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POSTER #1

INHIBITION OF SEC24D DECREASES EXOSOME RELEASE OF THE TUMOR SUPPRESSOR MIR-605 IN RENAL CELL CARCINOMA

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Background: MicroRNA 605 (miR-605) has been recently reported as a putative tumor suppressor and its overexpression decreases tumor cell proliferation, migration and clonogenicity. To date, the role of miR-605 in renal cell carcinoma (RCC) has not been investigated. We recently showed the decrease of circulating miR-605 in serum of clear cell renal cell carcinoma (ccRCC) patients who responded to the treatment with the histone deacetylase (HDAC) inhibitor vorinostat, and the VEGF blocker bevacizumab. The current study was designed to investigate the expression of miR-605 and understand the mechanism(s) responsible the extracellular release of miR-605 using RCC cells. **Methods:** 786-0 cells were treated with and without vorinostat for 24h and condition media were collected, briefly centrifuged to settle the cells and debris, and processed to isolate exosomes using the ExoQuick exosome isolation kit (Systems Biology, CA). Purified exosomes and 786-0 cells were used to isolate RNA and further prepared cDNA to utilize for quantitative RT-PCR analysis. Expression of miR-605 in exosomes and cells was determined by Quantitative RT-PCR using TaqMan MicroRNA Assays with miR-605 primers obtained from Applied Biosystems, NY. To determine the role of secretory protein 24 family member D (SEC24D), a catalytic component of coat protein complex (COPII) involved in the secretory pathway, 786-0 cells treated with vorinostat were used to determine SEC24D expression by QRT-PCR and Western blot analysis. TCGA data was used to determine correlation of SEC24D expression clear cell renal cell carcinoma patients' survival. **Results:** Vorinostat treatment resulted in a significant decrease of miR-605 expression in exosomes and increase (100 fold) of intracellular expression. Furthermore, the increased intracellular miR-605 was associated with the inhibition of SEC24D mRNA and protein expression in 786-0 cells treated with vorinostat. Cancer Genomic data analysis of c-Bioportal from MSKCC of TCGA revealed the overall poor survival of ccRCC patients with alteration of SEC24D. **Conclusion:** Taken together, our preliminary data suggest that the HDAC inhibitor vorinostat inhibits SEC24D and exosome mediated extracellular secretion of miR-605 in RCC cells. These results suggest that vorinostat treatment retained intracellular miR-605 that target genes involved in cell survival and proliferation in RCC. Further studies will evaluate circulating miR-605 as a predictive biomarker to determine the efficacy of vorinostat in ongoing trials with RCC patients.

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POSTER #2

ESTABLISHING A NOVEL PERITONEAL CARCINOMATOSIS MOUSE MODEL FOR SURVIVAL OUTCOME ASSESSMENT OF ESOPHAGEAL ADENOCARCINOMA

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Background: Esophageal adenocarcinoma (EAC) has become the predominant type of esophageal cancer in United States. Overall 5 year survival rate of EAC is below 20% and most patients present with locally advanced or widespread metastatic disease, where current treatment is largely ineffective. Therefore, new therapeutic approaches are urgently needed. Improvement of esophageal adenocarcinoma patient outcome requires well-characterized animal models in whichto evaluate novel therapeutics. In this study we aimed to establish a peritoneal dissemination esophageal adenocarcinoma xenografts mouse model that would support survival outcome analyses.

Methods: To find the best candidate cell line from 7 esophageal adenocarcinoma cell lines of various origin, we injected them intraperitoneally and subcutaneously into severe combined immunodeficiency (SCID) mice and examined the tumor progression and survival outcomes. Human esophageal adenocarcinoma cell lines of Caucasian/Hispanic origin ESO26, OE33, ESO51, SK-GT-2, OE19, OACM5.1C and Flo-1 originating from gastroesophageal junction, distal esophagus and gastric cardia/fundus were injected intraperitoneally/subcutaneously into SCID mice. The peritoneal/xenograft tumor formation and mouse survival were compared among different groups.

Results: All cell lines injected subcutaneously formed tumors within 3 months at variable rates. All cell lines except OACM5.1C formed intraperitoneal tumors within 3 months at variable rates. Mean animal survival with peritoneal dissemination was 108 days for ESO26 cells ($5X10^6$), 50 days for OE33 cells ($5X10^6$), 88 days for ESO51 cells ($5X10^6$), 76 days for SK-GT-2 cells ($5X10^6$), 55 days for OE19 cells ($5X10^6$), 45 days for OE19 cells ($10X10^6$) and 82 days for Flo-1 cells ($5X10^6$). Interestingly, all mice injected with OE19 cells (7/7, $5X10^6$ and 5/5, $10X10^6$)had bloody ascites and metastatic implants at the gastric cardia, porta hepatis and liver. The mean survival time of these animals was significantly shorter (45 days for $10X10^6$ cells).

Conclusion: Peritoneal esophageal adenocarcinoma xenografts were successfully established after intraperitoneal injection of OE19 cells and this animal model of peritoneal dissemination for survival outcome will provide a useful survival outcome assessment model for evaluation of cancer therapeutics in experimental esophageal adenocarcinoma.

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POSTER #3

TO QUANTIFY A DNA PHOTOLESION POTENTIALLY INVOLVED IN SKIN CANCER DEVELOPMENT VIA LC/MS AND IMMUNOASSAY

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The dominant DNA photo-damage product detected in UV irradiated bacterial endospores is 5-thyminyl-5,6-dihydrothymine, which is commonly referred to as the spore photoproduct (SP). SP accounts for > 95% DNA photolesion formed in UV irradiated spores; its formation in other species including humans has not been investigated mainly due to the lack of effective SP analysis means. Moreover, it requires 14 steps to chemically synthesize SP which is needed as the standard for SP analysis; such a lengthy synthesis represents an almost insurmountable barrier preventing a biological laboratory from conducting SP studies.

Taking advantage of our strong synthetic ability, we have carried out the SP synthesis. Moreover, using tri-deuterium labeled dinucleotide d_3-TpT, we prepared the d_3-SP via solid state photoreaction. After digesting the genomic DNA using an enzyme cocktail, we added a known amount of d_3-SP as an internal standard to the DNA digest, which allows us to reliably quantify the unlabeled SP formed in the genomic DNA of UV irradiated keratinocytes and skin tissues via HPLC/MS assay using a triple quadrupole mass spectrometer. Our results indicate that SPs may account for as many as 10% of total thymine dimers formed in the human skin. Considering that SP was implied to be highly mutagenic, our data suggest that SP may be an important player in the development of skin cancer.

To further facilitate the SP photobiology studies, we conjugated an SP-containing oligonucleotide to a carrier protein again via chemical synthesis. We then used the oligonucleotide-protein conjugate as the antigen for mouse immunization to produce a monoclonal SP antibody. Our ELISA results show that our antibody is highly specific to SP, which opens the door for SP in situ studies via immunoassays including isolation of SP-containing DNA fragments by immunoprecipitation, which then enables the elucidation of SP formation sites in the human genome via high throughput sequencing. Such studies, when coupled with the bioinformatics analysis of the mutational data obtained from skin cancer patients, may allow us to reveal the exact roles of SP in the induction of skin cancer.

Furthermore, the LC/MS assay and antibody-based immunoassay developed for SP studies represent a general approach for the analysis of any given DNA lesion. Because mutations induced by DNA lesions are the major cause for cancer development, our research here may provide a general methodology to the field for the investigation of other DNA lesions.

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POSTER #4

TLR9 IS INDISPENSABLE FOR MR1-MEDIATED BACTERIAL ANTIGEN PRESENTATION

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Mucosal-associated innate T (MAIT) cells are conserved T cells that express a semi-invariant T cell receptor (TCR; Va7.2 in human and Va19 in mice). The development of MAIT cells requires the antigen (Ag) presenting major histocompatibility complex (MHC)-related molecule, MR1, as well as commensal bacteria, because germ-free or MR1-deficient animals have few MAIT cells. B cells were the first identified (and the most prominent) antigen presenting cells (APCs) for MAIT cells. Previous studies have shown that MR1 is ubiquitously expressed, but is mostly localized in the cytosol. B cells normally express very few MR1 molecules on the cell surface. Upon a bacterial infection, MR1 translocates to cell surface with loaded Ag, interacts with the invariant TCRa and activates MAIT cells. However, it remains unknown how MR1 molecules are processed and loaded with bacterial Ag in APCs. We have found that treating B cells with a TLR9 agonist CpG increases MR1 surface expression. The upregulation of surface MR1 was blocked by protein transport inhibitor Brefeldin A, suggesting that endocytic trafficking stimulated by TLR9 activation is important for the surface expression of MR1 in B cells. Further, knocking-down TLR9 expression by shRNA reduces MR1-mediated activation of MAIT cells. Overall, our results indicate that TLR9 is important for MR1-mediated bacterial Ag presentation. Further studies are needed to elucidate how TLR9 controls MR1 endocytic trafficking and bacterial Ag processing and/or loading onto MR1.

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POSTER #5

NOVEL BIOMARKERS AND MOLECULAR ALTERATIONS FOR BREAST CANCER INITIATION AND SUSCEPTIBILITY

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Background: Despite significant advances in diagnosis and treatment, breast cancer remains the leading cause of cancer-related death in women worldwide. Therefore, there is a *critical need* to identify molecular mechanisms responsible for cancer initiation and progression.
To date, there have been attempts to identify biomarkers that can predict progression of pre-malignant lesions (i.e. DCIS) to invasive carcinoma. Our study aims to identify earliest markers of breast cancer initiation.

Methods: We evaluated local (breast tissue) and systemic (serum/plasma) molecular alterations associated with breast cancer susceptibility using the unique resources available at the Komen Normal Tissue Bank at IUSCC. Human specimens (serum/plasma and breast tissue) donated by women before their cancer was clinically detectable were used to identify circulating biomarkers that are associated with breast cancer risk. Specimens from age-matched controls were used for comparison.Sera/plasma were analyzed for circulating miRNAs and telomeric level in the cell-free circulating DNA (cfDNA). We employed next generation sequencing to obtain transcriptome in the "normal" breast of women who eventually developed breast cancer and age-matched control normal breast.

Results/Discussion: Serum/plasma of women who developed breast cancer showed variation in circulating miRNAs as well as telomeric cfDNA levels as compared to the healthy control group. Among 385 microRNAs detected in circulation, 10 miRNAs were present at different levels in the susceptible group. In addition, susceptible group displayed reduced levels of plasma telomeric cfDNA and telomere shortening in breast epithelium.

Transcriptome analysis of microdissected breast epithelium and stroma revealed molecular alterations in the "normal" breast tissue associated with cancer development. Preliminary data suggest inflammatory response, transcription regulation and lipid metabolism as major mechanisms associated with breast cancer initiation.

Conclusion/Impact: Functional analysis of biomarkers identified in this study may help in cancer risk assessment and improvement of preventive therapy.

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POSTER #6

ALTERATION OF WARBURG METABOLISM AND NF-KB SIGNALING ENHANCES PANCREATIC CANCER X-RAY SENSITIVITY

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Pancreatic cancer has a survival rate of less than 5% five years after diagnosis. Treatment for pancreatic cancer consists of surgery, chemotherapy, and radiation therapy. Despite this multimodality approach local regional reoccurrence of pancreatic cancer is common. In many cases pancreatic cancer recurrence is due to the therapeutic resistance of pancreatic cancer cells. While the genetics of pancreatic cancers are highly complex, about 90% of all pancreatic cancers exhibit a constitutively active KRAS oncogene. Common characteristics of this mutation are constitutive activation of the pro-survival transcription factor NF-kB and increased activity of pyruvate dehydrogenase kinase. NF-kB controls cell survival, angiogenesis, and invasion while PDH kinase causes the shift commonly seen in cancer metabolism to aerobic glycolysis (Warburg metabolism). We hypothesized that by blocking the pro-survival signals from NF-kB with the inhibitor Dimethylamino-parthenolide (DMAPT) and by shifting the metabolism from Warburg metabolism back to oxidative metabolism by using Dichloroacetate (DCA that we can increase the effectiveness of radiation therapy in treating pancreatic carcinomas clinically. This study examined the effectiveness of DMAPT and DCA to induce cytotoxicity and increase radiation-induced cell killing in human pancreatic cancer cells AsPc-1, BxPc-3, and MIA PaCa-2. We found that dual treatment of the three pancreatic cancer cell lines: altered Warburg metabolism; decreased cell viability; did not significantly alter cell cycle distribution and increased radiation-induced cell killing after single and fractionated doses through inhibition of split dose repair. In summary, we have established that dual treatment with DMAPT and DCA is not only cytotoxic to pancreatic cancer cells but also significantly enhances radiation-induced cell killing of human pancreatic cancer cells in vitro.

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POSTER #7

MOLECULAR INSIGHTS OF PATHWAYS RESULTING FROM TWO COMMON PIK3CA MUTATIONS IN BREAST CANCER

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Abstract:

The phosphatidylinositol 3-kinase (PI3K) pathway is activated in ~70% of breast cancers. PIK3CA gene mutations or amplifications that affect the PI3K p110a subunit account for activation of this pathway in 20-40% of cases, particularly in estrogen-receptor alpha (ERa)-positive breast cancers. AKT family of kinases, AKT1-3, are the downstream targets of PI3K and these kinases activate ERa. Although several inhibitors of PI3K have been developed, none has proven effective in the clinic, partly due to an incomplete understanding of the selective routing of PI3K signaling to specific AKT isoforms. Accordingly, we investigated in this study the contribution of specific AKT isoforms in connecting PI3K activation to ERa signaling, and we also assessed the utility of using the components of PI3K-AKT isoform-ERa signaling axis as predictive biomarkers of response to PI3K inhibitors. Using a variety of physiologically relevant model systems with defined natural or knock-in PIK3CA mutations and/or PI3K hyperactivation, we show that PIK3CA-E545K mutations (found in ~20% of PIK3CA-mutant breast cancers), but not PIK3CA-H1047R mutations (found in 55% of PIK3CA-mutant breast cancers), preferentially activate AKT1. Our findings argue that AKT1 signaling is needed to respond to estrogen and PI3K inhibitors in breast cancer cells with PIK3CA-E545K mutation, but not in breast cancer cells with other PIK3CA mutations. This study offers evidence that personalizing treatment of ER-positive breast cancers to PI3K inhibitor therapy may benefit from an analysis of PIK3CA-E545K-AKT1-estrogen signaling pathways.

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POSTER #8

ROLE OF GFI1 TRANSCRIPTION FACTOR IN MYELOMA CELLS GROWTH AND SURVIVAL

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Multiple myeloma (MM) is a plasma cell malignancy that is currently incurable for the overwhelming majority of patients. We reported that MM cells induce expression of the transcriptional repressor, Growth independent factor 1 (Gfi1), in bone marrow stromal cells (BMSC) that results in prolonged suppression of osteoblast differentiation by repressing transcription of the *Runx2* gene. Since Gfi1 is an anti-apoptotic factor in other hematologic malignancies, we hypothesized that Gfi1 has an important pro-survival role in MM cells by blocking apoptosis and regulating MM cell growth and survival.
CD138+ cells isolated from MM patients and healthy donors and human MM cell lines were tested for Gfi1 expression levels and the effects of Gfi1 knock down (KD) on MM cell survival by transduction with pLKO.1-puro lentivirus vectors encoding Gfi1 or non-mammalian shRNAs. We characterized HDAC inhibitor (HDACi)-induced changes in p53 enrichment at the *Noxa*, *PUMA* and *p21*gene promoters by ChIP assays and the effects on acetylation of Gfi1 on its p53 binding capacity in MM cells treated withtrichostatin A and nicotinamide.

We found that Gfi1 is highly expressed, at the mRNA and protein level, in $CD138^+$ cells from MM patients and MM cell lines compared with $CD138^+$ cells from normal donors. Gfi1 expression was further increased in MM cells by exogenous IL-6, TNFa and sphingosine-1-phosphate (S1P). KD of *Gfi1* inhibited the growth and induced apoptosis of MM cells, as measured by increased mRNA levels of *Bax*, *PUMA*, *Noxa*, increased cleaved caspase 3 protein levels and decreased protein levels of Mcl-1 and c-Myc. Gfi1's inhibition of apoptosis resulted in part from binding of Gfi1 to p53 which blocked p53's access to its pro-apoptotic targets promoters. HDACi treatment inhibited Gfi1's suppression of apoptosis by acetylation of Gfi1 at the K292 residue, which prevented Gfi1-p53 binding and subsequent enrichment of p53 at *Noxa*, *PUMA* and *p21* promoters in MM cells. $CD138^+$ cells from MM patients also showed increased levels of *SphK1* mRNA compared with normal donors and *SphK1* mRNA levels and protein activity were further increased in MM cells by exogenous IL-6 and S1P. Further, direct co-culture of MM cells with BMSC enhanced *Gfi1,IL6* (3 fold) and *SphK1* (2.5 fold) mRNA levels in MM cells. Importantly, *Gfi1* KD in MM cells profoundly downregulated *SphK1* mRNA levels and reduced expression of phospho-SphK1, suggesting that Gfi1 enhances MM growth in part *via* increasing expression and activity of SphK1.

Taken together, our results suggest that Gfi1 may act as a key regulator of MM growth and survival through its regulation of p53 and SphK1 activity, and that targeting Gfi1 may be a novel therapeutic strategy for MM patients.

