to normoxic conditions. Migration was inhibited in both treatment arms; however, combination therapy under hypoxia showed higher inhibition. Conclusion: Combination therapy enhances the efficacy of doxorubicin and reduces tumor resistance under hypoxic conditions. Further investigations are underway to understand the cell cycle arrest in combination treatment contributing to the inhibition of cell proliferation. Significance: This result suggests that combination therapy-induced liver tumor sensitization and could be a promising step in transarterial therapy.

Miller, Bradley	Supervisor Lab	Sorokin, Andrey, PhD	Basic	p52Shc isoform of Shc1 controls breast cancer initiation and growth	MCWCC Pilot Grant, NIH grants R01 DK098159 and grant from the Wisconsin Breast Cancer Showhouse, Inc. and the Medical College of Wisconsin Cancer Center	Introduction: SHC1 proteins exists in three functionally distinct isoforms (p46SHC, p52SHC, and p66SHC) that serve as intracellular adaptors for several key signaling pathways in breast cancer. Testing the isoform-specific roles of SHC1 proteins in breast cancer initiation and progression has been inaccessible due to the lack of isoform-specific inhibitors or gene knockout models. Objective: To test the role of individual SHC1 isoforms in initiation and progression of breast cancer and identify novel targets for drug therapies. Methods: Human breast tissue from the MCW Tissue Bank and Human INSTA-Blot™ Breast Tissue OncoPair ready-to-use PVDF membranes were used to assess total SHC proteins expressions in human breast cancer and normal tissue. Staining for ER, PR and HER2 were performed using Dako EnVision FLEX mini Kit. To test the role of individual SHC1 isoforms we have generated the first isoform-specific gene knockout models for p52SHC and p66SHC, using germline gene-editing in rat strain. Whole mount staining of rat mammary glands was used to analyze the difference in mammary gland structure between wild type (WT) and genetically modified rats. Rats were dosed with DMBA by oral gavage to induce mammary tumors, and progression of tumor development was followed for 15 weeks. At 15 weeks tumors were excised and analyzed by RNA-seq to determine differences between tumors lacking p66SHC or p52SHC. Results: Expression of SHC1 proteins was found to be up-regulated in human breast carcinomas. The comparison of DMBA-induced rat tumors with normal rat mammary tissue also revealed increased expression of SHC1 isoforms. Compared with WT rats, genetic ablation of the p52SHC isoform significantly attenuated mammary tumor formation, whereas the p66SHC knockout had no effect. These data, combined with p52SHC being the predominant isoform that is upregulated in human and rat tumors, provide the first evidence that p52SHC is the oncogenic isoform of SHC1 gene products in breast cancer. Compared with WT tumors, 893 differentially expressed genes were detected in p52SHC KO tumors compared with only 18 genes in the p66SHCKO tumors, further highlighting that p52SHC is the relevant SHC1 isoform in breast cancer. Finally, gene network analysis revealed that p52SHC KO disrupted multiple key pathways that have been previously implicated in breast cancer initiation and progression, including ESR1 and mTORC2/RICTOR. Conclusions: Our data demonstrate that p52SHC isoform is the key driver of DMBA-induced breast cancer while the expression of p66SHC and p46SHC are not enough to compensate. Significance: Our data strongly suggest that p52SHC-dependent signaling is required for mammary tumor progression and suggest p52SHC may serve as node interlinking several critical signaling pathways implicated in breast cancer progression. Our finding that p52SHC appears to be a central hub regulating these pathways provides rationale for an exciting target for new drug therapies. Funding source: MCWCC Pilot Grant, NIH grants R01 DK098159 and grant from the Wisconsin Breast Cancer Showhouse, Inc. and the Medical College of Wisconsin Cancer Center