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POSTER #9

IL-33/ST2 TRIGGERING OF IL-9–SECRETING T CELLS: FROM PROTEOMICS TO THERAPEUTICS

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As one of the most validated immunotherapies to date, allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative option for high-risk hematological malignancies, particularly acute myeloid leukemia (AML). The immunotherapeutic activity of allo-HCT is known as the graft-vs-leukemia (GVL) activity. However, GVL activity is often accompanied by T-cell reactivity to allo-antigens in normal host tissues, which leads to graft-versus-host disease (GVHD), another major cause of death after HCT. Therefore, there is a great unmet need to improve the current process of allo-HCT through increasing the GVL activity and decreasing GVHD. We have shown that an elevated plasma level of soluble (s)ST2 in HCT patients is a risk factor for severe GVHD. ST2 blockade reduces sST2-producing T cells while maintaining protective membrane (m)ST2-expressing T cells such as type 2 T cells and regulatory T cells during aGVHD. A novel IL-9 producing T helper subset, Th9, expresses mST2. Furthermore, Th9 cells and IL-9 producing CD8 cytotoxic (Tc9) cells have higher antitumor activity than Th1 and Tc1 cells in melanoma models. Interestingly, we found that the addition of IL-33 during T9 differentiation ($T9_{IL\text{-}33}$) increased expression of mST2 and PU.1, a transcription factor that promotes IL-9 production in both CD4 and CD8 T cells. Adoptive transfer of $T9_{IL\text{-}33}$ cells with bone marrow cells in a murine model of HCT resulted in less severe GVHD compared to transfer of $T9_{IL\text{-}33}$ cells generated from $ST2^{-/-}$ or $IL\text{-}9^{-/-}$ T cells. Furthermore, cytolytic molecules implicated in anti-leukemic activity (granzyme B and perforin) were upregulated in WT $T9_{IL\text{-}33}$ cells while $ST2^{-/-}$ $T9_{IL\text{-}33}$ cells did not. WT $T9_{IL\text{-}33}$ cells also exhibited higher anti-leukemic activity when cultured with a retrovirally transduced MLL-AF9 leukemic cells in comparison to $ST2^{-/-}$ $T9_{IL\text{-}33}$ in in vitro cytolytic assays. *In vivo* GVL experiments with MLL-AF9 AML and adoptive transfer of $T9_{IL\text{-}33}$ cells resulted in increased survival compared to syngeneic mice, allo-HCT mice transferred with T1 cells, or T9 cells or $T9_{IL\text{-}33}$ cells generated from $ST2^{-/-}$ or $IL\text{-}9^{-/-}$ T cells. Human T9 cells are poorly studied. Here we demonstrate that IL-33 has the same impact on human T cells through enhancing IL-9 and Granzyme B production compared to T9 cells as well as demonstrated higher *in vitro* anti-leukemic cytolytic activity when incubated with MOLM14, an aggressive AML tumor cell line

expressing FLT3/ITD mutations. Importantly, CD8a expression was upregulated in WT $T9_{IL\text{-}33}$ (bothCD4 and CD8) cells in comparison to $ST2^{-/-}$ $T9_{IL\text{-}33}$ cells, and CD8a blockade with neutralizing antibody during allogeneic specific $T9_{IL\text{-}33}$ differentiation reduced cytotoxicity of both murine $T9_{IL\text{-}33}$, and human $T9_{IL\text{-}33}$ cells as compared to the cell blocked with isotype control, suggesting that CD8a was associated with MHC-restricted cytolytic activity in $T9_{IL\text{-}33}$ cells. Altogether, our observations demonstrated that adoptive transfer of $T9_{IL\text{-}33}$ cells represents a promising cellular therapy following HCT.

Basic Science ***Faculty***

POSTER #10

TARGETING THE MDM2-AKT SIGNALING NETWORK IN COMBINATION WITH TEMOZOLOMIDE IMPROVES EFFICACY IN ECTOPIC AND ORTHOTOPIC PATIENT-DERIVED GLIOBLASTOMA XENOGRAFT MODELS

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An elusive goal in the treatment of patients with glioblastoma multiforme (GBM) is the development of therapeutic strategies that prevent emergence of therapy-resistant tumor cells. Our laboratory has identified a novel combination therapy that targets the Akt-Mdm2 signaling network in the context of genotoxic temozolomide (TMZ) therapy. Inhibitors were specifically selected based on structural properties related to blood-brain barrier permeability, and pharmacokinetic profiles of plasma, normal brain, flank/ectopic brain tumors, and intracranial tumors. Isobolograms and combination indices indicated that Akt (GDC0068) and Mdm2 (RG7112) inhibitors either together or in combination with TMZ, resulted in synergistic inhibition of cell growth in both mtp53 and wt53 GBM primary patient cell lines (GBM10, GBM43, and MHBT32). When RG7112 and GDC0068 (at a ratio of 0.5:1) were combined with TMZ, strong synergism was evident at multiple dose-ratios. As downstream measures of blocking Akt and Mdm2, activation of the Forkhead box O-class 1/3a (FoxO) transcription factors and increased p53/p73 proteins were evident following TMZ+RG7112+GDC0068. For in vivo studies, an intermittent dosing regimen of TMZ+RG7112+GDC0068 was developed to avoid normal tissue toxicity. Mice with TMZ-resistant GBM43 ectopic tumors were treated 3 × weekly for 3 weeks. Combination of TMZ+RG7112+GDC0068 significantly delayed tumor progression compared to other treatments (n=8-9 per group, $p < 0.05$). TMZ+GDC0068 and TMZ+RG7112+GDC0068 significantly reduced tumor volume relative to TMZ, and on the last day with surviving TMZ+GDC0068-treated mice (2500 mm3 endpoint), tumor volumes were significantly reduced in TMZ+RG7112+GDC0068 compared to TMZ+GDC0068 ($p<0.05$). In mice with GBM43 intracranial tumors, survival was significantly increased by GDC0068+RG7112 (13 days), and further increased by TMZ+RG7112+GDC0068 (24 days) compared to TMZ alone. These data suggest that blocking the Mdm2-Akt signaling network in the context of TMZ-mediated DNA damage holds therapeutic promise. Studies to identify mechanism and appropriate biomarkers of response are in progress.

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POSTER #11

P62-ZZ DOMAIN SIGNALING INHIBITION PREVENTS MM CELL-INDUCED EPIGENETIC REPRESSION AT THE RUNX2 PROMOTER AND RESCUES OSTEOBLAST DIFFERENTIATION

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Multiple myeloma (MM) bone disease is characterized by lytic bone lesions that contribute to patient morbidity and mortality after patients are in complete remission. The mechanisms mediating this long-term osteoblast (OB) suppression are poorly understood. We hypothesized that MM cells induce epigenetic changes at the *Runx2* promoter in preOB bone marrow stromal cells (BMSC). We demonstrated that Gfi1, a transcriptional repressor of *Runx2* that is induced in BMSC by MM, directly binds to the *Runx2* promoter, recruiting chromatin corepressors and inducing epigenetic repression of *Runx2* in preOB, thereby preventing OB differentiation.

We reported that p62 (sequestosome-1) in BMSC is critical for the formation of MM-induced signaling complexes that mediate OB suppression, and found that an inhibitor of the p62 ZZ domain, XRK3F2, blunted MM cell-induced *Runx2* suppression and *Gfi1* induction in murine preOB. In vivo, XRK3F2 induced new bone formation and remodeling in the presence of high tumor burden without altering bones without tumor.

We tested if XRK3F2 prevents the Gfi1-mediated epigenetic suppression of *Runx2* observed following MM exposure. ChIP analysis of murine preOB exposed to MM ± XRK3F2 demonstrated that XRK3F2 prevented MM-induced *Runx2* promoter Gfi1 occupancy, recruitment of the chromatin corepressor HDAC1, and histone de-acetylation. Coculture experiments using human MM cells and murine preOB showed that XRK3F2 both prevents and reverses *Gfi1* upregulation. Importantly, long-term culture of primary MM patient BMSC with XRK3F2 increased acetylation at the *Runx2* promoter, allowing rescued osteogenic differentiation and mineral deposition.

We conclude that XRK3F2 blocks MM-induced signaling, reducing recruitment of Gfi1 and its corepressor HDAC1 to the *Runx2* promoter, and preventing MM-induced epigenetic suppression of *Runx2*. These results suggest that targeting p62-ZZ as a therapeutic strategy in MM may reverse *Gfi1* upregulation, rescuing MM-induced epigenetic suppression of *Runx2* in BMSC and healing MM bone lesions.

Basic Science ***Faculty***

POSTER #12

MEMBRANE HYPEREXCITABILITY OF DORSAL ROOT GANGLION NEURONS CAUSED BY ACUTE AND CHRONIC OXALIPLATIN TREATMENT

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Objectives: Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting neurotoxicity caused by chemotherapy drugs. CIPN is frequently associated with pain that significantly impairs the quality of life and work capability of cancer patients. Use of oxaliplatin for colorectal cancer is associated with a unique cold-sensitive acute pain in addition to the chronic pain, which is also seen for other CIPN-causing drugs. The objective of this work was to study the membrane excitability mechanisms contributing to acute and chronic pain caused by oxaliplatin in peripheral sensory neurons located at dorsal root ganglion (DRG).

Methods: The acute effects of oxaliplatin were studied by acute application of oxaliplatin to DRG neurons dissociated from adult C57BL/6 mice. To study the chronic effects of oxaliplatin, a total of four injections of oxaliplatin over one week were given to adult Sprague-Dawley rats. One week after the last injection, DRG neurons were dissociated. For both mouse and rat studied, small DRG neurons, most of them are nociceptive, were chosen for whole-cell patch clamp recording. Membrane excitability was recorded in current-clamp mode.

Results: Both acute and chronic treatment of oxaliplatin increased membrane excitability of small DRG neurons. Acute treatment depolarized the membrane potential, decreased the action potential threshold, and increased action potential numbers. The effects of acute treatment were completely reversed by retigabine, a FDA approved anti-epileptic drug and a KCNQ/M channel opener. In contrast to acute treatment, chronic treatment did not change resting membrane potential. However, chronic treatment decreased the voltage threshold of action potentials and caused spontaneous firing of action potentials that was not seen in control groups.

Conclusions: Depolarization of membrane potential of nociceptive DRG neurons might contribute to acute pain caused by oxaliplatin. Retigabine might reverse oxaliplatin-caused acute pain by hyperpolarization of membrane potential of nociceptive DRG neurons. Decreased voltage threshold of action potentials, which may be caused by sensitized threshold sodium channels, might contribute to the chronic pain caused by oxaliplatin treatment. Targeting membrane depolarization and threshold sodium channels might be a useful strategy to treat oxaliplatin-induced acute and chronic pain, respectively.

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Basic Science ***Faculty***

POSTER #13

MUSCLE DYSFUNCTION IN IMMUNE COMPETENT MICE WITH OSTEOLYTIC BREAST CANCER IN BONE IS ASSOCIATED WITH SKELETAL MUSCLE OXIDATION OF RYR1

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Cancer-associated muscle weakness is an important paraneoplastic syndrome for which there is currently no treatment. Using a mouse model of human breast cancer bone metastases (MDA-MB-231) we have shown that bone-derived TGF-ß, released as a result of tumor-induced osteolysis is responsible for skeletal muscle weakness. Weakness is due to an increase in oxidation of skeletal muscle ryanodine receptor/calcium release channel (RyR1) via NADPH oxidase 4 (Nox4) leading to sarcoplasmic reticulum (SR) calcium leak.

To determine if similar bone-muscle interactions were evident in immune competent mice with tumor-induced bone destruction, we tested muscle function in the 4T1 mouse model of breast cancer in bone. 4T1 cells injected directly into the tibia of Balb/C mice led to osteolytic bone destruction, weakness and cachexia. *In vivo f*at and lean content were decreased in mice with 4T1 osteolytic cancer in bone. Forelimb grip strength was reduced and *ex vivo* contractility of the extensor digitorum longus (EDL) muscle showed a significant reduction in specific force in tumor bearing mice compared with non-tumor bearing controls. *Ex vivo* force measurements, biochemistry, and gene expression, were performed on muscle contralateral from the tumor-bearing limb to avoid interference from tumor infiltration and to determine if these effects were systemic. Biochemical analysis revealed that RyR1 on the SR, a key protein involved in skeletal muscle excitation-contraction coupling, was oxidized and depleted of the stabilizing subunit, calstabin1. SMAD3 phosphorylation was increased in muscle from mice with 4T1 osteolytic cancer in bone, suggesting a role for TGF-ß. Nox4 is a TGF-ß target and a constitutively active source of reactive oxygen species (ROS) in muscle. Nox4 mRNA expression and Nox4-RyR1 binding was increased in muscle, providing a potential source of RyR1 oxidation. These data show that immune competent and immune deficient mice share similarities in skeletal muscle weakness in the setting of osteolytic breast cancer in bone.

Basic Science ***Faculty***

POSTER #14

ANDROGEN RECEPTOR EXPRESSION IS ASSOCIATED WITH SUNITINIB RESISTANCE IN RENAL CELL CARCINOMA MODELS

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Background: Androgen receptor (AR) expression has been reported in renal cell carcinoma but its biological role remains elusive. Sunitinib is a potent anti-angiogenic drug approved for the treatment of advanced renal cell carcinoma (RCC). However, eventually RCC tumors develop drug resistance. We have hypothesized that tumor cells resistance to sunitinib is associated with kinome reprograming and anti-apoptotic genes upregulation. To date, there is no report on the association between AR expression and resistance to TKI such as sunitinib in RCC. Our study was designed to investigate the role of AR in sunitinib resistance in RCC. We used our previously reported sunitinib resistant ccRCC cells and patient derived xenograft models. **Methods:** Human RCC cell lines; 786-0, 786-0R (sunitinib resistant), C2, C2R (sunitinib resistant), ACHN and Caki 2 were utilized to determine sensitivity or resistance to sunitinib. Patient derived xenograft (PDX) models of advance and metastatic RCC and RCC cell lines, sensitive and resistant tumors were used to detect AR expression by qRT-PCR, immunohistochemistry and Western blot analysis. Reverse phase protein array (RPPA) was used to assess 249 protein including AR in sunitinib sensitive and resistant tumors. **Results:** Our qRT-PCR data showed an increase by 1000 folds in mRNA levels of AR in our sunitinib resistant cell lines. Similarly, RPPA data revealed AR to be increased in sunitinib resistant RCC PDX tumors. This observation was confirmed by Western blot analysis. **Conclusion:** Overall our data suggest the potential role of AR and its association with resistance to sunitinib. Ongoing studies are testing the *in vitro* and *in vivo* combination treatment of RCC models with sunitinib and AR antagonists.

Basic Science ***Graduate Student***

POSTER #15

THE ROLE OF WNT5A SIGNALING PATHWAY IN EPITHELIAL OVARIAN CANCER PROGRESSION

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Ovarian cancer (OvCa) is the fifth leading cause of cancer death among US women and has the highest mortality rate of all the gynecologic tumors. Unlike other cancers, OvCa rarely metastasizes hematogenously. Instead, the transformed ovarian epithelial cells disseminate from the primary site into the peritoneal cavity in form of single cells or multicellular aggregates. The peritoneal fluid facilitates the attachment of the disseminating cells to peritoneum and omentum, where they establish their metastatic sites.

WNT5A is a non-canonical WNT ligand that is critical in normal development. WNT5A binds to different receptors and activates several downstream signaling pathways that regulate cell growth, polarity, differentiation and migration. In cancer, WNT5A can act as a tumor suppressor or an oncogene. The role of WNT5A in OvCa is still questionable, as some studies show that WNT5A induces ovarian cancer progression, while other studies state that WNT5A is a tumor suppressor that induces senescence in ovarian cancer cells. Besides the contradicting data about WNT5A in ovarian cancer, its activated pathways and mechanisms in ovarian cancer cells are largely unknown.

In this study, we aim to elucidate the roles played by WNT5A in epithelial ovarian cancer cells and explore its activated pathways. In our data, we show the presence of high levels of WNT5A in ascites from ovarian cancer patients with advanced stages. We also used human ovarian epithelial cell lines OVCAR3, OVCAR5 and OVCAR8 that have different endogenous levels of WNT5A and its binding receptors. WNT5A induces ovarian cancer cells invasion in trans-well invasion assay. WNT5A also induces the formation of filopodia in ovarian cancer cells, which is an important event in ovarian cancer cell migration. Moreover, WNT5A signaling regulates the expression of genes correlated with metabolism, inflammation and invasion.

Overall, our data suggests that WNT5A plays an oncogenic role in epithelial ovarian cancer cells. More experiments exploring WNT5A activated pathways and effects in epithelial ovarian cancer cells are underway.

Basic Science ***Graduate Student***

POSTER #16

INVESTIGATING THE ROLE OF THE SHH PATHWAY IN MYOGENESIS AND REGULATION OF ADULT SKELETAL MUSCLE HOMEOSTASIS

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Skeletal muscle atrophy can occur with inactivity, malnutrition, or in chronic diseases such as cancer, organ failure, and AIDS. Muscle wasting reduces quality of life, decreases response to therapy, and contributes directly to mortality. Our lab has substantial data implicating the Sonic Hedgehog (Shh) pathway as a causal factor in muscle wasting. However, the mechanisms by which the Shh pathway regulates muscle homeostasis have yet to be determined.
In vitro data using C2C12 myotubes showed that agonists/inhibitors of the Shh pathway decreased/increased the number of nuclei per myotube respectively. This suggests that the pathway may play a role in determining the differentiation potential of myoblasts. *In vivo*, Ptch1 haploinsufficient ($Ptch1^{+/lacZ}$) mice exhibit increased pathway activity in the skeletal muscle. Analysis of these mice revealed a hypotrophic muscle phenotype. Interestingly, $Ptch1^{+/lacZ}$ mice have significantly reduced grip strength compared to wild-type littermates (WT), which progressively worsens with age. This indicates that increased pathway activity may lead to a functional impairment of muscle. Shh is a pathway known to regulate stem cell proliferation and differentiation. Primary myofibers were isolated from $Ptch1^{+/lacZ}$ and WT mice and stained for the satellite cell marker Pax7. Immunofluorescence analysis showed that $Ptch1^{+/lacZ}$ mice had far fewer Pax7 positive cells per myofiber. RNA-Seq analysis revealed a significant downregulation of genes associated with the cell proliferation (cyclin D1, Ki-67) in Ptch1 haploinsufficient mice. Moreover, genes coding for proteins of the differentiated muscle phenotype, including myosin heavy chain 1 and alpha actinin, also were decreased. Taken together, the data establishes that Ptch1 and the Shh signaling pathway play a role in maintaining muscle homeostasis. Understanding the mechanism by which these processes are regulated will be critical in determining the pathways therapeutic potential for diseases associated with muscle wasting.

Basic Science ***Graduate Student***

POSTER #17

GLYPICAN-1 AS A MEDIATOR OF WNT SIGNALING IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC), a highly metastatic and aggressive malignancy, is the fourth leading cause of cancer death in the United States with a grim 5 year survival rate of 7-8%. Although several predominant mutations and dysregulated signaling pathways have been identified in PDAC, the precise mechanisms that drive tumor progression remain unclear. The canonical Wnt signaling pathway, which plays a pivotal role in normal embryonic development and tissue renewal, has also been implicated in several different types of human cancers. While mutations in the Wnt pathway are surprisingly rare in PDAC, aberrant activation of canonical Wnt signaling has been observed in an estimated 65% of human pancreatic tumors. Wnt7b, one of the 19 human Wnt ligands, is strongly overexpressed in PDAC and has been characterized as a potent mediator of pathogenic Wnt signaling in pancreatic cancer. In addition to excess Wnt7b, a heparan sulfate proteoglycan known as glypican-1 (GPC1) is overexpressed in PDAC and augments PDAC progression and metastasis by acting as a co-receptor for many heparin-binding growth factors. Glypicans have been shown to contain binding domains that resemble the Frizzled family of Wnt receptors and have been demonstrated to modulate Wnt signaling activity, suggesting a functional link between these two seemingly disparate entities. The demonstrated functional interaction of Wnts and glypicans, in addition to the known overexpression of Wnt7b and GPC1 in PDAC, suggest that Wnt7b and GPC1 may be acting in concert to augment PDAC progression. Accordingly, as the interaction of Wnt7b and GPC1 has never been investigated in any context, we have initiated a study to test the hypothesis that Wnt7b and GPC1 act synergistically to promote PDAC progression. First, we utilized CRISPR/Cas9 genome editing technology in T3M4 and AsPC-1 pancreatic cancer cells, which exhibit strong canonical Wnt signaling as well as high levels of both Wnt7b and GPC1, to generate clonal cell lines lacking Wnt7b, GPC1, or both Wnt7b and GPC1. These cell lines are being evaluated for differential Wnt signaling, proliferation, migration, invasion, and gene expression profiles. We also plan to investigate the interaction of GPC1 and Wnt7b *in vivo* using a syngeneic mouse model. The discovery of a meaningful functional link between Wnt7b and GPC1 would reveal a previously unknown interaction in PDAC and may provide novel therapeutic targets in PDAC.

Basic Science ***Graduate Student***

POSTER #18

POLYPHENOL-MEDIATED EPIGENETIC REACTIVATION OF TUMOR SUPPRESSOR GENE SEMA3A IN BREAST CANCER CELLS

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Alterations in DNA methylation occur in cancer, and may underlie silencing of genes with tumor suppressor functions. Reversing DNA methylation, thus potentially reactivating genes that combat cancer, constitutes a promising anti-cancer strategy. Interestingly, studies have indicated that certain dietary polyphenols, such as resveratrol (RSV) present in grapes, exert anti-cancer effects through epigenetic regulation of gene expression. However, those studies have been limited to candidate genes, and comprehensive and mechanistic insights are missing.

In the present study, following genome-wide DNA methylation analysis with Illumina 450K BeadChip array, we identified CpG sites within regulatory regions of tumor suppressor genes that are hypomethylated upon treatment of breast cancer cells with resveratrol. Non-invasive MCF10CA1h and invasive MCF10CA1a human breast cancer cell lines were used as an experimental model. Pyrosequencing and QPCR were performed to assess respectively methylation and expression of selected genes.

We identified 990 hypomethylated CpG sites in MCF10CA1h and 1,146 hypomethylated CpG sites in MCF10CA1a cells upon 9-day treatment with 15μM resveratrol as compared with control untreated cells (differential methylation=-0.05, nominal $p<0.05$, limma t-test). Those CpG sites corresponded to approximately 650 genes that were predominantly associated with tumor suppressor function in cancer. Based on magnitude of decreased methylation, location of a change in regulatory gene region, and gene candidacy as an important tumor suppressor, we selected *SEMA3A* as resveratrol target for further investigation. As the array data indicated, resveratrol led to reduction in methylation of *SEMA3A* promoter region. The 52% decrease in methylation of *SEMA3A* was confirmed by pyrosequencing in MCF10CA1a breast cancer cells.This coincided with 23% up-regulation of *SEMA3A* expression. In addition, the analysis of the effects on DNA methyltransferases (DNMTs) demonstrated that resveratrol decreases expression of *DNMT3A*. Down-regulation of this *de novo* DNA methylating enzyme may mediate decrease in methylation within tumor suppressor genes upon resveratrol exposure. Further studies will investigate the role of DNMT3A in methylation events at the promoter of *SEMA3A*.

These results demonstrate a role for polyphenol-mediated epigenetic modifications in reactivation of tumor suppressor genes in breast cancer and pave the way for further studies on the mechanisms behind these changes. It provides novel insight and supports epigenetic-targeting strategies as an effective anti-cancer approach.

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Basic Science ***Graduate Student***

POSTER #19

DETERMINING THE ROLE OF ELF1 IN PROSTATE CANCER

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Various members of the ETS transcription factor family have been shown to undergo chromosomal rearrangements in prostate tissue and are involved in tumor formation and progression. The characterization of the roles for these oncogenes in prostate tissue is well studied; however, the functions of the normally expressed ETS proteins in prostate tissue are not well understood. Genomic and phenotypic assays, along with bioinformatic analyses, will be combined to gain insight into the role of ELF1 in the prostate. Preliminary results indicate that ELF1 may act as a tumor suppressor within the prostate and compete with oncogenic ETS. Comparisons between the oncogenic ETS and ELF1 can then be used to better understand the mechanisms of how the ETS transcription factor family regulates oncogenicity and could help pave the way for therapeutics to inhibit prostate tumor progression through this family.

Basic Science ***Graduate Student***

POSTER #20

IMPAIRED SPINDLE ASSEMBLY CHECKPOINT IN VIVO PROMOTES MDS/AML IN A NOVEL MOUSE MODEL OF FANCONI ANEMIA

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The Fanconi anemia (FA) signaling network prevents tumorigenesis by protecting genomic integrity. Biallelic germline mutations in any of the FA genes cause Fanconi anemia, an inherited syndrome of genomic instability, bone marrow failure, and cancer predisposition. Heterozygous inborn mutations in certain FA genes increase the risk of breast/ovarian cancers, and somatic mutations of FA genes occur in malignancies in non-Fanconi patients. Thus, disruption of FA signaling promotes tumorigenesis in inherited genetic syndromes and in the general population.

The FA network functions as a genome gatekeeper throughout the cell cycle. In interphase, this network prevents mutagenesis by facilitating DNA damage repair upon exposure to genotoxic insults. FA signaling has also recently been implicated in many aspects of cell division, including the spindle assembly checkpoint (SAC) that ensures high-fidelity chromosome segregation. However, the *in vivo* contribution of abnormal mitosis to malignant transformation of FA-deficient cells remains unknown.

To determine whether error-prone chromosome segregation upon loss of FA signaling contributes to tumorigenesis, we generated a novel murine FA model by genetically weakening the SAC in the FA-deficient background. These mice exhibited increased chromosomal instability evidenced by elevated red blood cell micronucleation, increased frequency of chromosome missegregation in microscopy-based cytome assays, and augmented bone marrow karyotype instability. Importantly, unlike FA or SAC control mice, the FA-SAC mice were prone to premature death due to development of myelodysplasia and AML, recapitulating the manifestations of human Fanconi anemia.

This study provides *in vivo* evidence supporting the essential role of compromised chromosome segregation in the development of myelodysplasia and leukemia due to impaired FA signaling. Our findings may have implications for future therapies against FA-deficient chromosomally unstable cancers.

Basic Science ***Graduate Student***

POSTER #21

CHARACTERIZING BONE METASTASIS-DERIVED CELLS IN A 3D CO-CULTURE SYSTEM

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Bone is a frequent target of breast cancer metastasis, so addressing the paths by which breast cancers metastasize is incredibly important. Here we utilize two breast cancer-derived cell lines, tumor-derived MDA-MB-231 cells (TMD) and bone metastasis-derived MDA-MB-231 cells (BMD), to characterize behavioral differences that are associated with metastasis and to identify genes involved in allowing a tumor cell to metastasize to bone.

The two cancer cell types were compared under a variety of assays. First, a wound healing assay was performed to measure the differences in their migration, and TMD was found to migrate more quickly than BMD. Second, cancer cells were co-cultured with MC3T3-E1 osteoblast-like cells, and BMD cells were found to form smaller cancer colonies than TMD. Third, MC3T3 osteoblast-like cells were cultured with cancer cells to form 3D spheroids, and the BMD cells were found to integrate better in the spheroid. Finally, a gene microarray was utilized to identify genes that were differentially expressed in TMD and BMD cells. Two genes of interest, CDH12 and SSX1, were identified to be tested via RNA silencing in TMD and BMD cells.

Here we found that TMD cells were more migratory than BMD cells, while BMD cells readily integrated into spheroids composed of bone cells. Using 3D co-culture of bone and cancer cells can shed more light on the interactions involved in metastasis that 2D assays are unable to detect. Genes of interest were identified using RNA microarray. Future work will validate the role of these genes in the bone metastasis.

Basic Science ***Graduate Student***

POSTER #22

LINKING POLYPLOID MITOSIS TO CHROMOSOMAL INSTABILITY

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Chromosomal instability (CIN) is defined as increased rates of whole chromosome mis-segregation during mitosis. It is a common feature in cancer cells and is proposed to be associated with tumorigenesis, increased invasiveness, and resistance to cancer therapies. Polyploid cells are one hallmark of cancer, but the mechanisms that induce polyploidy and how they contribute to oncogenesis and CIN are not well understood. We found that MDA-MB-231 cells or HeLa cells could be induced into a polyploid state by treatment with SU6656, a drug recently shown to be a potent Aurora B inhibitor. Drug treatment resulted in mitotic spindles that were multi-polar and that contained high numbers of chromosomes. Upon drug washout, cells resumed mitosis, but this division was highly aberrant resulting in lagging chromosomes, chromosome bridges and failed cytokinesis. Based on FISH analysis of chromosome 2 and chromosome 7, we found that polyploid daughter cells have a heterogeneous karyotypic pool, indicating that these polyploid mitotic cells are highly chromosomally unstable. Previous studies have shown that several key mitotic genes are down-regulated in cells with increased ploidy caused by induction into the endocycle. Consistent with this idea, we found that the mitotic kinesin-14, HSET, was down-regulated in these cells upon return to mitosis. Cell lines that overexpressed HSET reduced multi-polarity in polyploid cells, consistent with observations showing that HSET is responsible for centrosome clustering in cells with centrosome amplification. FISH analysis revealed that HSET overexpression narrowed the variation in karyotypes seen with drug treatment alone. These results suggest that restoring expression of down-regulated genes in these polyploid cells may provide a mechanism to restore chromosomal stability. Overall our results suggest that polyploid cancer cells induced by mitosis inhibition are highly chromosomal unstable, causing a heterogeneous karyotypic pool in polyploid daughter cells, which may accelerate adaption to external stresses. Thus, polyploidy reversion in cancer cells may represent a survival mechanism for cancer cells in response to cancer therapies and may be a major contributor to disease relapse.

Basic Science ***Graduate Student***

POSTER #23

GAIN-OF-FUNCTION MUTANT P53 DRIVES THE DEVELOPMENT OF PRE-LEUKEMIC HEMATOPOIETIC STEM CELLS THROUGH REGULATING EPIGENETIC PATHWAYS

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Acute myeloid leukemia (AML) is an aggressive blood cancer with poor prognosis. AML is thought to arise from leukemia stem cells (LSCs); however, recent evidence suggest that the transforming events may initially give rise to pre-leukemic hematopoietic stem cells (HSCs), preceding the formation of fully transformed LSCs. Pre-leukemic HSCs have been shown to contribute to normal blood development and harbor a selective growth advantage compared to normal HSCs. Recently, acquired somatic gain-of-function (GOF) p53 mutations were identified in the blood of aged healthy individuals as well as AML patients, suggesting that p53 mutations may be early events in the pathogenesis of AML. Thus, it is important to investigate the role of mutant p53 in leukemia initiation and progression.

We found that some GOF mutant p53 proteins, including $p53^{R248W}$, $p53^{Y220C}$, and $p53^{R273H}$, enhanced the self-renewal potential of normal HSCs without affecting terminal differentiation. Further, we discovered that HSCs expressing mutant p53 show enhanced clonal expansion following genotoxic stress. These findings demonstrate that HSCs expressing mutant p53 share several key features of pre-leukemic HSCs. Although p53 mutations are limited in AML, p53 mutations do co-exist with mutations of epigenetic regulator ASXL1 in AML. Somatic *ASXL1*mutations occur in 10-30% of patients with myeloid malignancies and are associated with adverse outcome. To determine the synergy between mutant p53 and ASXL1 deficiency in leukemogenesis, we generated $p53^{R248W/+}$ $Asxl\text{-}1^{+/-}$ mice. We found that the expression of mutant p53rescued the self-renewal defect of $Asxl\text{-}1^{+/-}$ bone marrow cells in both primary and secondary transplantation assays, suggesting that mutant p53 may cooperate with Asxl-1 deficiency in the formation of leukemia stem cells.

To investigate how GOF p53 regulates HSC self-renewal, we performed transcript profiling assays to compare gene expression in HSCs isolated from $p53^{+/+}$ and $p53^{R248W/+}$ mice. We found that mutant p53 regulates the expression of several epigenetic regulators, including *EZH1*, *EZH2*, and *SETD2*, in HSCs. In addition, we found that there were increased levels of H3K27me3 and decreased levels of H3K36me3 in $p53^{R248W/+}$ HSCs compared to that of the $p53^{+/+}$ HSCs. Given that none of these epigenetic regulators are modulated by WT p53 in HSCs, we demonstrated that mutant p53 modulates epigenetic regulators in HSCs. To understand how mutant p53 enhances the self-renewal potential of ASXL1-deficient HSCs, we performed H3K27me3 ChIP-seq analysis in hematopoietic stem and progenitor cells (HSPCs) from $p53^{+/+}$, $p53^{R248W/+}$,$Asxl1^{+/-}$, and $p53^{R248W/+}Asxl1^{+/-}$mice. We found that $Asxl1^{+/-}$ HSPCs exhibited significantly low levels of H3K27me3 at promoters, and these defects were rescued in the mutant p53 background, demonstrating that mutant p53 maintains global H3K27me3 in the absence of ASXL1.

Collectively, we demonstrated that gain-of-function mutant p53 drives the development of pre-leukemic HSCs by a novel mechanism involving disruption of the epigenetic pathways.

Basic Science ***Graduate Student***

POSTER #24

CONCOMITANT TARGETING OF MET AND EGFR SIGNALING IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States, and is expected to become the second leading cause by 2030. Due to its late stage at clinical presentation and its highly aggressive nature, PDAC patients have a poor prognosis with a median survival of 6-7 months. In addition to frequent major driver mutations (KRAS, SMAD4, TP53, and CDKN2A) and a plethora of other low-frequency mutations, PDAC is associated with overexpression of tyrosine kinase receptors including EGFR and MET and their corresponding ligands. Bioinformatics analysis of the PDAC data set from The Cancer Genome Atlas (TCGA) revealed that ~8% of PDAC patients harbor upregulated MET gene expression. Furthermore, EGFRgene expression was upregulated in ~4% of patients. Importantly, patients that possessed alterations in METhad a statistically significant shorter survival than patients without alterations in MET. Addition of cabozantinib (MET inhibitor) and erlotinib (EGFR inhibitor) enhanced chemosensitivity to gemcitabine in 3-dimensional culture. Treatment with gemcitabine alone had no effect on survival of mouse models of PDAC. By contrast, gemcitabine together with cabozantinib and erlotinib (G.E.C) extended survival significantly in three different mouse models of PDAC. Importantly, gene set enrichment analysis of tumors from mice harboring Krasand TP53R172Hmutations (KPC) showed significant enrichment of MET and EGFR signatures compared to wild-type mice. We therefore sought to determine the effect of cabozantinib and erlotinib treatment without gemcitabine in KPC mice. This targeted therapy combination prolonged KPC mouse survival compared with gemcitabine alone. These results suggest that therapy targeting both MET and EGFR could improve survival of PDAC patients whose tumors exhibit activation of these pathways.

Basic Science ***Graduate Student***

POSTER #25

SYSTEMS BIOLOGY APPROACH TO OBTAIN SIGNIFICANT MODULES OF IMMUNE THERAPY AND COLORECTAL CANCER

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Colorectal cancer (CRC) is the second leading cause of cancer death in the United States. There has been a lot of research around genes influencing CRC, despite its extensive understanding on the genetic perspective and the emergence of drugs targeting these genes, the tumor progression could be hardly mitigated. However, immune therapy has recently been observed to be effective in CRC treatment and diagnosis. This study focuses on developing a statistically validated multi-feature analytical approach to identify immuno-oncology targets. The features considered in this study were gene expression, DNA methylation, concepts from literature and immuno-cancer pathways. The network algorithm will identify the potentially relevant immuno-oncology modules of CRC. For the study level-3 data (7.2 gigabytes) of gene expression and DNA methylation was obtained from The Cancer Genome Atlas. Around 9951 genes were identified to be significant from the gene expression data analysis and 19788 genes significant in DNA methylation data. The CRC and Immuno-oncology concepts were manually annotated from 50 peer reviewed articles. The output of the preliminary analysis could predict 95 concepts annotated to the 1587 significant genes and were integrated into the network. The top rank concepts in terms of genes associated were '*apoptosis*', '*transforming growth factor*', '*protein arginine methyltransferase*', '*carcinoembryonic antigen*' and '*methyl binding protein*'. The genes annotated with highest number of concepts were '*PRMT5*', '*CSF2*', 'CFLAR' and '*MLH1*'. These genes were observed in the literature as targets of CRC.