Norwood Toro, Laura	Research Scientist I	Beyer, Andreas, PhD	Basic	Adverse Effects of Chemotherapy on Human Microvascular Function	NIH R01 HL133029, We Care Foundation Grant; MCW-Cardiovascular Center Pre-PPG grant, Advancing a Healthier Wisconsin – Redox Biology Grant.	Introduction: One of the most devastating side effects of cancer treatment is chemotherapy (CT)-induced heart failure, which results in significant morbidity for patients. Cardiovascular (CV)-related side effects of CT are known to involve excess reactive oxygen species. However, the underlying mechanisms are disputed. To date little to no evidence exists that describe the direct effect of CT on the microcirculation, and none of these studies were performed in humans. Microvascular (MV) dysfunction is a known early indicator of numerous CV disease phenotypes. Objective: The goal of this study was to evaluate whether CT treatment affects human MV function. Methods: To study the effect of CT on the MV system, flow mediated dilation (FMD) was evaluated in freshly isolated human microvessels from healthy patients that were ex vivo treated with various CT drugs (trastuzumab - TZ, doxorubicin - Dox, lapatinib - Lb, or imatinib - Ib). Mitochondrial DNA (mtDNA) damage was assessed using semi-quantitative PCR comparing amplification of a small fragment to a large fragment in the mitochondrial genome. Results: Ex vivo treatment of microvessels with any of the four CT drugs reduced nitric oxide (NO)-mediated dilation compared to untreated controls. TZ decreased FMD, and Dox treatment eliminated endothelial dilator capacity. TZ, Lb, and Ib treatments maintained MV dilation via a compensatory increase in mitochondrial H2O2-mediated dilation. Smooth muscle dependent dilation to papaverine was not affected by any of the treatments. Analysis of mtDNA damage from HUVEC cells treated with Dox showed an increase in mtDNA damage, and mitochondria-targeted Endo III treatment prevented Dox-mediated loss of dilation. These studies suggest a role for mtDNA damage in the Dox phenotype. Conclusions: Our studies demonstrate for the first time the effect of CT drugs on human MV function and may lead to methods of preventing additional adverse CV side effects from CT. Significance: Click here to enter text. Funding source: This work was supported by NIH R01 HL133029, We Care Foundation Grant; MCW-Cardiovascular Center Pre-PPG grant, Advancing a Healthier Wisconsin – Redox Biology Grant.
Parchur, Abdul, PhD	Postdoctoral Fellow	Joshi, Amit, PhD	Basic	Site-selective Plasmonic Photothermal Therapy of Colorectal Cancer Liver Metastasis	Research Affairs Committee, and Wisconsin Breast Cancer Showhouse	Introduction: While nanoparticles (NP) deposit in tumors following systemic intravenous (IV) administration, the delivered dose to tumor is typically low, which can adversely impact the efficacy of photothermal therapy (PTT). Interventional image guided site-selective delivery of NP to the liver circulation via portal vein/hepatic artery can improve the tumor dose. Theranostic plasmonic-magnetic-nanoparticles (TPNs) with combined MR and X-ray contrast, in addition to PTT capabilities, can enable effective pre-procedure therapy planning via computer simulation of light and bioheat transport, as well as provide real-time image guidance for the delivery of NP and therapeutic light. Objective: We report TPNs composed of Gold (Au) core and Gd2O3:Yb/Er shell with PTT capability, X-ray contrast, and T1-MR contrast, and demonstrate the increased delivery to tumors via portal vein injections in a rat model of colorectal liver metastasis (CRLM) generated with CC-531 cell implantation into the liver. Photothermal therapeutic response was also compared with clinical microwave therapy under similar conditions. Methods: Au nanorods resonant at 830 nm were synthesized and encapsulated with Gd2O3:Yb/Er shell and PEGylated, resulting in formation of sub-100 nm TPNs. TPNs were characterized using transmission electron microscopy (TEM) and zeta potential measurements. T1 relaxivity of TPN was determined in a Bruker 9T MRI scanner and rat imaging was performed on GE 7T human MRI scanner, while X-ray contrast was calibrated at 60 kV on a Pxinc's X-RAD SmART scanner using a cone beamCT. Six WAG/RijCmcr rats (350-450g) implanted with CRLM tumors were selected for the IACUC approved experiments. TPNs (0.5 mL, 1013 NP/mL) were injected either locally into the liver via portal vein or systemically via tail vein. Rats were imaged with T1 MR scans immediately