Basic Science ***Graduate Student***

POSTER #26

EVALUATION OF BRUTON'S TYROSINE KINASE AND PI3K P110DELTA IN MUTANT SHP2-INDUCED JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is a fatal childhood myeloproliferative neoplasm in which myeloid cells are overproduced and hematopoietic progenitors are hypersensitive to granulocyte-macrophage colony stimulating factor (GM-CSF). The only curative treatment is allogeneic bone marrow transplant, but even this rigorous therapy yields only a 50% relapse-free 5-year survival. A majority of patients have hyperactive Ras signaling, with the most common mutation being somatic gain-of-function (GOF) mutations in *PTPN11*, which encodes the protein tyrosine phosphatase Shp2. Previous work in the Chan lab has shown that the PI3K catalytic subunit p110δ is needed for both Akt and Erk hyperactivation, and promotes GOF Shp2-induced GM-CSF hypersensitivity and hyperproliferation, contributing to the progression of JMML. Based on the significant role of p110δ in promoting GOF Shp2-induced leukemia, we investigated potential tyrosine kinases that can cooperate with p110δ to promote Akt and Erk activation and lead to hyperproliferation of myeloid cells. Bruton's Tyrosine Kinase (BTK) has been identified as a critical molecule in lymphoid malignancies and the BTK inhibitor, ibrutinib, has been approved for use in patients with B cell leukemias. From studies done in the context of B cell receptor signaling, it is known that BTK is activated downstream of p110δ and then signals to PLC¿2 and PKC to promote ERK activation. However, BTK also phosphorylates B cell adaptor for PI3K (BCAP), allowing phospho-BCAP to bind to the regulatory p85α subunit and promote activity of PI3K. Therefore, we hypothesize that BTK signaling enhances p110d hyperactivation in a BCAP-dependent manner, thus promoting GOF Shp2-induced leukemia. BTK is hyperphosphorylated in bone marrow-derived macrophages with a mutant Shp2 knock-in. Using specific BTK and p110d inhibitors provided by AcertaPharma, we found that proliferation of GOF Shp2-expressing cells and phosphorylation of Akt and ERK are decreased cooperatively by the two inhibitors. Although there was no effect on PLC¿2 phosphorylation with either of the two inhibitors, we found that BCAP phosphorylation is increased in mutant Shp2 macrophages, suggesting BCAP as an alternative downstream target of BTK to signal to Akt and ERK. These studies are timely and significant, as the AcertaPharma inhibitors are currently being tested in combination for safety and efficacy in chronic lymphocytic leukemia patients, and therefore have the potential to be a novel treatment in patients with JMML.

Basic Science ***Graduate Student***

POSTER #27

THE MECHANISMS OF OXIDATIVE DAMAGE-INDUCED LOCALIZATION OF MISMATCH REPAIR PROTEIN HETERODIMER MSH2-MSH6, AND EPIGENETIC PROTEIN DNA METHYLTRANSFERASE 1 TO CHROMATIN

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Abstract

Elevated levels of reactive oxygen species at sites of chronic inflammation cause oxidative DNA damage and contribute to tumorigenesis. Tumors have many aberrant epigenetic alterations, many of which affect the expression of tumor suppressor genes. Epigenetic changes occur at sites of chronic inflamation, however it is not known how these epigenetic alterations are initiated. We hypothesize that the recruitment of epigenetic proteins to sites of oxidaitve damage through their interaction with mismatch repair (MMR) proteins are important for initiating epigenetic alterations. Here we demonstrated that after hydrogen peroxide treatment, MMR proteins MSH2 and MSH6 become localized to sites of oxidative DNA damage and this results in the recruitment of epigenetic silencing proteins such as DNA methyltransferase 1 (DNMT1) to chromatin, thereby reducing the expression of tumor suppressor genes. However, it is not known what mechanism drives oxidative damage-induced MSH2 and MSH6 nuclear localization and their chromatin binding. We demonstrated that removing the sequence of any one of the three nuclear localization signals (NLSs) in the MSH6 disordered region reduces the oxidative damage induced-nuclear localization of MSH2 and MSH6, suggesting the NLSs are important for hydrogen peroxide-induced nuclear translocation of MSH2 and MSH6. We also demonstrated that inhibiting Jak2 reduced the hydrogen peroxide-induced chromatin binding of MSH2, MSH6 and DNMT1. This finding suggests phosphorylation of MSH2 and/or MSH6 by Jak2 may be important for their interaction with oxidative DNA damage and reducing such phosphorylation by using Jak2 inhibitors may aboragate the interaction between MSH2/6 and DNA, thereby reducing the localization of DNMT1 to chromatin. Understanding the mechanism by which MSH2, MSH6 and DNMT1 become localized to chromatin after oxidative damage will potentially allow us to develop treatment strategies to reduce the initiation of aberrant epigenetic alterations during chronic inflammation and therefore reduce tumorigenesis in people with chronic inflammatory diseases.

Basic Science ***Graduate Student***

POSTER #28

THE ONCOGENE ZNF217 MAY BE REGULATED BY CELLULAR LOCALIZATION

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The oncogene and transcription factor ZNF217 is overexpressed in 20-30% of breast cancers. Its overexpression correlates strongly with poor prognosis in patients and causes accelerated tumor progression, metastasis, and chemoresistance in vivo. While several studies have begun to examine downstream targets of ZNF217, no studies have looked at the regulation of localization of this protein. The localization of ZNF217 may also have value as a biomarker to predict patient prognosis.

As determined by both immunohistochemistry and western analysis, we find that ZNF217 protein is predominantly nuclear but can be cytoplasmic in some human breast cancer tumors and cell lines. Smaller isoforms of ZNF217 are also present in human breast tumors and cell lines, but the importance of these smaller isoforms is still unknown. Our current efforts focus on further elucidating the underlying mechanisms of the localization of ZNF217 and in understanding the role of alternative ZNF217 isoforms in breast cancer progression. Using truncation mutants, we will determine regions of ZNF217 required for localization. Time-lapse microscopy of ZNF217 fluorescent fusion proteins will be used to determine if ZNF217 localization changes over the course of the cell cycle. The consequences of mislocalization of ZNF217 will also be studied.

Due to its aberrant localization in some human breast tumor samples, the localization of ZNF217 has the potential to be used as a clinical biomarker. Using the localization of ZNF217 in human breast tumors and the corresponding patient data, we can determine the usefulness of the localization of ZNF217 as a biomarker. The localization of ZNF217 may be used to predict patient prognosis, survival, or recurrence. Identifying the mechanism and regulation of the localization of ZNF217 may bring about novel drug targets for tumors that overexpress ZNF217 and cause poor prognosis in patients. The mechanism of regulation of the localization of ZNF217 may be the basis of a biomarker assay used in patients for personalized treatment strategies.

Basic Science ***Graduate Student***

POSTER #29

KNOCKING OUT MYD88 SIGNALING IN DONOR T CELLS ALLEVIATES GVHD

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Allogeneic hematopoietic cell transplantation (HCT) is a potential curative option for those affected by cancers of the bone marrow and blood through the beneficial effect of the graft-versus leukemia (GVL) activity. However, graft-versus-host disease (GVHD) hinders the efficacy of allogeneic HCT as a curative treatment. Both the harmful GVHD and the beneficial GVL effect are mediated by T cells from the donor. Attempts to separate GVL and GVHD have yet to reach fruition.

MyD88 signaling has recently been shown to be important both in Th1 and Th17 responses, as loss of MyD88 signaling leads to loss of proliferative ability in response to antigen as well as production of IFN¿ and IL17 (Schenten D., 2014). In GVHD models knocking out MyD88 in host hematopoietic antigen presenting cells (APCs) has no effect on GVHD (Li H., 2011). The role of MyD88 signaling in donor T cells, however, has yet to be elucidated. We hypothesized that knocking out MyD88 in donor T cells will have a protective effect in GVHD by reducing pathogenic T cell proliferation and cytokine expression. Here, we show that knocking out MyD88 in the donor T cell compartment reduces GVHD severity and mortality in multiple models of GVHD. The amount of the proinflammatory cytokine IFN¿ found in the plasma of mice receiving MyD88 KO T cells was significantly lower than in mice receiving WT T cell. Also, the percentage of IFN¿$^+$ T cells in the intestines, the target organ in GVHD most associated with mortality, was significantly lower in the mice receiving MyD88 KO T cells. This difference seems to be independent of proliferation, migration, and apoptosis. It is also independent of IL1R signaling, as experiments transplanting IL1R KO donor T cells showed no difference in either GVHD severity or mortality compared to recipients of WT T cells. The decrease in GVHD is dependent on MyD88 signaling in CD4 T cells and not CD8 T cells. This may be due to the decrease in production of GM-CSF by CD4 T cells without MyD88 signaling. Using CSF2 (gene for GM-CSF) KO mice as donors and transplanting KO CD4 T cells with WT CD8 T cells would help to discern whether or not GM-CSF production by CD4 T cells is actually responsible for this decrease in GVHD severity and mortality. Using an MLL-AF9 cells developed in our lab, MyD88 KO T cells, primed through a mixed lymphocyte reaction, showed no difference in cytolytic activity. I will use these MLL-AF9 cells to confirm these results in vivo. I will also use the MyD88 small molecule inhibitor ST2825 as a therapeutic approach toward reducing GVHD while sparing GVL.

Basic Science ***Graduate Student***

POSTER #30

CANCER CELL-MICROENVIRONMENT INTERACTIONS UPREGULATE ETS1 EXPRESSION AND PROMOTE METASTATIC COLONIZATION IN OVARIAN CANCER

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Metastatic colonization of organs in the peritoneal cavity in ovarian cancer (OC) begins with the attachment and interaction of cancer cells with the surface mesothelium. Juxtacrine and paracrine interactions drive changes in gene expression that allow for cancer cells to successfully adapt to their new microenvironment and form metastatic tumors. These gene expression changes can be induced by alterations in the activity of transcription factors (TFs). Indeed, deregulation of several families of TFs has been implicated in OC. The ETS family of TFs can promote cellular migration and invasion through induction of various genes, including matrix metalloproteases. Using a 3D culture model that mimics the early steps of metastasis, we investigated the expression of ETS TFs in metastasizing OC cells, and found that ETS1 was the most upregulated. Using a tissue microarray, we determined that ETS1 is also upregulated in human ovarian cancer samples compared to normal fallopian tube epithelium. Validating the importance of this finding, we found that high ETS1 expression correlates with poor patient prognosis. In *in vitro* experiments, knocking down ETS1 in OC cells reduced migration, proliferation, and colony formation. We also observed reduced invasion through and colonization of the 3D culture. Overexpression of ETS1 in OC cells had the opposite effect. Knocking out ETS1 in OC cells using CRISPR/CAS9 caused a reduction in tumor mass in mouse xenografts. Both ChIP-seq and RNA-seq were performed to look for direct downstream targets of ETS1. ETS1 was found to promote the expression of a number of genes involved in EMT, and also strongly induced focal adhesion kinase (FAK) expression. FAK inhibition yielded the same functional effect as ETS1 knockdown. Overexpression of FAK rescued the functional effect of ETS1 knockout, further suggesting that FAK is downstream of ETS1. These results suggest that interactions between OC cells and the microenvironment induce ETS1 expression, and that this increase in ETS1 enhances the ability of OC cells to form metastatic colonies. Furthermore, FAK is one of the key mediators of this metastasis-promoting ability of ETS1.

Basic Science ***Graduate Student***

POSTER #31

ACTIVATION OF A NOVEL FORM OF REGULATED NECROSIS TO ELIMINATE EXTRACELLULAR MATRIX DETACHED EPITHELIAL CELLS

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The overwhelming majority of breast cancer deaths are caused by the metastasis of cancer cells from the primary tumor to distant sites in the body. For cancer cells to successfully metastasize, they must: detach from the primary tumor, move out of the original tissue into the circulatory or the lymphatic system, travel to a new site followed by arresting their movement, and then extravasate to begin colonizing a secondary site. When normal epithelial cells detach from the extracellular matrix (ECM), they induce caspase-dependent cell death which is known as anoikis. Anoikis can therefore serve as a criticalbarrier to metastasis as cancer cells are exposed to limited and variable matrix conditions during each step of the metastatic cascade. However, our previous studies suggest that anoikis evasion is not sufficient to protect ECM-detached cells from cell death. Using MCF10A mammary epithelial cells, we have previously shown that ECM-detachment induced metabolic changes can compromise the survival of detached cells, although the precise mechanism controlling cell death remains unclear. Here, we present data suggesting that ECM-detached cells can also be eliminated by regulated necrosis (RN), a genetically programmed, caspase-independent form of necrosis that is morphologically indistinguishable from classical necrosis. In general, the molecular mechanisms involved in RN are poorly understood. With this in mind, the current most appreciated subtype of RN, termed necroptosis, is dependent upon TNFα, RIP1, RIP3, MLKL, and PGAM5 to execute cell death. These data have been gathered in attached cells. Of note, our data suggest that ECM-detachedcells are being eliminated by a mechanism that is dependent on the kinase activity of RIP1K. Further studies have shown an increase in the expression of CYLD, a deubiquitinating enzyme that specifically removes ubiquitin chains from RIP1, in turn stabilizing both RIP1 expression and activation. Upon analyzing other dependent executioners of necroptosis, we have strikingly found that TNFα, RIP3, and MLKL are all dispensable for RN to occur in ECM-detachment. Furthermore, using a variety of molecular tools, such as 3D cell culture, we have found that PGAM5 is necessary for the execution of ECM-detachment induced RN. These findings uncover a novel and distinct RN pathway and highlight the need to more thoroughly understand RN as well as the mechanisms employed by ECM-detached cells to antagonize RN and promote their survival in detachment.

Basic Science ***Graduate Student***

POSTER #32

NFKB INDUCING KINASE- A NOVEL DRIVER OF SCHWANN CELL TRANSFORMATION IN NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis Type II (NF2) is an autosomal dominant cancer predisposition syndrome which results from germline haploinsuffiency at the *NF2* locus. NF2 patients almost invariably suffer from bilateral vestibular schwannomas (VS) and are at a greatly increased risk for the development of other tumors of neural crest derived tissues such as meningiomas, ependymomas, and astrocytomas. The *NF2* gene codes for the protein, Merlin (Moesin-Ezrin-Radixin-Like Protein). Tumor formation is believed to be the result of loss of heterozygosity at the *NF2* locus, leading to total loss of Merlin in the tumor progenitor cells. Sporadic vestibular schwannomas can develop in individuals who do not harbor germline mutations at the *NF2* locus. But, greater than 90% of these tumors have biallelic disruption of *NF2*, further cementing the critical role of loss of Merlin in Schwann cell oncogenesis. Although vestibular schwannomas are largely benign, their growth can compromise auditory, vestibular, and facial nerve function, causing significant morbidity in affected patients. Current frontline therapy for the treatment of VS is surgical resection. Depending on the extent to which the VS has infiltrated surrounding structures, the need for surgical margins can further compromise auditory, vestibular, and facial nerve function and surgery does not address the underlying pathophysiology of NF2 to prevent tumor recurrence. In order to explore the cell signaling pathways by which loss of Merlin drives oncogenic transformation and to develop new therapeutic targets for NF2, our lab has developed a murine model of NF2 in which the phenotype of the mice largely recapitulates the clinical pathology observed in NF2 patients.

The cell signaling pathways by which Merlin acts to exert its tumor suppressive effects are not fully understood. But using our NF2 mice as well as human VS tissue, we have demonstrated that loss of Merlin results in hyperactivation of the NF-kB pathway. Probing deeper into the individual components of NF-kB signaling, we have found that both in human vestibular schwannoma samples and schwannomas in our mice, there is an accumulation of a catalytically active 55kD fragment of NF-kB Inducing Kinase (NIK). This 55 kD NIK fragment can drive non-cannonical NF-kB signaling. When overexpressed in wildtype Schwann cells and transferred into the sciatic nerve of immunodeficient mice, the 55kD fragment is sufficient to drive schwannoma genesis, even in the presence of functional Merlin. This 55kD fragment lacks a C-terminal negative regulatory domain found in the full length protein and we hypothesize that loss of Merlin in Schwann cells leads to accumulation of the 55kD NIK fragment and constitutive NF-kB activation. In turn, NF-kB hyperactivation drives Schwann cell transformation and tumor formation. This work highlights NIK as a novel potential therapeutic target for the treatment of Neurofibromatosis Type II.

Basic Science ***Graduate Student***

POSTER #33

A SPECIFIC CO-ACTIVATOR INTERACTION DEFINES THE ONCOGENIC MECHANISM OF ETS FACTORS REARRANGED IN PROSTATE CANCER

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In 50-70% of prostate cancers, chromosomal rearrangements result in the overexpression of a subset of ETS family transcription factors (ERG, ETV1, ETV4, or ETV5) that are not expressed in the normal prostate. Aberrant expression of one of these oncogenic ETS genes causes prostate adenocarcinoma in mouse models in the presence of a constitutively active PI3K pathway. The objective of the present study is to identify therapeutically targetable co-factors which cooperate with oncogenic ETS. Pull-down of co-factors from nuclear extracts using purified ERG followed by mass spectrometry analysis identified a novel co-activator. We then tested 21 ETS proteins for their ability to bind this co-activator. Strikingly, the same four ETS proteins that cause prostate cancer were the only ETS proteins that interacted with this co-activator, indicating a specificity mechanism. Further, the interaction between oncogenic ETS and this co-activator was necessary for transcriptional activation, prostate cell migration, anchorage independent growth and xenograft tumor formation. These findings indicate that this specific protein-protein interaction is a key to the oncogenic function of ETS family transcription factors in prostate cancer.