after injection for portal vein group, and at 4, 24, and 72 h for the tail vein group. DynaCT imaging was acquired by injecting 0.5 mL of TNP (3x1014 NP/mL). Images were analyzed for TPN uptake, and excised tissue was imaged with TEM to verify TPN uptake in cancer cells. COMSOL Multiphysics 9.3a was used to solve Lorentz equations using Mie theory to simulate Gd2O3:Yb/Er coated gold nanorod NIR absorption by controlling layer shape and thickness. Furthermore, thermal contours in tissue for both microwave and photothermal ablation were calculated using Penn's bioheat equations. Results: PEGylated TPN demonstrated both X-ray and MR contrast in a dose linearly dependent manner. The T1 relaxivity at 9T was found to be ~1.1x108 mM–1S–1 in terms of TPN concentration. The average TPN size was 75 nm and zeta potential ~4.8 mV indicating long circulation potential. Uptake of the TPN into the liver via site-selective vs systemic delivery methods was compared through analysis of cross-sectional MR images pre and post-delivery. Local delivery resulted in ~3.2 times increase in tumor dose. CT enhancement was also observed in the tumors post TPN administration. Furthermore, the ex vivo TEM images of CRLM tumor tissue indicated TPN uptake in tumor cells following site-selective delivery, as well as consistency in TPN shape and lack of aggregation in vivo, indicating potential for effective PTT. Computational simulations confirmed that TPNs have an absorption resonance around 810-nm and TPN mediated photothermal ablation, restricted thermal therapy envelop to tumors, which was not the case for microwave ablation, where the thermal contours only tracked the probe position. Conclusions: TPN with X-ray and MR imaging contrast can specifically target CRLM tumors via portal vein injections, and enable high-efficiency PTT. Interventional image guided portal vein injections are superior to systemic tail vein injections for NP delivery to CRLM. The ability of controlling the nanoparticle distribution helps to control local temperature distribution in tissue during therapy. Photothermal ablation with plasmonic nanoparticles is an efficient method for treatment of liver tumors.

Schlaak, Rachel	Research Assistant	Bergom, Carmen, MD, PhD	Basic	Identifying Hereditary Factors That Modulate Radiation-Induced Cardiac Toxicity	MCW Cancer Center Pilot Grant; Michael H. Keelan, Jr., MD, Foundation Grant; Mary Kay Foundation Award Grant No. 017-29; Nancy Laning Sobczak, PhD, Breast Cancer Research Award; Susan G. Komen, American Cancer Society Institutional Research Grant 86-004-26; and Grant 8KL2TR000056.	Introduction: Over half of all cancer patients receive radiation therapy (RT) as part of their cancer treatment. Radiation can improve cancer outcomes, but toxic normal tissue side effects may occur causing morbidity and mortality. Radiation-induced cardiac toxicity has been reported in patients with thoracic tumors, such as lung and breast cancers. However, there are currently no biomarkers or protective agents to predict or protect against radiation-induced cardiotoxicity. Objective: The purpose of this study was to use animal models to assess RT cardiac toxicity, followed by genetic mapping of radiation sensitivity, to identify new biomarkers that predict cardiac toxicity from RT. Methods: Salt-sensitive (SS) and Brown Norway (BN) rats were selectively bred to produce SS.BN3 consomic rats, which are genetically identical to SS rats except chromosome 3 is inherited from the BN rat. We have previously shown that SS rats exhibit enhanced cardiac toxicity compared to SS.BN3 rats (Schlaak et al., 2019). Congenic mapping was used to identify regions on chromosome 3 modifying radiation sensitivity. Adult female SS, SS.BN3, and congenic rats (CG.1, CG.2, CG.3 and CG.4) received image-guided localized whole-heart RT of 24 Gy (AP and 2 lateral fields, weighted 1:1:1). Echocardiograms with strain analysis were performed at baseline, and 3 and 5 months. Also, RNA-seq with IPA analysis and immunohistochemistry was performed on SS and SS.BN3 left ventricle tissue. Results: Congenic rat strain CG.4 exhibited enhanced cardiac toxicity compared to the SS.BN3 rats. At 5 months post-RT, only the SS and CG.4 rats displayed cardiac hypertrophy ($p<0.01$). The chromosomal region unique to the SS and CG.4 rat strain is 25 MB, which displays enhanced radiation sensitivity compared to SS.BN3. RNA-seq gene expression analysis demonstrated mitochondrial dysfunction genes were significantly increased in SS vs BN3. 331 genes are located in the 25 MB region of interest, with 93 genes (<0.9% of genome) differentially expressed. Immunohistochemistry also showed increased mast cells in the SS.BN3 left ventricle tissue at 10 weeks post-RT compared to SS ($p<0.05$). Conclusions: Similar to SS rats, the CG.4 rats are more sensitive to cardiac radiation than the SS.BN3 rats, indicating a role for heritable factors in determining radiosensitivity. Gene expression analysis showed numerous potential targets on the 25 MB region on chromosome 3, including genes involved in mitochondrial dysfunction, sirtuin signaling, notch signaling, and apoptosis. The increase of mast cells in SS.BN3 left ventricle tissues compared to SS tissue may also be contributing to the observed differences in heart damage. Significance: Genetic mapping will aid in narrowing the causative target(s) to improve potential therapies. This project has the potential to enhance the effectiveness and toxicity profile of RT in cancer. Funding source: MCW Cancer Center Pilot Grant; Michael H. Keelan, Jr., MD, Foundation Grant; Mary Kay Foundation Award Grant No. 017-29; Nancy Laning Sobczak, PhD, Breast Cancer Research Award; Susan G. Komen, American Cancer Society Institutional Research Grant 86-004-26; and Grant 8KL2TR000056.
Sun, Yunguang, MD, PhD	Assistant Professor	Rui, Hallgeir, MD, PhD	Basic	NSG-Pro: a next generation model for breast cancer patient-derived xenografts	NIH/NCI R01CA188575, MCW ACS-IRG	Introduction: Patient-derived xenograft (PDX) models of breast cancer have existed since 1975, yet the inability to grow human estrogen receptor (ER) breast tumors in rodent hosts precluded studying the majority of patients that ultimately progress to lethal metastatic disease. We demonstrate that the failure of current PDX hosts is due to the inability of mouse prolactin (Prl) to cross-react with the human prolactin receptor (hPrlR), a required endocrine factor for the survival, growth, and metastatic progression of ER+ PDX. Objective: We hypothesize that the lack of hPrl in the mouse PDX host strain deprives human ER+ luminal breast cancers of a necessary endocrine factor that supports breast tumor engraftment, growth, metastasis, and therapeutic response. Notably, introducing hPrl into a PDX host strain would be predicted to have little or no negative impact on ER-/PR- or HER2+ breast PDX biology, suggesting that a humanized-prolactin PDX host strain increase engraftment of luminal ER+ breast cancer and maintain the high engraftment rates of other PDX subtypes. Methods: We engineered NSG-Pro mice by introducing hPrl to host NSG mice. These mice were used to implant clinically dissected human breast cancer tissue. Transplantable PDX lines were studied with drug response experiments such as Tamoxifen, Parp1 inhibitor or Her2 humanized antibody Trastuzumab. Also, RNA seq and WGS were performed to explore the pathways and mutations contributing to Tamoxifen resistance. Results: NSG-Pro-mice showed >50% engraftment rates for ER+ and ER- PDX. These transplantable PDX models recapitulate the clinical features of its corresponding human breast cancer. RNA seq and WGS analysis revealed elevated Her2 and Parp1 in Tamoxifen-resistant sublines may be potential Tamoxifen resistance mechanisms. Targeting Parp1/Lig3 greatly inhibited resistance tumor growth. Trastuzumab blocks the growth of resistance tumors effectively but drugs resistance eventually develops. Conclusions: In summary, our study showed that NSG-Pro-mice was the first PDX platform with >50% engraftment rates for ER+ and ER- PDX. Moreover, PDX models developed in NSG-Pro-mice reliably recapitulate human cancer biology. NSG-Pro unlocks the potential for developing precision therapy plans in patients that were previously inaccessible to PDX modeling and opens a gateway for breast PDX modeling to be broadly applied to a burgeoning era of genomic medicine and precision therapy. Significance: NSG-Pro-mice provide powerful patient avatars for precision medicine to identify molecular vulnerabilities and interrogate biology and drug responsiveness of ER-positive human breast cancers. Funding source: NIH R01, Cancer Center ACS seed grant