Basic Science ***Graduate Student***

POSTER #34

CADHERINS MODULATE OVARIAN CANCER CELL AND MULTICELLULAR AGGREGATE INVASIVENESS

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Epithelial ovarian carcinoma (EOC) metastasizes by shedding of cancer cells from the primary tumor into the peritoneal cavity where they form multicellular aggregates (MCAs). These free-floating metastatic units along with single cells subsequently adhere to peritoneum, migrate through mesothelial cell layer into submesothelial matrix and proliferate into secondary tumor masses. EOC progression is accompanied by an increase of E-cadherin (Ecad) expression at early stages of metastasis and abundant expression of N-cadherin (Ncad) later in the disease. The contribution of MCA dynamics to the metastatic success and role of cadherins in MCA formation, survival in ascitic fluid and further fate at the secondary anchorage site remain largely unknown. In attempt to mimic and trace cell/aggregate anchorage and invasion into submesothelial matrix, we performed live imaging of fluorescently tagged epithelial type (Ecad+) OvCa433-RFP and mesenchymal type (Ncad+) DOV13-GFP single cells and MCAs during the 3-dimentional rat tail collagen I (3D RTCI) invasion process. Study revealed dramatic differences between the ability of Ecad+ and Ncad+ ovarian cancer cells and MCAs to migrate through the 3D RTCI gel constructs. Both Ncad+ DOV13 and Ecad+ OvCa433 single cells, as well as OvCa433 MCAs, retained superficial localization on top of a collagen layer. On the contrary, DOV13 MCAs demonstrated considerable plasticity and lateral motility, quick dispersal on top of the collagen layer with subsequent penetration into the 3D matrix within 72hrs. Strikingly, rarely seeded DOV13 single cells overtime established a cell network on top of RTCI and further became capable of invading the underlying matrix layers, stably keeping connections with the adjacent cells dispersed in the upper layer. To test the possibility of Ncad+ “invading leaders” paving the way for the rest of the cells and facilitating the movement of non-invasive Ecad+ cell population through the existing microtracks, we conducted collagen invasion live imaging utilizing a mixture of invasive DOV13 and non-invasive OvCa433 cells and MCAs. Intriguingly, the two lines have eventually formed completely separate cell networks. Subsequently, DOV13 cells successfully invaded in RTCI, whereas OvCa433 cells stayed isolated on top in a dispersed monolayer and didn't follow through microtracks. These preliminary data support the hypothesis that MCA dynamics and subsequent fate at the secondary anchorage site may largely depend on cadherin composition. Our findings suggest that even theoretically invasive, Ncad+ cells require well established interactions with neighboring cells linked to the surface in order to migrate down. If such type of collective behavior is the case at the EOC metastatic site, then intraperitoneally floating Ncad+ aggregates occur under timing advantage over the single cells due to pre-formed cell-cell connections and thus, therapeutic approach aimed at MCA disruption and blocking cell-cell junction re-creation is reasonable to test.

Basic Science ***Graduate Student***

POSTER #35

NOVEL ROLE OF MIR-29 IN PANCREATIC CANCER AUTOPHAGY AND ITS THERAPEUTIC POTENTIAL

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Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most lethal human malignancies with a five-year survival rate of 8% and is often undiagnosed until it has metastasized. These advanced tumors display resistance to current therapeutic modalities. The lack of effective therapies and early detection reinforces the need to understand molecular mechanisms associated with PDAC to develop novel therapies. We found consistent downregulation of miR-29 in pancreatic cancer cells, and its restored expression sensitized chemotherapeutic resistant pancreatic cancer cell lines to gemcitabine, leading to reduced cancer cell viability and increased cytotoxicity. Furthermore, reintroduction of miR-29 blocked autophagy flux, evidenced by an accumulation of autophagosomes and autophagy substrate, p62, and decreased autophagosome-lysosome fusion. In addition, miR-29 decreased ATG9A and TFEB expression, which are critical for autophagosome trafficking and lysosomal function respectively. Subsequent knockdown of ATG9A or TFEB expression alone or in combination resulted in inhibition of autophagy similar to miR-29 overexpression. Finally, miR-29 reduced migration, invasion, and anchorage independent growth of pancreatic cancer cells. Collectively, our findings indicate that miR-29 functions as a potent autophagy inhibitor that sensitizes pancreatic cancer cells to gemcitabine and decreases their invasive potential. Our data provides evidence for the use of miR-29 as a novel therapeutic agent to target PDAC.

Basic Science ***Graduate Student***

POSTER #36

THE IMPACT OF PARITY ON THE METASTATIC SUCCESS OF OVARIAN CANCER

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Ovarian cancer (OvCa) is the most fatal gynecological cancer, frequently going undetected until metastatic and often fatal stages of the disease. OvCa follows a unique form of metastasis, spreading throughout the peritoneal cavity. Epidemiologic data suggest that parity reduces the risk of developing OvCa, with more births providing greater protection. Despite the association of parity with a decreased OvCa incidence, very few studies have explored the relationship between parity and metastasis. A recent study compared metastatic success to the omentum in 12-month-old parous and 5-month-old virgin C57BL/6 mice, reporting that parous mice are less susceptible to metastasis due to parity-associated differences in the immune compositional profile in the omental fat band (Cohen et al. 2013). This study compared mice of different ages and did not report specific numbers of pregnancies. To further investigate the role of parity number in OvCa metastasis, we designed a study where three age-matched C57BL/6 groups were evaluated: nulliparous (P0), parous 1 (P1), and parous 3 (P3) mice. We tested the effect of parity on metastatic success *in vivo* with an allograft study using the C57BL/6 syngeneic ID8 mouse ovarian surface epithelial cell line. ID8 RFP-tagged cells (10^6) were intraperitoneally injected into P0, P1 and P3 mice. The mice were imaged once a week starting at 5 weeks post injection and sacrificed for dissection at 8 weeks post injection. Live imaging data suggested OvCa metastasis was less efficient in the P3 animals compared to the other cohorts. After dissection, abdominal organs were imaged *ex vivo* and tumor burden was quantified. In contrast to the results of Cohen et al. that utilized a different syngeneic cell line, no significant difference in metastasis to the omentum in the parous animals was detected. P3 animals displayed significantly less metastasis to the gonadal fat depots, suggesting that gonadal adipose in multi-parous animals is a unique microenvironment, resilient to metastasis. To investigate responsible factors, we isolated RNA from periovarian adipose of healthy non-tumor bearing P0, P1 and P3 mice and carried out RNAseq. Among the pathways enriched in the RNAseq dataset, immune response, cell adhesion and developmental pathways were prominent.

Basic Science ***Graduate Student***

POSTER #37

THE IMPACT OF AGE ON THE METASTATIC SUCCESS OF OVARIAN CANCER

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Ovarian cancer (OvCa) is the leading gynecological malignancy in women in the United States. OvCa metastasizes uniquely, spreading through the peritoneal cavity and generating widespread metastatic sites. The vast majority of OvCa cases occur in women over 40 and the median age at diagnosis is 63 (SEER). Despite age being a significant risk factor for the development of OvCa, there is a paucity of studies addressing the role of aging in OvCa metastasis. To our knowledge, there are no reports utilizing old mice to investigate the effects of age on metastasis *in vivo*. We designed a study using a C57BL/6 model of aging where young (Y) mice are 3-6 months of age and aged (A) mice are 20-23 months of age, corresponding to young (20-30 years) and aged (60-67 years) humans. Using the C57BL/6 syngeneic ID8 mouse ovarian surface epithelial cell line, we tested the effect of aging on metastatic success *in vivo.* An allograft study was carried out with Y and A mice that were intraperitoneally injected with $3.7x10^6$ ID8 RFP-tagged cells. The mice were imaged once a week starting at 4.5 weeks post injection and were sacrificed for dissection at 8 weeks post injection. Live imaging suggested OvCa metastasis was more efficient in the aged animals than in the young animals. After dissection, the abdominal organs were imaged *ex vivo* and tumor burden was quantified. The aged mice displayed heavier tumor burden in the gonadal fat compared to the young. Interestingly, no difference in metastasis to the omentum was detected.To investigate why gonadal fat is more receptive to metastasis in the aged animals, periovarian adipose from 4 young and 4 aged healthy non-tumor bearing mice was isolated for RNAseq analysis. Several immune pathways involving B cells were found to be significantly upregulated in the RNA from aged animals. Studies will be conducted to elucidate the status of B cells in aging periovarian adipose, including immunohistochemistry for CD45 and other B cell markers upregulated in the RNAseq dataset.

Basic Science ***Graduate Student***

POSTER #38

CLASSIFICATION OF GLIOBLASTOMA MULTIFORME (GBM) SUBTYPES BY GENE EXPRESSION LEVEL USING MACHINE LEARNING ALGORITHMS

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Classification of Glioblastoma Multiforme (GBM) subtypes by Gene Expression level using Machine Learning algorithms

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Glioblastoma multiforme (GBM) is the common and most malignant of the glial tumors. GBM is classified into four subtypes: Classical, Mesenchymal, Proneural and Neural. The diagnosis and determination of therapy response of a cancer patient can be effectively done by identifying its subtypes. However, previous classification method (Prediction Analysis for Microarrays - PAM50) are clinically-based and have limited diagnostic ability. This study focuses to predict and classify the samples of GBM using Machine learning algorithms. For the current study, 173 samples were considered for an automated subtypes. A scoring technique was used (PAM50) and a new was developed that compares the average gene expression value for a particular gene across different subtypes. The scoring was defined as (i) Gene Expression score (CM1) = avg(x)-avg(y)/1+(max(y) -min(y)) ; (ii) Gene Expression score (CM1') = avg(x)-avg(y)/1+(avg(max(y))-avg(min(y)))where 'x' and 'y'are GBM subtypes. Principal component analysis was also performed for each subtype and also for combinations. Top discriminative genes identified using CM1 score, CM1' score and Principal Componentamong all subtypes were used for classification. These genes were loaded to Weka software suit separately and evaluated for the prediction using different classifiers like J48, Random forest, Decision tree etc.., and 10 fold classification. On using the top ten discriminative genes from all subtypes for classification, gave high number of false positives. When triplet combinations of subtypes were evaluated the false positive decreased substantially for the (i) Group1: Classical, Mesenchymal, Proneural; (ii) Group 2: Classical, Neural, Proneural. From the above preliminary observed results, it can be concluded that Mesenchymal and Neural have higher similarity. This is hypothesized as a causative effect of occurrence of same set of genes in both subtypes. Significant genes for each of the subtypes were identified for 95% confidence interval using the unpaired t-test. The number of significant genes identified were 267, 345, 317, and 195 for Classical, Mesenchymal, Neural and Proneural respectively.

Basic Science ***Graduate Student***

POSTER #39

ONCOGENIC RAS DIFFERENTIALLY REGULATES METABOLISM AND ANOIKIS IN EXTRACELLULAR MATRIX-DETACHED CELLS

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In order for cancer cells to survive during metastasis, they must overcome anoikis, a caspase-dependent cell death process triggered by extracellular matrix (ECM) detachment, and rectify detachment-induced metabolic defects that compromise cell survival. However, the precise signals used by cancer cells to facilitate their survival during metastasis remain poorly understood. We have discovered that oncogenic Ras facilitates the survival of ECM-detached cancer cells by using distinct effector pathways to regulate metabolism and block anoikis. Surprisingly, we find that while Ras-mediated phosphatidylinositol (3)-kinase signaling is critical for rectifying ECM-detachment-induced metabolic deficiencies, the critical downstream effector is serum and glucocorticoid-regulated kinase-1 (SGK-1) rather than Akt. Our data also indicate that oncogenic Ras blocks anoikis by diminishing expression of the phosphatase PHLPP1 (PH Domain and Leucine-Rich Repeat Protein Phosphatase 1), which promotes anoikis through the activation of p38 MAPK. Thus, our study represents a novel paradigm whereby oncogene-initiated signal transduction can promote the survival of ECM-detached cells through divergent downstream effectors.

Basic Science ***Graduate Student***

POSTER #40

RAS-MEDIATED REGULATION OF CYTOCHROME C-INDUCED CASPASE ACTIVATION IS DEPENDENT ON THE STATUS OF EXTRACELLULAR MATRIX ATTACHMENT

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Hyperactivating mutations in Ras are found in a significant percentage of cancers, with a particularly high frequency in pancreatic and colon carcinomas. The hyperactivation of Ras drives a vast number of distinct downstream signaling pathways that play an important role in disease progression. One outcome of overactive oncogenic Ras signaling is the inhibition of anoikis, a form of apoptotic cell death caused by detachment from the extracellular matrix (ECM). It is well-established that one mechanism of anoikis inhibition by Ras involves impaired mitochondrial cytochrome c release. In addition, here, we have found that Ras-mediated anoikis inhibition can also occur downstream of mitochondrial cytochrome c release. Our data suggest that anoikis inhibition following cytochrome c release is not dependent on changes in the abundance of the Apaf-1, pro-caspase-9, or pro-caspase-3. Instead, our data suggest that Ras signaling leads to an inhibition in the ability of caspase-9 to bind Apaf-1 which thereby inhibits proper formation of the apoptosome and caspase activation. Interestingly, in stark contrast to the inhibition of cytochrome c-induced apoptosis in ECM-detached cells, the overexpression of oncogenic Ras in ECM-attached cells results in enhanced sensitivity to exogenous cytochrome c. This sensitization was found to be due to upregulation of apoptosomal proteins (e.g. Apaf-1, pro-caspase-9) in an ERK/MAPK dependent manner. In aggregate, our data suggest that in addition to inhibiting the release of cytochrome c in both attachment and detachment, oncogenic Ras drives additional mechanisms that prevent apoptosome formation and caspase activation in detachment. Furthermore, our data support a model whereby ECM-attached cells containing oncogenic Ras mutations could be selectively eliminated by cytochrome c or agents that mimic its action.

Basic Science ***Graduate Student***

POSTER #41

DOPPLER SPECTROSCOPY OF INTRACELLULAR MOTION TO ASSESS CHEMOTHERAPEUTIC EFFICACY IN ESOPHAGEAL CANCER

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Biodynamic imaging is a new 3D tissue optical imaging modality that measures intracellular Doppler light scattering to assess the efficacy of applied chemotherapeutics. Ex vivo living biopsy samples, as thick as a millimeter, can be volumetrically imaged in real time using laser ranging and coherent detection as the tissue responds to applied therapies. The therapeutic drug mechanism of action has physiological effects that alter the motions of constituents inside the living cells and hence produce telltale Doppler spectral signatures that relate to the effectiveness of the drug. This functional imaging approach could have important applications for personalized selection of chemotherapy for cancer care.

A pilot project is currently being conducted to test biodynamic imaging for the efficacy of cancer treatment of esophageal cancer. Over ten patients have been enrolled to date in the clinical study, all patients presenting with esophageal cancer. Pinch biopsies are acquired at the time of diagnosis and parts are selected for biodynamic assessment. A biopsy is divided into 32 samples that are immobilized in multi-well plates, while maintaining sample tissue health. The therapies tested in this study are cisplatin + 5FU versus carboplatin + Taxol. The combination therapies and the monotherapies are tested in parallel, along with negative controls. The data consist of a 4 hour baseline to establish the baseline Doppler spectrum. After the drug is applied, the samples are monitored for 9 hours. The change in the Doppler spectra are converted to drug-response spectrograms, which are analyzed for biodynamic biomarkers. Data analysis uses principle of feature vectors and clustering to identify samples with positive (beneficial) response to therapy relative to samples with negative (nonbeneficial) response to therapy. In this esophageal trial, a comparison is made between the two combination therapies, each containing platinum. In all enrollment cases, the patients were given carboplatin + Taxol along with radiation therapy. Ongoing studies will correlate biodynamic biomarkers against the clinical outcome to test the ability for biodynamic profiling to select cancer chemotherapy.

Basic Science ***Graduate Student***

POSTER #42

AN UNEXPECTED ROLE FOR STROMAL MMP3 DURING BREAST CANCER

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The tumor microenvironment is composed of multiple cell types and extracellular proteins that interact with the cancerous epithelial cells. Matrix metalloproteinases (MMPs), are an example of a family of extracellular zinc-dependent endopeptidases that regulate the microenvironment around epithelial cells. Their role in cancer is not only constrained to extracellular matrix degradation for tumor invasion, but also play key roles in signaling pathway regulation. MMP3/stromelysin-1 is an MMP that functions during breast development and cancer progression. To date, MMP3 transcript expression has been detected in the stromal cells in the mammary gland but in both the epithelial and stromal cells in breast cancer. To assess MMP3 localization, we are currently staining normal mammary glands and mammary tumor tissue sections by immunohistochemistry.

The role of stromal and epithelial MMP3 has not been distinguished in published studies. To determine the requirement for MMP3 in breast cancer progression, we transplanted mammary epithelial cancer cells (V_0PyMT) into the mammary glands of MMP3 knockout and heterozygous syngeneic mice. We quantified the tumor burden of both primary and metastastic tumors over time and discovered that elimination of stromal MMP3 increased the primary tumor burden, suggesting an inhibitory role for stromal MMP3 in cancer progression. This data suggest that stromal MMP3 inhibits, but is not required for, the development of the primary breast tumor. However, in contrast to our primary tumor data, we interestingly discovered that stromal MMP3 was required for visible lung metastases. We are determining if stromal MMP3 affects the tumor cells' ability to enter the bloodstream by tail vein injecting MMP3 Het and KO mice to assess if macrometastasis is due to intravasation or post-intravasation effects during the metastatic cascade.

This study will help to elucidate new mechanisms through which MMP3 functions within particular microenvironmental contexts during breast cancer progression.

Correia AL, Mori H, Chen EI, Schmitt FC, Bissell MJ. The hemopexin domain of MMP3 is responsible for mammary epithelial invasion and morphogenesis through extracellular interaction with HSP90ß. *Genes & Development*. 2013;27(7):805-817. doi:10.1101/gad.211383.112

Basic Science ***Graduate Student***

POSTER #43

INHIBITING THE TRANSCRIPTIONAL ACTIVATION FUNCTION OF ONCOGENIC ETS FACTORS IN PROSTATE TUMORS

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The ETS family of proteins is comprised of 27 transcription factors that bind DNA via a conserved DNA binding domain. Four of these factors—ERG, ETV1, ETV4, and ETV5—make up the oncogenic ETS class, as expression of these factors promote prostate cancer phenotypes. The goal of my project is to identify a way to inhibit the function of these oncogenic ETS factors, without affecting other ETS family members. We recently identified a co-activator that specifically interacts with the four oncogenic ETS proteins, but not other ETS family members. This ETS/co-activator interaction promotes prostate cancer phenotypes such as migration, clonogenic survival, and tumor formation. Therefore, our overall objective is to identify and develop a specific inhibitor of the ETS/co-activator interaction to treat the 50-70% of prostate cancer patients with ETS positive tumors. We have already determined the region of ERG and ETV5 essential for co-activator binding. My preliminary data indicates a broad region of the co-activator required for ERG binding. To determine a minimum interaction region, I am preforming a variety of purified protein pull down assays with co-activator truncations and point mutations. The minimum interaction region of the co-activator will be used in an Alpha-LISA drug screen to determine candidate inhibitors of oncogenic ETS/co-activator interactions.