Udhane, Vindhya, PhD	Postdoctoral Fellow	Nevalainen, Marja, MD, PhD	Basic	Enzalutamide-Induced Jak2-Stat5 Feed-Forward Loop Promotes Therapy-Resistant Prostate Cancer Growth and Provides an Exploitable Molecular Vulnerability	National Cancer Institute (NCI)/National Institutes of Health (NIH) Research Project Grant (2RO1CA11358-06), an NCI/NIH Exploratory/Developmental Research Grant (1R21CA178755-01) and Advancing a Healthier Wisconsin (#5520368) to M.T. Nevalainen. D.T. Hoang is supported by an NCI/NIH Predoctoral Individual National Research Service Award (NRSA) Fellowship (1F31CA180626-01).	Introduction: Androgen deprivation remains the cornerstone for the management of advanced prostate cancer (PC). The second-generation anti-androgen, enzalutamide (ENZ), is FDA-approved for castrate-resistant (CR) PC and targets androgen receptor (AR) activity in CRPC. Despite initial clinical activity, acquired resistance to ENZ arises rapidly and most patients develop terminal disease. Stat5a/b (Stat5), which is both a signaling protein and a nuclear transcription factor is known to sustain viability of PC cells and to induce growth of PC xenograft tumors in vivo. Blockade of Stat5 signaling induces apoptotic death of PC cells, suppresses growth of both xenografted and autochthonous PC tumors as well as clinical patient-derived PCs ex vivo in culture. Conversely, overexpression of active Stat5 has been shown to induce proliferation of PC cells in vitro and growth of PC tumors in mice. Stat5 induces metastatic progression of PC, as evidenced by Stat5 promotion of metastasis formation in vivo, and induction of hallmarks of EMT and stem-like cancer cell properties through induction of Twist1 and BMI1 expression in PC. In 30-40% of advanced CRPCs, the chromosome 17 locus encompassing STAT5A and STAT5B genes undergoes amplification resulting in increased Stat5 protein levels. Notably, high nuclear Stat5 protein expression at the time of the initial PC treatment predicted recurrence of the disease in three independent cohorts totaling 1,035 patients. The predictive role of active Stat5 for clinical PC progression to lethal CR state corroborates involvement of Stat5 in PC progression in preclinical PC models. The findings of Stat5 as a PC growth promoter and a predictor of PC recurrence implies involvement of Stat5 in development of CRPC, which led us to hypothesize that Jak2-Stat5 signaling sustains viability of PC cells following disruption of AR signaling by ENZ. Objective: To investigate the significance of Jak2-Stat5 signaling in resistance of PC to ENZ. Methods: Levels of Jak2 and Stat5 activation were evaluated by immunohistochemistry, immunoprecipitation and western blotting in PC cells, xenograft tumors and in patient-derived PCs ex vivo in 3D tumor explant culture. Jak2 and Stat5 were suppressed either by genetic knockdown using lentiviral expression of shRNA or pharmacologically by Jak2 and Stat5 inhibitors. Levels of mRNA were assessed by QPCR and gene expression profiling. A unique PC xenograft mouse model (CWR22Pc), which mimics PC clinical progression in patients, was utilized to assess in vivo responsiveness of primary and ENZ resistant PCs to inhibition of Jak2/Stat5 as a second-line treatment. Patient-derived clinical PCs were tested for responsiveness to Stat5 blockade ex vivo in tumor explant cultures. Results: We demonstrate, for the first time, that ENZ induces a robust increase in Stat5 activation in PC cells in vitro, in xenograft tumors in vivo and in patient-derived PCs during ENZ treatment. Mechanistically, ENZ-liganded AR induced rapid and sustained Jak2 phosphorylation in PC cells through a process involving Jak2-specific phosphatases PTPε and SHP2. ENZ-induced Jak2 activation leads to Stat5 phosphorylation, and a formation of a feed-forward loop in PC cells, where active Stat5 increases Jak2 mRNA and protein levels. We further demonstrate that activated Stat5 promotes growth of PC cells during ENZ treatment and, at the same time, inhibition of Stat5 as a second-line treatment induces extensive death of PC cells surviving ENZ treatment. Pharmacological Stat5 blockade inhibited castrate resistant growth of PC xenograft tumors after ENZ resistance developed and induced further death after ENZ treatment in patient-derived PCs ex vivo in tumor explant cultures. Mechanistically, residual PC cells which survived genetic knockdown of AR could be eliminated by suppression of Stat5, implying a presence of Stat5-regulated, AR-independent growth mechanisms. In summary, this work supports the new concept of a critical role of a hyperactive Jak2-Stat5 signaling loop in promoting resistance of PC to ENZ. Pharmacological Jak2-Stat5 inhibition may provide an effective therapy for Stat5-positive advanced PC in combination with ENZ or after ENZ fails. Conclusions: ENZ-liganded AR induces sustained Jak2 and Stat5 phosphorylation in PC leading to a formation of a positive feed-forward loop, where activated Stat5, in turn, induces Jak2 mRNA and protein levels contributing to further Jak2 activation. Collectively, this work introduces the novel concept of a pivotal role of Jak2-Stat5 signaling in mediating resistance of PC to ENZ which represents a readily targetable molecular vulnerability in ENZ-resistant Stat5-positive PCs.
Wedemeyer, Michael	Research Assistant	Volkman, Brian, PhD	Basic	Engineering an Orthogonal Chemokine:GPCR Pair to Guide Modified Cell Therapy		Introduction: Cellular therapy has boomed in the past decade, rising to combat potent and complex diseases such as cancer. Today, modified T-cells are being used in clinical trials to eliminate cancerous tumors and have been FDA approved for the treatment of Lymphoma. These techniques offer great promise in treating a spectrum of cancers but are held back by a common issue: cell targeting and retention. Chemotactic cytokines (chemokines) are the body's most elegant system for directing the movement of therapeutic cells toward sites of immunological importance. Objective: The goal of this project is to spatiotemporally control cell migration and localization by engineering an orthogonal chemokine:GPCR pair that functions independent of the native chemokine system. Methods: To achieve this goal, we have developed a strategy to utilize atomic-level structures to create detailed homology models of chemokine:GPCR complexes using the Rosetta software suite. Through a system of computer-assisted rational design and directed evolution, novel receptors are being designed for exclusive activation by novel chemokines. Engineered proteins are being purified recombinantly and assessed for orthogonal chemotactic activity in receptor transfected Jurkat T-cells. Results: Using previous structural and functional data, a panel of 16 chimeric chemokines were designed based on five human chemokines: CCL5, CCL20, CXCL11, CXCL12, and CX3CL1. Chimeric chemokines successfully disrupted chemotactic signaling in native receptors, but first and second-generation chimeric receptors were unable to promote migration at relevant concentrations. To guide protein design, a protocol to construct physically realistic models of chemokine bound GPCRs was developed and benchmarked against recently published crystallographic co-complexes. The global root-mean-square deviation (RMSD) of Cα atomic positions was below 4Å, with key activation motifs approaching the resolution limit of the published structure (2.37Å and 2.20Å, respectively). Conclusions: A major underlying principle used in the first-generation design was the traditional “2-site” understanding of chemonkine:GPCR binding and activation. This data supports a more nuanced interaction that cannot be fully exploited without innovative techniques to understand the atomic minutia. Integrating in vitro and in silico information, third-generation chimeric chemokines are being designed to trigger directed migration exclusively through engineered chemokine receptors. Significance: Chemokines play a critical role in the adaptive immune system and the metastasis of many cancers. This work has improved our understanding of chemokine:GPCR interactions, developed tools to investigate chemokine specificity, and built toward an orthogonal chemotactic pair that may improve the capability of cell therapies to target specific tissues.