Basic Science ***Graduate Student***

POSTER #44

ADAR3 INHIBITS RNA EDITING AT THE Q/R SITE OF GLURB TRANSCRIPTS THOUGH DIRECT BINDING

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RNA editing is a naturally occurring cellular process in which RNA-editing enzymes modify transcripts in a site-specific matter. Cancer transcriptomes often exhibit altered RNA editing and several editing events have been directly linked to cancer progression. Editing at one adenosine of a transcript encoding the glutamate receptor subunit B (GluRB) changes the amino acid encoded from glutamine to arginine, thus this is referred to as the Q/R site. This modification alters the calcium permeability of the glutamate receptor, having profound effects on cellular physiology. 100% of GluRB transcripts are edited in the human brain; and, loss of editing at this site results in death of the organism. Interestingly, a reduction of GluRB editing has been observed in glioblastoma patients and has been shown to contribute to increased tumorigenesis. However, a reduction in GluRB editing does not directly correlate with a reduction in the expression level of its primary editing enzyme, ADAR2. The goal of these studies is to identify cellular factors that regulate editing of GluRB and to determine the molecular mechanism of that regulation. Recently, an inactive ADAR family member in *C. elegans* has been shown to regulate RNA editing; interestingly, humans express an inactive ADAR family member as well, ADAR3. We hypothesized that ADAR3 may regulate RNA editing in humans, focusing on editing of GluRB mRNA, as defects in its editing status have been implicated in glioblastoma progression.

Overexpressing ADAR3 in a glioblastoma-derived cell line with low endogenous expression of ADAR3, we have monitored RNA editing of GluRB transcripts. We have established that ADAR3 expression inhibits RNA editing in U87 and NHA cell lines. To interrogate the biological mechanism of ADAR3 inhibition of RNA editing, I took a two-pronged approach, expressing ADAR3 with mutations in annotated domains to determine its mode of action and immunoprecipitating ADAR3 to elucidate protein and RNA interactions. Our findings indicate that the dsRNA binding domains of ADAR3 are required for inhibition of RNA editing and that ADAR3 binds directly to GluRB mRNA transcripts. As the GluRB transcript is specifically edited by ADAR2 at the Q/R site, we suggest that ADAR3 directly competes with ADAR2 for binding to GluRB transcript, inhibiting RNA editing. To investigate the clinical relevance of these findings, we measured ADAR2 and ADAR3 expression and GluRB editing in 10 glioblastoma tumors and matched adjacent brain tissue. We found that GluRB editing is reduced and that ADAR3 is overexpressed in many of the tumor samples compared to the normal adjacent tissue, suggesting aberrant ADAR protein expression in glioblastoma tumors that is correlated with reduced GluRB editing. Our results suggest that the relative level of ADAR3 and ADAR2 protein expression in glioblastoma tumors can together influence the level of GluRB editing.

Basic Science ***Graduate Student***

POSTER #45

METHIONINE RESTRICTION ALTERS FUNCTIONAL POLARIZATION OF MACROPHAGES IN A MURINE MODEL OF PROSTATE CANCER

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Background: Epidemiological studies link prostate cancer (CaP) to dietary intake. Recent research has shown that individuals consuming a Western diet, high in protein, have higher circulating IGF-1 (insulin-like growth factor 1) which feeds into the nutrient sensing mTOR pathway. This evolutionarily conserved pathway is central to the development of advanced stage and castration resistant CaP. Our previous published studies have shown that dietary protein restriction inhibits tumor growth via mTOR pathway alteration and reduces circulating IGF-1 levels *in vivo*. The current study hypothesizes that dietary methionine restriction will alter the functionality of immune cells enhancing the ability of the immune system to respond to cancerous insults.

Methods: Mice were fed low (7% protein) or control protein (21% protein) diets. In a prevention study mice consumed modified diets for four weeks prior to being inoculated with CaP tumors. In a simultaneous intervention study mice were inoculated with CaP tumors first then four weeks later were placed on the modified diets.

Results: In the low protein diet group, we observed that pro-tumor M2 polarized macrophages were significantly reduced in the tumor microenvironment (TME) for both prevention ($p=0.012$) and intervention diet ($p=0.0003$) studies as compared to the control diet, in addition to observation of mTOR inhibition and IGF-1 reduction. Besides protein content in diet, we also analyzed the amino acid composition. Multiple groups have shown that methionine restriction (MR) inhibits mTOR activation, alters the immune system, and prolongs the survival of rodents. The mechanism by which MR impacts the innate immune system is still poorly understood. In our preliminary *in vitro* studies, we observed that modification of a single amino acid (AA), such as methionine, is sufficient to inhibit activation of both the mTOR pathway and M2 polarization (Arginase1 qRT-PCR of two samples in quadruplicate ($p=0.0732$)), while increasing the presence of M1 macrophage marker iNOS ($p=0.2689$) in bone marrow derived macrophages (BMDMs). Thus, we hypothesize that modulating specific amino acids in the diet is sufficient to alter macrophage expression of M1/M2 characteristics, and their functions in the TME.

Conclusions: Preliminarily data shows that altering one specific AA intricately linked to the mTOR pathway is sufficient to change macrophage polarization status both at the protein and gene expression levels, suggesting that dietary alteration of a single AA is capable of altering macrophage function. The results of the study provide the basis for translational use of dietary means to alter the immune system and improve the therapeutic effects of immunotherapies.

Basic Science ***Graduate Student***

POSTER #46

THE SELECTIVE CLASS I HDAC INHIBITOR ENTINOSTAT ENHANCES THE ANTITUMOR EFFECT OF PD-1 INHIBITION IN A SYNGENEIC ORTHOTOPIC MURINE MODEL OF RENAL CELL CARCINOMA

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Background: Recent advances in immunotherapy have highlighted the antitumor effects of immune checkpoint inhibition. Novel anti-PD-1/PD-L1 immunotherapies have been shown to effectively overcome tumor avoidance of immune surveillance in several tumor types including renal cell carcinoma. Our group has recently shown that the selective class I HDAC inhibitor entinostat is effective in suppressing regulatory T cells and enhancing immunotherapies in murine renal and prostate models, RENCA and Myc-Cap respectively. In this study we have evaluated the combination of entinostat with an anti-PD-1 antibody in the RENCA renal cell carcinoma model.

Methods: 32 BALB/c female mice were implanted with the syngenic, orthotopic, renal cell carcinoma mouse model, RENCA – luciferase tagged – at day -8. Treatment (8 mice /group) with anti-mouse-PD-1 (aPD-1; 10mg/kg twice a week, I.P.), entinostat (5mg/kg 5 days a week), or combination of the two was begun at day 1. Bioluminescence imaging was performed at days -1, 9 and 19 to assess the orthotopic tumor growth. End point tumor weights were taken to assess the effect of combination treatment.

Results: Analysis of tumor growth showed a reduction of bioluminescence across the three time points in the combination group as compared to the vehicle and single agent treatments. Additionally, end point analysis of tumor weights revealed an overall reduction in the size of the tumors in the entinostat/anti-mPD-1 combination group (88% inhibition) as compared to the vehicle ($p=0.0002$), aPD-1 alone (25% inhibition) ($p=0.0181$), and entinostat alone (63% inhibition)($p=0.0481$) groups. Examination of the status of the infiltrating immune cells of the tumor microenvironment via flow cytometry, qRT-PCR, immunohistochemistry, and/or immunofluorescence analysis is ongoing.

Conclusions: Our preliminary results suggest that the immunomodulatory activity of the selective class I HDAC inhibitor entinostat may enhance the antitumor effect of PD-1/PD-L1 inhibition and provide the rationale for the clinical testing of this novel combination in patients with RCC.

Basic Science ***Graduate Student***

POSTER #47

MECHANISMS OF DIVERGENT ONCOGENIC FUNCTIONS OF THE HIGHLY HOMOLOGOUS ETS FACTORS, ETS1 AND ETS2.

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ETS1 and ETS2 are homologous and ubiquitous downstream effectors of RAS/ERK signaling. Some studies find a common oncogenic function for ETS1 and ETS2, while others indicate that ETS2 is a tumor suppressor. However, no mechanistic basis for differences between ETS1 and ETS2 is known. We recently found that ETS1 promotes oncogenic phenotypes such as cell migration in prostate cancer cells, while ETS2 represses these phenotypes. We have now identified a mechanistic difference. RNA-seq and ChIP-seq, using a CRISPR/Cas9 ETS1 deletion for specificity, indicate that ETS1 and ETS2 bind the same targets but regulate genes in opposite directions. Chimeric dissection of ETS2 identified a repression domain that mediates a specific co-repressor interaction that is not conserved in ETS1. Importantly, this co-repressor is highly expressed in cancers where ETS2 is a tumor suppressor and at low levels in cancers where ETS2 is an oncogene, thus providing a potential explanation for diverging functions.

Basic Science ***Graduate Student***

POSTER #48

PHOSPHORYLATION OF SERINE 165 ON YBX1 MEDIATES NF-¿B ACTIVATION IN COLON CANCER

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Y-box binding protein 1 (YBX1) is a multifunctional protein known to facilitate many of the hallmarks of cancer. Elevated levels of YBX1 protein are observed in a variety of cancers, making it an excellent marker as well as a potential therapeutic target in cancer. The connection between YBX1 and the important nuclear factor kB (NF-kB) however has never been previously reported. Here, we show that overexpression of wild type YBX1 (wtYBX1) activates NF-kB, suggesting that YBX1 is a potential NF-kB activator. Furthermore, using mass spectrometry analysis, we identified novel phosphorylation of serine 165 (S165) on YBX1. Overexpression of the S165A-YBX1 mutant in either 293 cells or colon cancer HT29 cells showed dramatic reduction in NF-kB activation as compared to that of wtYBX1, confirming that S165 phosphorylation is critical for the activation of NF-kB by YBX1. We further show that expression of the S165A-YBX1 mutant dramatically decreased the expression of downstream NF-kB-inducible genes, reduced cell growth, and compromised tumorigenic ability in colon cancer cells, as compared to wtYBX1. Taken together, we provide the first evidence that YBX1 functions as a tumor promoter via NF-kB activation, and phosphorylation of S165 of YBX1 is critical for this function. Therefore, our important discovery may lead to blocking S165 phosphorylation as a potential therapeutic strategy in colon cancer patients.

Basic Science ***Graduate Student***

POSTER #49

A NOVEL BONE BIOREACTOR USED TO MODEL BONE METASTASIS EX VIVO

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Metastatic breast cancer tumors, rather than the primary tumors themselves, contribute to patient death. At death, roughly 73% of women with breast cancer have bone metastases, which are incurable. Therefore, understanding what drives cancer to metastasize to bone and identifying treatments that eliminate bone metastasis are essential to improving the survival and quality of life of cancer patients with metastasis to bone. The current methods used to study bone metastasis are restricted to *in vitro* tissue culture models and to *in vivo* animal models, both of which have several limitations. The *in vitro* tissue cultures lack the 3-D environment of heterogeneous cell types of the bone and marrow, and *in vivo* animal models often are limited by the confounding primary tumor burden and also are not applicable to rapid screening aimed at targeting bone metastases. **In this interdisciplinary project, we use a novel bone bioreactor to culture mouse bone explants, study bone metastases, and develop therapies to help breast cancer patients that have developed bone metastases.** The objective of this research is to develop an experimental system that preserves the 3-D environment and heterogeneous culture conditions (bone, marrow, and cancer cells) within the physiological context of an intact bone environment and apply the technology to develop faster screening techniques than the ones available in current animal models

We propose to use this model to understand fundamental questions of bone metastases and to test therapies prior to use in patients. We will validate the bioreactor as a means to understand the stages of metastatic tumor colonization, progression, and response to therapies. After validation in a murine model, our bioreactor will make it possible to study metastatic cancer progression temporally and independently from primary tumor growth. Later, we will use the bone bioreactor to study the effects on human bone coming from human orthopaedic surgical procedures. Because this system is amenable for investigating bone colonization by multiple cancer types, this study also has general application beyond breast cancer. Due the usage of bone explants and vibrational technology that is currently available to patients, this study has high translational value.

Basic Science ***Graduate Student***

POSTER #50

TUMOR DERIVED IL6 PROMOTES SKELETAL MUSCLE ATROPHY AND CACHEXIA IN PANCREATIC DUCTAL ADENOCARCINOMA.

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Cachexia, or weight loss in cancer, increases morbidity and mortality. In pancreatic ductal adenocarcinoma (PDAC), >85% of patients will suffer weight loss with many dying of cachexia. Interleukin-6 (IL6) is increased in the blood of patients with PDAC and correlates with weight loss and mortality. PDAC tumors often stain heavily for IL6 in stromal cells, with a subset exhibiting staining within tumor cells. IL6 is part of the IL6 family of cytokines and induces signaling by binding its specific a-receptor and the ligand receptor complex then binds to the gp130 receptor on cell membranes, causing transphosphorylation of the Janus Kinase. Three primary pathways are activated by IL6, including STAT3, ERK, and PI3K/Akt. This study aims to investigate the role of tumor-derived IL6 and subsequent activation of the STAT3 pathway in PDAC cachexia.

We developed a murine model of PDAC cachexia using orthotopic injection of tumor cells isolated from the genetic PDAC model LSL-**K**rasG12D:LSL-Tr**p**53R172H:Pdx1-**C**re (KPC). Tumors derived from diverse KPC cell lines induce muscle wasting and increase cachexia associated with increased IL6 expression levels. Notably, the KPC32908 cell line expresses the greatest amount of IL6 compared to wildtype cells. To determine the roles of tumor-cell-derived IL6 in PDAC cachexia, the *Il6* gene in KPC32908 cells was mutated using CRISPR/Cas9 to induce loss of expression. DNA sequencing of the CRISPR target site verified mutagenesis. QPCR verified substantial reduction in *Il6* mRNA expression (>80% decrease) in the KPC-IL6 Null (IL6 null) clone. Western blotting showed conditioned media (CM) from KPC32908 parental (KPCp) and CRISPR Negative Control (CNC) cells increased phosphorylation of STAT3 in mouse myotubes, while IL6 null CM did not. Mean minimum myotube diameter after treatment with KPCp, CNC CM was significantly less than those treated with IL6 null CM (9.85 μm;9.78 μm;14.43 μm respectively, $p<0.01$).

In vivo results showed animals injected with KPCp and CNC cells versus those injected with IL6 null cells had a significant loss of average fat (-48.8%; -30.6%; -13.5% respectively $p<0.001$), and lean tissue (-18%; -6.3%; +7.02% respectively, $p<0.001$) measured with Echo MRI and significantly reduced muscle mass versus IL6 null (Gastrocnemius: -32.9%; -37.9% and Quadriceps: -25.4%; -19.6% respectively, $p<0.001$). Average IL6 serum levels were significantly increased in KPCp and CNC groups versus the IL6 null group measured with ELISA (191.4 pg/ml; 282.07 pg/ml; 37.17 pg/ml respectively, $p<0.001$). Phosphorylation of STAT3 in the quadriceps muscle of KPCp and CNC groups was markedly increased versus IL6 null group. Moreover, tumor-derived IL6 could be required for both cachexia and tumor growth as the KPCp and CNC groups had significantly larger normalized tumor mass compared to the IL6 null group (0.10; 0.04; 0.01 respectively, $p<0.01$). These results suggest a reduction in the cachexiogenic potential of IL6 null tumor cells.

Basic Science ***Graduate Student***

POSTER #51

REGULATION OF THE ONCOGENIC FUNCTION OF ERG IN PROSTATE CELLS BY PHOSPHORYLATION

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A chromosomal rearrangement that results in aberrant expression of the transcription factor ERG is observed in ~50% of prostate cancers. ERG activates a transcriptional program responsible for cell migration, epithelial to mesenchymal transition (EMT), angiogenesis, and cell survival. Expression of ERG coupled with constitutive activation of the PI3K pathway results in the transition from prostatic intraepithelial neoplasia to invasive adenocarcinoma. However, the molecular mechanisms that are responsible for this transition are not well understood. In the present study, we have identified a mechanism in which phosphorylation of ERG leads to dissociation of a transcriptional co-repressor and increased oncogenic transcriptional activity. Phosphonull mutants of ERG exhibit a stronger association with the co-repressor, diminished ability to activate target genes, and loss of cell migration. In order to identify genome wide targets of ERG and the transcriptional co-repressor, Chromatin Immunoprecipitation with massively parallel DNA sequencing (ChIP-seq) was conducted on the ERG phophonull mutant. In a related study, we have preliminary evidence which suggests that our identified transcriptional co-repressor switches to a transcriptional co-activator upon phosphorylation. We are currently performing biochemical and functional assays to further investigate the relationship of ERG with this co-repressor and its effect on transcription and oncogenic activity. Overall this study provides insight to the signaling pathways responsible for oncogenic activity of ERG positive prostate cancer.

Basic Science ***Graduate Student***

POSTER #52

IDENTIFICATION OF IMMUNO-ONCOLOGY CROSSTALK PATHWAYS IN LUNG ADENOCARCINOMA

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Identification of Immuno-Oncology Crosstalk Pathways in Lung Adenocarcinoma

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Identifying dysregulated pathways from the high throughput data for biomarker detection is the rate limiting step in the complex diseases cure. Pathways don't perform alone; they interact with each other through the overlapping genes. This phenomenon is known as crosstalk of pathways. The aim of the study is develop a methodology to find the highly interacting (cross-talk) immune-oncological pathways and their drug-gene-pathway modules which can be further validated *in-vivo* using Lung Adenocarcinoma (LUAD) as a case study. The reference pathway cross-talk matrix is built using the KEGG Knowledgebase, which consists of the 302 KEGG pathways associated with 6996 genes. The LUAD gene expression data available in The Cancer Genome Atlas (TCGA) is used for the study. The data of 32 patients was used in the study and of these, 9 patients were treated with immunotherapy drugs. A set of 3018 significant genes associated with 296 pathways [*C.I.* =95%, *p-value* <=0.05] are identified in this dataset, and a disease crosstalk matrix is constructed. Each cell in the matrix gives the cross-talk score of the pathways computed using the formula: . The interaction among the significant genes (3018 genes) in the crosstalk pathways were identified using the BioGrid physical gene-gene interaction map and a gene interaction network (10102 interaction) is generated. The significant genes in the network are annotated to their drugs as given in the clinical data of TCGA. The drug-gene-pathway modules of LUAD are identified using Seed-Based-Network Propagation Algorithm. These modules give the profile of the highest cross-talk pathways of LUAD that can be studied further for alternative drug targets. The study identified T-cell receptor signaling pathway and B cell receptor signaling pathway of LUAD have high crosstalk scores with Erbb Signaling pathway (18.67, 15.15) Vegf signaling pathway (17.77, 22.45); Osteoclast differentiation (16.35, 14.89).

Basic Science ***Graduate Student***

POSTER #53

EPIGENETIC TARGETING OF DNMT1 IN ADIPOCYTES INHIBITS HIGH-GRADE SEROUS OVARIAN CANCER CELL MIGRATION AND INVASION THROUGH TIMP3 UPREGULATION

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Rationale: Ovarian cancer metastasis is commonly found in the omentum and others have found that adipocytes play a significant role in tumor progression. Obese adipose tissue has been shown to have increased methylation levels due to increased DNMT1 levels and activity. We have previously shown that combination treatment of high-grade serous ovarian cancer cells with a DNMT1 inhibitor (either decitabine or guadecitabine/SGI-110) plus cisplatin treatment reduced tumor progression by hypomethylating promoters and re-expressing tumor suppressor genes *in vivo* and in high-grade serous ovarian cancer clinical trials. Based on these observations, it was of interest to test whether DNMT1 inhibition could reverse adipocyte methylation, alter adipokine secretion, and decrease migration and invasion of ovarian cancer cells towards adipocytes.

Methods: Adipocytes were treated with DMSO (control) or low-dose guadecitabine (100nM daily for 5 days). SKOV3, Kuramochi, OVCAR4, or OVCAR8 ovarian cancer cells were seeded in Boyden migration and invasion chambers and allowed to move towards adipocytes for 8 and 16h, respectively. EMT marker expression (SLUG, fibronectin, and TWIST1) was assessed by qRT-PCR. Adipocyte-conditioned media was used to culture ovarian cancer cells in clonogenicity assay and a human adipokine array (R&D Systems) was performed.

Results: Guadecitabine treatment of adipocytes decreased (P<0.05) migration of OVCAR4 and OVCAR8 (35% and 40%, respectively, compared to control), and a 50% decrease (P<0.05) in invasion towards adipocytes after guadecitabine treatment was observed for OVCAR4, OVCAR8, and Kuramochi cells. Expression of EMT markers SLUG, fibronectin, and TWIST1 decreased after guadecitabine treatment. Conditioned media from guadecitabine-treated adipocytes decreased (P<0.05) clonogenic survival by 18% compared to control adipocyte-conditioned media. Increased secretion of LIF (lipoprotein lipase inhibitor) and TIMP3 (metalloproteinase inhibitor) after guadecitabine treatment (1.6- and 1.8-fold increase, respectively) was observed based on adipokine array and verified by qRT-PCR. Treatment with recombinant TIMP3 (50nM) decreased invasion of OVCAR8 (63%, P<0.001) and OVCAR4 cells (73%, P<0.05).

Conclusion: Guadecitabine treatment of adipocytes alters adipokine secretion resulting in decreased cancer cell migration and invasion. The presence of hypomethylating agent therapy in the tumor microenvironment may not only have platinum resensitizing effects on cancer cells but additionally alter adipokine secretion leading to decreased ovarian cancer metastasis.

Basic Science ***Graduate Student***

POSTER #54

CSF1R MER-CRE-MER: A MOUSE MODEL TO STUDY THE CLONAL CONTRIBUTION OF DEVELOPMENTAL LINEAGES TO JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile Myelomonocytic Leukemia (JMML) is a fatal pediatric myeloproliferative neoplasm (MPN) that can arise *in utero*. JMML has a clonal origin within hematopoietic stem and progenitor cells (HSPCs) that acquire somatic mutations in the GM-CSF signaling pathway. The LoxP-STOP-LoxP PTPN11^{E76K} mouse is a validated model of this disease. Following Cre mediated recombination, PTPN11$^{E76K/+}$ mice recapitulate the two defining molecular features of JMML: i) growth hypersensitivity of myeloid progenitors to GM-CSF and ii) hyperactive RAS-ERK signaling. However, previous studies have expressed PTPN11^{E76K} in overlapping hematopoietic lineages with near-complete efficiency. As a result, previous work has not addressed the clonal origin of JMML. Furthermore, the contribution of individual developmental lineages to JMML is unknown. In this report, we describe an inducible mouse model that uniquely activates the PTPN11^{E76K} mutation in 3 distinct hematopoietic lineages with near-clonal efficiency.

The colony stimulating factor 1 receptor (CSF1R) is expressed on differentiated myeloid cells and its signaling is crucial for their differentiation, proliferation, and survival. Fortuitously, CSF1R is also expressed on HSPCs at distinct developmental stages, including yolk sac EMPs, fetal liver HSCs, and adult HSCs. Using the inducible CSF1R Mer-Cre-Mer+ ROSA$^{YFP/+}$ mice, we validated that tamoxifen-injection at E8.5, at E14.5, and at 5week of age could activate 25% of EMPs, 1.0% of fetal liver HSCs, and 1.0% of adult HSCs, respectively.

To evaluate the clonal contributions of lineage-specific HSPCs to JMML, we bred CSF1R Mer-Cre-Mer+; ROSA$^{YFP/+}$; PTPN11$^{E76K/+}$ (CYE) mice that express PTPN11^{E76K} in adult HSCs following tamoxifen injection at 5 weeks of age. Genomic PCR analysis confirmed that YFP+ cells expressed the PTPN11^{E76K} construct, whereas YFP- cells expressed only PTPN11^{WT}. Peripheral blood analysis showed CYE mice have increased YFP+ cells and that these cells are skewed towards the CD11b+ myeloid lineage compared to littermate controls (Cre+ ROSA$^{YFP/+}$; PTPN11$^{+/+}$). Subsequently, only YFP+ HSPCs from CYE animals, and not YFP- HSPCs, had growth hypersensitivity and hyperactive RAS-ERK signaling – the two defining features of JMML. Finally, CBC analysis of CYE animals showed progressively increasing leukocyte counts, demonstrating progression of MPN and suggesting that these animals will succumb to their disease.

In conclusion, we have demonstrated that timed injection of tamoxifen in CSF1R Mer-Cre-Mer+ animals results in near-clonal and lineage-restricted HSPC labelling in distinct hematopoietic lineages. Furthermore, we show this system can be used to model clonal MPN development using a defined JMML mutation: PTPN11^{E76K}. Finally, our system allows the study of interactions between mutant and normal hematopoiesis in the same

mouse by comparing YFP+ and YFP- cells. Given these characteristics, our CYE model will address the distinct developmental contributions of yolk sac EMPs, fetal liver HSCs, and adult HSCs to disease development in our JMML mouse model.

Basic Science ***Graduate Student***

POSTER #55

FANCONI ANEMIA PATHWAY SAFEGUARDS INTERPHASE AND MITOSIS

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Fanconi anemia (FA/BRCA) tumor suppressor signaling network controls multiple genome-housekeeping checkpoints, from DNA replication and repair in interphase to high-fidelity chromosome segregation during mitosis. However, the role of error-prone mitosis due to loss of FA signaling in the maintenance of genome integrity has not been determined. To address this clinically relevant issue, we dissected the origins of genomic instability in FANCA-/- patient cells. We detected increased mitotic errors during human FANCA-/- hematopoiesis. Quantitative micronucleus assays revealed that both interphase DNA damage and mitotic errors significantly contribute to genomic instability in primary FANCA-/- patients fibroblasts. Functional studies revealed decreased microtubule nucleation in FANCA-/- patient cells, providing a mechanistic link between loss of FANCA and mitotic spindle dysfunction. Our findings support the model of the FA/BRCA signaling functioning as an gatekeeper of genomic integrity throughout interphase and mitosis.

Basic Science ***Graduate Student***

POSTER #56

EPIGENETIC PRIMING POTENTIATES IMMUNE CHECKPOINT INHIBITORS IN OVARIAN CANCER

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Background

Ovarian cancer (OC) progression is accompanied by the establishment of stable and transcriptionally repressive epigenetic modifications. An important mechanism of immune evasion is represented by epigenetic silencing of tumor antigens (NY-ESO-1, Muc16 and MAGE). We hypothesize that by reversing DNA methylation, DNA methyl transferase inhibitors (DNMTIs) restore the expression of such antigens, potentiating anti-tumor immune response. The targeting of immune checkpoints regulated by programmed cell death protein-1 (PD-1) signaling represents a novel therapeutic strategy in cancer, including in OC. Here we set out to measure the anti-tumor effects of epigenetic priming in combination with PD-1/PDL-1 blockade in OC preclinical models.

Methods

The ID8 intraperitoneal (ip) immunocompetent syngeneic mouse model was used to measure the effects of the novel DNMTI guadecitabine (SGI-110, Astex Pharmaceuticals Inc) and PDL1 blockade. The experimental groups consisted of non-specific IgG (control), guadecitabine 2mg/m^2 sq bi-weekly, murine anti-PDL1 inhibitory antibody (10mg/kg) bi-weekly and combination of guadecitabine with anti-PDL1 antibody (n=6 mice/group, 3 week treatment). Immune cells collected from the ascites and spleens of tumor bearing mice were either directly processed or co-cultured with ID8 cells for 48 hours. Cells were immuno-phenotyped by flow cytometry. In OC cells treated with guadecitabine, changes in gene expression were analyzed by real time-PCR.

Results

Combination of PD1 blockade with guadecitabine significantly decreased primary tumor formation ($P<0.05$) and malignant ascites accumulation ($P<0.0001$) compared with the control group in ID8 tumor bearing mice. CD8+ cells isolated from ascites and spleens demonstrated increased expression of exhaustion markers (PD1+ and CTL4+) and decreased expression of activation markers (CD40 and MHC-I) in the control (IgG) group compared with the SGI-110 and anti-PDL1 inhibitory antibody alone or in combination treatment groups. Treatment with DNMTIs significantly increased the expression of tumor antigens Muc16, Mage A2, A11, and NY-ESO 1 ($p<0.01$) in several OC cell lines.

Conclusions

Guadecitabine in combination with anti-PDL1 antibody induced striking anti-tumor effects in an immunocompetent OC syngeneic model by activating cytotoxic T-cells. These data support clinical strategies utilizing epigenetic priming using DNMTI in combination with immune checkpoint inhibitors.

Basic Science ***Medical Student***

POSTER #57

SCHWANNOMA FORMATION IS UNCHANGED BY ABOLISHMENT OF EIF4E PHOSPHORYLATION IN MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis type 2 (NF2) is an inherited tumor predisposition syndrome that occurs as a result of germline hemizygosity for the *NF2* tumor suppressor, leading to the development of multiple schwannomas and meningiomas. There are currently no effective medical treatments for vestibular schwannoma, owing in part to an incomplete understanding of the normal function of *NF2* and the biochemical derangements that occur as a result of its loss. To address this, we used an shRNA kinase library to compare the relative effect of single kinase knockdowns on growth of *NF2*-deficient versus *NF2*-sufficient human meningioma cells. Of the candidates, knockdown of MAP kinase-interacting protein kinase 2 (Mnk2) achieved lowest depletion values in two separate shRNA clones. Mnk2 is known to phosphorylate eukaryotic elongation factor 4E (eIF4E), whose overexpression and phosphorylation has been correlated with poor outcomes in several other cancers. Relative to normal eighth cranial nerve tissue, spontaneous and NF2-associated human vestibular schwannomas demonstrated increased levels of both p-eIF4E and total eIF4E by western blot. We performed a genetic intercross of $Nf2^{flox/flox}$;PeriostinCre mice with $eIF4E^{S209A}$ transgenic mice. $Nf2^{flox/flox}$;PeriostinCre mice (*Nf2*-deficient in the Schwann cell lineage) develop schwannomas of the dorsal root ganglia (DRG) and cranial nerves as well as progressive sensorineural hearing loss. The $eIF4E^{S209A}$ mouse strain has a global point mutation of eIF4E that prevents its phosphorylation; this mutation produces no discernable phenotype. DRG volume and histology were primary readouts of schwannoma formation. Whole tissue lysates of trigeminal ganglia were used to characterize protein levels Nf2, p-eIF4E, and total eIF4E by western blot. Hearing thresholds were determined using click auditory brainstem response (ABR). There were no significant differences in overall survival between genotypes. Compared to *Nf2*-sufficient mice, 8-month-old *Nf2*-deficient mice had significantly increased DRG size with histologic features of schwannoma regardless of eIF4E allele expression. Schwannomas of $eIF4E^{S209A}$ were significantly larger than those expressing the $eIF4E^{WT}$ allele. Although hearing thresholds were significantly increased in *Nf2*-deficient mice expressing the $eIF4E^{WT}$ allele compared to their *Nf2*-sufficient controls, this difference was not present in the $eIF4E^{S209A}$ mice based on the presence of *Nf2*. While we cannot exclude the possibility that Mnk2 has eIF4E-independent effects in *Nf2*-deficient cells, our data suggest that abolishment of eIF4E phosphorylation is not sufficient to prevent schwannoma formation.

Basic Science ***Medical Student***

POSTER #58

RECOMBINANT DEK ENHANCES THE EX VIVO EXPANSION OF HUMAN AND MOUSE HEMATOPOIETIC STEM CELLS IN A CXCR2- AND HSPG-DEPENDENT MANNER

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DEK is a nuclear DNA-binding protein that has been implicated in the regulation of transcription, chromatin remodeling, and mRNA processing. Endogenous DEK regulates hematopoiesis, as bone marrow (BM) from $DEK^{-/-}$ mice manifest increased hematopoietic progenitor cell (HPC) numbers and cycling status and decreased long-term (LT) and secondary hematopoietic stem cell (HSC) engrafting capability (Broxmeyer et al., 2012, Broxmeyer et al., 2013). We now show that rmDEK is myelosuppressive *in vitro* in an S-phase specific manner and reversibly decreases numbers (~2 fold) and cycling status of HPC *in vivo*, with $DEK^{-/-}$ mice being more sensitive than control mice to this suppression. In contrast, *in vivo* administration of rmDEK to wild type and $DEK^{-/-}$ mice enhanced numbers of phenotypic LT-HSC. This suggests that exogenous DEK may enhance HSC numbers by blocking production of HPCs. We thus assessed effects of DEK on the *ex vivo* expansion of human $CD34^{+}$ cord blood (CB) and mouse Lin^{-} BM cells stimulated with SCF, Flt3 ligand, and TPO. DEK significantly enhanced *ex vivo* expansion of HSC by ~3 fold when compared to cells expanded without DEK. As rmDEK can bind to heparin sulfate proteoglycans (HSPG), become internalized, and then remodel chromatin in non-hematopoietic cells *in vitro* (Kappes et al., 2011; Saha et al., 2013), we assessed effects of DEK on the heterochromatin marker H3K9Me3 in the nucleus of mouse Lin^{-} $Sca1^{+}$ $cKit^{+}$ (LSK) BM cells by imaging flow cytometry. DEK enhanced the presence of H3K9Me3 in the nucleus of $DEK^{-/-}$ LSK cells, indicating that rmDEK can be internalized by LSK cells and mediate heterochromatin formation. Neutralizing HSPG blocked the inhibitory effect of rmDEK on colony formation as well as the expansion of HSC in *ex vivo* expansion assays. Upon finding that DEK has a Glu-Leu-Arg (ELR) motif, similar to that of CXC chemokines such as IL-8, we hypothesized that DEK may manifest at least some of its actions through CXCR2, the receptor known to bind and mediate the actions of IL-8 and MIP-2. First, we confirmed expression of CXCR2 on the surface of HSC and HPC and then determined if neutralizing CXCR2 could block DEK's function in HPC and HSC. BM treated *in vitro* with rmDEK, rhIL-8, or rmMIP-2 inhibited colony formation. However, pretreating BM with neutralizing CXCR2 antibodies blocked the inhibitory effect of these proteins. Blocking the ability of DEK to bind to CXCR2 also inhibited the expansion of HSC in an *ex vivo* expansion assay. This suggests that DEK binds to CXCR2, HSPG or both to mediate its function on HPC and HSC, enhancing HSC but decreasing HPC numbers. Therefore, DEK may be a crucial regulatory determinant of HSC/HPC function and fate decision that is utilized to enhance *ex vivo* expansion of HSC.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #59

THE PAPILLOMAVIRUS E2 PROTEIN BINDS TO ORC2 AND IMPAIRS MAMMALIAN ORIGIN LOADING

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THE PAPILLOMAVIRUS E2 PROTEIN BINDS TO ORC2 AND IMPAIRS MAMMALIAN ORIGIN LOADING

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Mammalian DNA replication is initiated by the origin recognition complex (ORC) and mediated by the pre-replication complex (pre-RC). The papillomavirus (PV) genome is maintained as a double stranded DNA episome in infected cells. To initiate replication, the viral E2 protein binds to and recruits the E1 DNA helicase at the viral origin. The PV genome replication program is linked to the life cycle of the host and involves three stages: initial amplification from a single episome to a few copies per cell, a cell cycle linked maintenance phase, and a differentiation dependent late stage where the genome is amplified to thousands of copies. The role of ORC and other pre-RC factors during PV replication had yet to be elucidated. We demonstrate that human (HPV-31) and bovine (BPV-1) E2 proteins bind to the ORC2 protein but not ORC1. E2 bound ORC2 directly and E1 was excluded from this complex. Surprisingly, the ORC2 protein was not detected at the HPV origin in cells that maintained HPV-31 and HPV-16 episomes. SiRNA and shRNA knockdown of ORC2 enhanced PV replication in both HPV-31 and BPV-1 transient replication models and in keratinocytes that stably maintain HPV-16 episomes. ORC2 silencing also enhanced HPV-31 replication in keratinocytes upon differentiation. To investigate the biological implications of E2 binding to ORC2, we observed that ORC2 occupancy at a known mammalian origin of replication was impaired in cells that stably expressing HPV-31 and HPV-16 E2 proteins compared to their parental controls. Our data suggest that E2 interaction with ORC2 protein does not serve to activate E1 and E2 dependent viral licensing; rather high levels of E2 that occur during differentiation dependent amplification can restrict pre-ORC assembly at mammalian origins that would otherwise effectively compete for host replication complexes.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #60

LPA AND ZIP4 ARE INVOLVED IN CANCER STEM CELL-LIKE ACTIVITIES IN OVARIAN CANCER

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LPA and ZIP4 are involved in cancer stem cell-like activities in ovarian cancer