Yeo, Chay Teng	Graduate Student	Corbett, John, PhD	Basic	The regulation of oxidative metabolism by nitric oxide protects β-cells from DNA damage-induced cell death	NIH/NIDDK-R01DK052194, NIH/NIAID R01 AI044458 and a gift from the Forest County Potawatomi Foundation.	Introduction: Nitric oxide, generated in β-cells in response to cytokines, induces DNA damage that activates DNA damage response (DDR) and eventually leads to apoptosis if damage is too extensive. We showed that nitric oxide, while inducing double-strand DNA breaks (DSBs), also inhibits DDR signaling and protects β-cells from DNA damage-induced apoptosis. The ability of nitric oxide to inhibit the DDR signaling is selective to β-cells and correlates with a loss in ATP levels, while non-β-cells are able to maintain both ATP levels and DDR signaling when treated with nitric oxide. Objective: We tested the hypothesis that β-cell metabolism enables nitric oxide-dependent inhibition of DDR and protection from DNA damage-induced cell death. Methods: INS 832/13, MEFs, and HepG2 were cultured in a full media supplemented with 10% FBS. Proteins were extracted by lysing cells in laemmli buffer, and were determined via Western blot. Nucleotides (ATP and NAD+) were extracted from treated cells by perchloric acid precipitation and quantified by HPLC analysis. Metabolomics analysis was performed to determine change in metabolites from treated cells harvested in 80% methanol containing C13-labeled internal standards, and measured via a 1200 Infinity Series HPLC in-line with a 6430 QqQ mass spectrometer. Extracellular flux analysis (Oxygen consumption rate and extracellular acidification rate) was performed on a Seahorse XF96 analyzer. Cell death was determined by measuring incorporation of the fluorescent SYTOX Green nucleic acid stain. Results: β-cell metabolism is characterized by the tight coupling of glycolysis to mitochondrial oxidative metabolism. Nitric oxide is known to inhibit mitochondrial oxidative metabolism (e.g. aconitase in TCA cycle and complex IV of electron transport chain (ETC)). Under inhibition of mitochondrial oxidation by nitric oxide, β-cells are not able to compensate for their loss in ATP by increasing glycolytic metabolism. In contrast, non-β-cells compensate for the mitochondrial inhibition by increasing glycolytic metabolism to maintain their ATP pool. Consistent with the action of nitric oxide, we showed that rotenone, an inhibitor of complex I of ETC, also inhibit DDR signaling and decrease ATP levels in β-cells but not in non-β-cells. Importantly, when non-β-cells are forced to generate ATP via mitochondria, nitric oxide inhibits DDR signaling, decrease ATP levels, and protects cells from DNA damage-induced apoptosis. Conclusions: Our findings suggest that the tight coupling of glycolysis to mitochondrial oxidative metabolism in β-cells allows nitric oxide to decrease ATP levels and subsequently inhibit DDR signaling, and eventually protects β-cells from DNA damage-induced apoptosis. Significance: Targeting DDR signaling in cancer cells is exploited for cancer treatment. These findings may provide mechanistic insights into the potential role of intermediary metabolism as a contributor to the efficacy of DNA damaging agents in killing cancer cells. Funding source: National Institute of Diabetes and Digestive and Kidney Diseases grant DK-052194, National Institute of Allergy and Infectious Diseases, grant AI-044458 and a gift from the Forest County Potawatomi Foundation.