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Overcoming drug-resistance and specifically targeting cancer stem cells (CSC) represent major challenges in high grade serous ovarian cancer (HGSOC) treatment. Lysophosphatidic acid (LPA) is a growth factor (an oncolipid) for HGSOC. Responses to LPA are mediated primarily by their plasma membrane bound G-protein coupled receptors (GPCRs; $LPAR_{1-6}$). In addition, LPA has been identified as a ligand for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR¿¿¿¿). Through genetic, cell biological, and biochemical analyses, we revealed novel LPA regulatory roles in EOC side population (SP) cells (capable of excluding Hoechst 33342 outside the cells), which have been shown to be an enriched source of CSC and progenitor cells in EOC. LPA significantly increased SP and $CD44^{+}CD117^{+}$ double positive cell populations in HGSOC cell lines PE01, PE04, and OVCAR3. LPA dose- and time-dependently up-regulated the zinc transporter ZIP4 (gene name Slc39a4) in these cell lines. Regulation of *ZIP4* expression was exclusively studied in the context of zinc previously. Other factors regulating ZIP4 expression are essentially unknown. We have revealed that LPA is a novel regulator of ZIP4 in HGSOC, which is mediated by PPAR¿¿¿¿. We have established ZIP4- and PPAR¿¿¿¿-knockout PE04 and OVCAR3 cell lines using the Cas-9 nuclease to facilitate RNA-guided site-specific DNA cleavage (CRISPR) system. ZIP4 was functionally involved in CSC-related cellular activities including proliferation, anoikis-resistance, colony-formation, spheroid-formation, drug-resistance, and LPA-induced SP in HGSOC cells. Importantly, ZIP4-KO blocked ~ 90% of LPA's effect on SP. In summary, LPA and ZIP4 were involved in CSC-like activities in HGSOC.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #61

DEVELOPMENT OF XERODERMA PIGMENTOSUM GROUP A (XPA)-DNA INTERACTION INHIBITORS FOR CANCER THERAPY

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Targeting DNA repair and the DNA damage response for cancer therapy has recently gained increasing attention with inhibitors of the PARP enzyme showing a therapeutic efficacy in various cancers. The utility of DNA repair inhibitors can be expanded by their use in combination treatment with DNA damaging chemotherapeutics. During the course of our ongoing research, we have identified NER inhibitors targeting the DNA binding activity of the Xeroderma Pigmentosum Group A (XPA) protein, a critical component for both transcription coupled and global genomic NER. In addition, XPA protein is required for the removal of all types of DNA lesions repaired by NER. Clinical validation of XPA has been obtained where high expression of XPA in lung, ovarian and lung cancer results in decreased efficacy of platinum therapy. Considering the importance of XPA in the DNA damage recognition process and the potential to enhance the therapeutic efficacy of platinum based anticancer drugs, we have identified X-80 class of compounds in the original screen and it displayed reasonable potency in fluorescence polarization DNA binding assays. More recent structure activity relationships (SAR) of this class of compounds has identified a series of drug-like analogs that display a >50-fold increase in potency and excellent specificity. Analyses of the SARs define the chemical and structural features that impact the interaction with XPA, cellular permeability and contribute to selectivity. Data demonstrate that the X80 class of inhibitors do not interact with DNA but directly bind the XPA protein. These data demonstrate the potential in developing novel adjuvant anticancer therapeutics that target XPA-DNA interactions. Further structure-activity relationships (SAR) of lead compound is underway to develop highly potent XPA inhibitors for preclinical settings.

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Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #62

A NOVEL WAY TO SENSITIZE PANCREATIC CANCER CELLS FOR GEMCITABINE TREATMENT

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies, with a dismal median survival of 6 months. Gemcitabine is still the first-line treatment for patients with PDAC, and its combination with albumin-bound paclitaxel has shown clear therapeutic advantage. We hypothesize that strategies to increase gemcitabine sensitivity may be effective in PDAC treatment. In this study, we report that cancer stem cell regulator Sox2 is highly induced in acquired gemcitabine resistant pancreatic cell lines, and down-regulation of Sox2 sensitizes cancer cells to gemcitabine treatment both in the cultured cells and in mouse models. Down-regulation of Gli1/2 was associated with reduced Sox2 expression, decreased expression of cancer stem cell markers. We found Gli1/2 proteins in association with a Sox2 promoter sequence with a putative Gli binding site, suggesting a direct regulation of Sox2 by Gli1/2. We further showed that down-regulation of Gli1 or Gli2 was as effective as Sox2 knockdown in sensitizing cancer cells to gemcitabine treatment. The relevance of Gli1/2-Sox2 signaling axis to PDAC was shown by the association of high Sox2 expression with poor survival in stage II PDAC patients. Taken together, our data indicate an important role of non-canonical hedgehog signaling in regulation of gemcitabine sensitivity, and down-regulation of Gli1/2 transcription factors, together with gemcitabine, may be effective in pancreatic cancer treatment.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #63

SRC PHOSPHORYLATED MDM2 REQUIRES MDMX TO ACT AS A NEDDYLATING LIGASE

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Mdm2 is an oncoprotein and, along with its family member MdmX, have been shown to be elevated in human cancers. Like Mdm2, MdmX, is a RING containing protein, yet it lacks intrinsic E3 ligase function. Mdm2 has been shown to facilitate ubiquitination as a monomer, a homodimer, and as a heterodimer with MdmX depending on the cellular stress. Our recent report establishes that under growth conditions that activate Src, Mdm2 functions as a neddylating enzyme. Whether MdmX is necessary forMdm2 to neddylate p53 has yet to be shown. Here we demonstrate that Src phosphorylation results in increased levels of MdmX, increased binding between Mdm2 and MdmX, and increased neddylation of MdmX and p53. Interestingly, the lack of MdmX (in transient assays or in shRNA cell lines) results in decreased neddylation of p53. These data support a critical role for MdmX as part of the Mdm2 neddylating complex.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #64

IMPACT OF MESOTHELIN EXPRESSION ON THE METASTATIC SUCCESS OF OVARIAN CANCER

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Ovarian cancer is the most lethal gynecological cancer in U.S. women. Poor 5-year survival rates (<30%) are due to presentation of most women at diagnosis with advanced stage disease with widely disseminated intraperitoneal metastasis. However, when diagnosed before metastatic propagation the overall 5-year survival rate is >90%. Metastasizing tumor cells grow rapidly and aggressively attach to the mesotheliumof all organs within the peritoneal cavity, including the parietal peritoneum and the omentum, producing secondary lesions.Mesothelin (MSLN), a 40kDa glycoprotein that is over expressed in many cancers including ovarian and mesotheliomas is suggested to play a role in cell survival, proliferation, tumor progression and adherence. However, the biological function of mesothelin is not fully understood as MSLN knockout mice do not present with an abnormal phenotype. Conversely, MSLN has been shown to bind to the ovarian cancer antigen, CA-125, and thought to play a role in the peritoneal diffusion of ovarian tumor cells. Taking into consideration the potential importance of MSLN/CA-125 binding in ovarian tumor metastasis within the peritoneum, MSLN wild type (WT) and knockout (KO) mice were used to explore the role of mesothelin on the susceptibility of ovarian tumor cells to adhere to the mesothelium of the organs in the peritoneal cavity. An e*x vivo* peritoneal assay, using CA-125 positive human ovarian tumor cells OVCAR8-GFP and peritoneal explants from MSLN WT and KO mice demonstrated a decrease in OVCAR8-GFP cell adhesion to peritoneal tissues from MSLN KO mice compared to MSLN WT mice. Furthermore, allograft tumor studies using MSLN WTand KO mice injected intraperitoneally with fluorescently-tagged syngeneic murine ovarian cancer cells (ID8-RFP) was performed. Disease progression was evaluated post injection by fluorescent *in vivo* imaging prior to end point dissection (~8 weeks). Abdominal organs were dissected, imaged *ex vivo* and organ-specific tumor burden was quantified by tumor area. Tumor burden was significantly decreased in the liver and omentum of MSLNKO mice compared to MSLN WT mice. Together, the results demonstrate a loss of mesothelial cell-ovarian tumor cell adhesion in the omentum and peritoneum of mice that do not express MSLN.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #65

PRECLINICAL ANALYSIS OF NOVEL SMALL MOLECULE INHIBITORS OF REPLICATION PROTEIN A (RPA), IN PATIENT DERIVED SPHEROID MODELS OF HIGH GRADE SEROUS OVARIAN CANCER

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Objectives. Three-dimensional (3D) culture models are emerging in preclinical drug sensitivity analyses and are particularly relevant in high grade serous ovarian cancer (HGSOC), where metastatic cells often form spheroids within ascites prior to surface deposition. The use of primary cells harvested from the ascites of patients with HGSOC also better represents the heterogeneity inherent to this malignancy. Platinum (Pt) based chemotherapy is a key component in the treatment of HGSOC and the formation of Pt-DNA damage ultimately induces cell death. Replication protein A (RPA) plays a crucial role in the repair of Pt-DNA adducts through the nucleotide excision repair (NER) and homologous recombination repair (HRR) pathways, which are major contributors to cellular resistance. Therefore, we investigated the effectiveness of RPA inhibition alone and in combination with cisplatin in patient derived spheroid models of HGSOC.

Methods. Malignant cells were harvested from ascites of patients with HGSOC, cultured and seeded into hanging drops, allowing spheroid formation. The anti-cancer activity of novel RPA inhibitors (RPAi) was tested alone and in combination with cisplatin using sequential and concurrent treatment regimens.

Results. Ascites derived HGSOC cells were reliably cultured and spheroids were generated. IC_{50} values for Pt and RPAi were variable as a function of patient origin, but values were similar to those obtained by conventional two-dimensional culture. When treated sequentially or in combination, synergy was seen with Pt and RPAi.

Conclusions. Multicellular spheroids derived from HGSOC ascites can be reliably generated for use in preclinical drug sensitivity assays. Spheroids are sensitive to Pt and RPAi. RPAi showed both single agent activity and synergy with Pt.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #66

EXPLORING THE MECHANISMS OF EPIGENETIC ALTERATIONS DURING INFLAMMATION-DRIVEN TUMORIGENESIS

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Inflammation plays a pivotal role in the initiation and promotion of cancer. Indeed, oxidative stress caused by inflammation can lead to genetic mutations and epigenetic changes that promote tumorigenesis. The genetic abnormalities leading to cancer have been well studied whereas less is known concerning the role of epigenetic changes. Interestingly, there is a global loss of DNA methylation in cancer along with concurrent hypermethylation of distinct promoter CpG islands. Hypermethylation of the promoters of tumor suppressor genes (TSGs) can lead to their silencing ultimately contributing to cancer. How epigenetic alterations are initiated is not known, but we hypothesize that oxidative stress caused by inflammation is involved. Accordingly, we are using a mouse model to test the hypothesis that inflammation causes DNA damage and recruitment of epigenetic proteins to damaged chromatin leading to silencing of genes in tumors. When Min mice, which are heterozygous for mutant *adenomatous polyposis coli* (*APC*), are infected with the bacterium enterotoxigenic *Bacteroides fragilis* (ETBF), tumors develop in their distal colons. Preliminary results demonstrate that ETBF promotes enrichment of epigenetic proteins at the promoters of several TSGs. Furthermore, ETBF causes genome-wide methylation changes in tumors that form at sites of inflammation. Specifically, ETBF causes a global loss of DNA methylation in tumors along with concurrent hypermethylation of many promoter-associated CpG islands, including CpG islands associated with TSGs. Previous work in vitro indicated that mismatch repair (MMR) proteins are involved in recruitment of epigenetic proteins to sites of DNA damage. We tested whether this finding holds true in our mouse model. ETBF-treated mice lacking expression of the MMR protein *Msh2* in their intestinal epithelium have reduced recruitment of epigenetic proteins to chromatin compared to ETBF-infected wildtype mice. Moreover, tumors from ETBF-infected *Msh2*-deficient/Min mice had fewer DNA methylation changes compared to tumors from ETBF-infected wildtype/Min mice. These findings have begun to uncover a novel mechanism underlying inflammation-induced tumorigenesis, namely the involvement of MMR machinery in promoting epigenetic changes via the recruitment of epigenetic proteins to sites of DNA damage.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #67

DPP4 TRUNCATED COLONY STIMULATING FACTORS MANIFEST DISTINCT REGULATORY FUNCTION COMPARED TO THEIR FULL LENGTH FORMS AND DPP4 IS ALTERED BY, AND MODULATES SENSITIVITY TO, EPHOSS. .

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Hematopoietic stem cells (HSC) reside in hypoxic niches (~1-4% O_2), yet, HSC studies are typically performed using cells isolated in ambient air (~20% O_2). Inhibiting ambient air exposure/harm of cells, which we termed Extra Physiologic Oxygen Shock/Stress (EPHOSS), by collecting/processing human cord blood (hCB) or mouse bone marrow (mBM) stem cells in hypoxia (3% O_2), enhances recovery of phenotypic/functional long-term repopulating HSC (LT-HSC) and is mechanistically linked, in part, to the mitochondrial permeability transition pore (MPTP), Reactive Oxygen Species (ROS) and cyclophilin D. We hypothesized that Dipeptidyl Peptidase 4 (DPP4), an enzyme that N terminally cleaves and modifies the function of select proteins leading to alterations in homing/engraftment of HSC, may be altered by EPHOSS and involved in EPHOSS effects on HSC. Proteomic and bioinformatics analysis identified 1) many unexpected intracellular/secreted proteins with DPP4 truncation (T) sites and 2) specific, as well as overlapping, modifications in phosphorylated and differential protein signaling of T-cytokines (GM-CSF and IL-3) compared to their full length (FL) forms leading to alternative regulation of signaling/function in normal and leukemic cells. T-GM-CSF and T-IL-3 had enhanced receptor binding compared to their FL forms with significant, and reciprocal, blunting of functional activity of both factors *in vitro* and *in vivo*. To investigate effects of DPP4 on EPHOSS, and vice versa, mBM was harvested (air/ hypoxia) with a DPP4 inhibitor (DPA), or from DPP4 K/O mice. This resulted in significant increases in the number of phenotypic LT-HSC (p=.017) in air, suggesting that DPP4 inhibition blunts EPHOSS mediated loss of phenotypic LT-HSC. Also, the percentage of DPP4+ cells was increased in primitive fractions of mBM or hCB, (LSK ~15%, LSKCD150 ~40%, CD34+CD38- ~10%, CD34+CD38-CD45RA-CD90+CD49F+ ~40% p=.007) and further enhanced 15-20% when cells were isolated in hypoxia (p=.005). Unexpectedly, Cyclosporin A, a cylophilinD/MPTP inhibitor did not increase the number of DPP4+ cells as was seen with hypoxic harvest. Further, LT-HSC ROS levels (mitochondrial/total) were not diminished in DPA or DPP4 K/O groups harvested in air despite the increase in phenotypic LT-HSC over air harvest alone, thus suggesting a non ROS/MPTP mechanism. In conclusion, DPP4 expression/activity serves heretofore unknown roles in the regulation/signaling of multiple protein types as well as with respect to hematopoiesis and cellular responses to EPHOSS.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #68

MDM2 SILENCING PROMOTES TUMOR-INITIATING CELLS IN OSTEOSARCOMA AND BREAST CANCER

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Chemotherapy has made remarkable strides in the past century to alleviate tumor burden across most cancer types, yet it remains ineffective at achieving complete tumor remission. To improve chemotherapy efficacy and patient survival, novel drugs will require a greater mechanistic understanding of tumor initiating cell (TIC) regulatory pathways. Intratumoral heterogeneity is a major obstacle for conventional targeted treatment modalities, which select for aggressive phenotypes with acquired drug resistance and TIC enrichment. Here we focus on elucidating the role of Mdm2 in mediating TIC levels in osteosarcoma (MG-63, Saos2) and breast cancer (MDA 231, MDA 468, and BT474) cell lines. We demonstrate Mdm2 silencing by ShRNA results in increased levels of Nanog, Oct4a, and Sox2, and diminished expression of ERa and ERß by western blot. We observed an increase in the immunophenotypic expression of CD133 in ShMdm2 osteosarcoma and breast cancer cell lines by flow cytometry. Our data provides the first evidence that loss of Mdm2 results in increased TIC population. Further analysis will examine the affects of TIC using small molecular inhibitors to Mdm2. This insight will contribute to the molecular foundations defining the role of Mdm2 in TIC and will determine if targeting Mdm2 therapeutically is a prognostic indicator for recurrence.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #69

MDM2 REGULATES INTRACELLULAR PATHWAYS IN TBNC AND OSTEOSARCOMA THAT AFFECT OSTEOCLASTOGENESIS

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Advanced cancer cells infiltrate and colonize new organ microenvironments through transformation of the dynamic metastatic niche. In bone, tumor cells secrete soluble factors that distort and manipulate normal bone remodeling leading to osteolytic lesions and inflammation. Clinically, primary high-grade bone sarcomas and secondary metastatic cancers exhibit elevated levels of murine double minute 2 (Mdm2), the principal cellular antagonist and E3 ubiquitin ligase of tumor suppressor p53. We demonstrate a novel function of mdm2, independent of p53, to drive osteolytic disease influenced by the signaling cascade of RANKL, ICAM1, and RANTES. To elucidate the direct influence of Mdm2 in cancer cells on osteoclast differentiation, several cell lines were created to overexpress or silence Mdm2. We found that osteoblast-like osteosarcoma (MG63, Saos2) and metastatic breast cancer cell lines (MDA468, MDA231, TMD231, T47D, and BT474) lead to significant increased osteoclast differentiation, proliferation, and bone resorption. Co-cultures of Mdm2 over-expressing cancers and normal monocytes resulted in increased numbers of osteoclasts. In cancer cell lines where Mdm2 is silenced, we observed lower number of osteoclasts. The presence of Mdm2 in cancer cells also increased osteoclast resorption in an *in vitro* organ model. To determine the effects of ICAM1, we used an ICAM antibody to block ICAM1 in the media and showed a decrease in osteoclastogenesis. Understanding the role of Mdm2 in osteoclastogenesis may be amendable to translational benefit in disease models of advanced bone resorption and bone metastases.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #70

ATG16L1 AND COLON CANCER METASTASIS

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Colorectal cancer (CRC) is the third leading cause of cancer- related death in the United States, with metastatic stage IV CRC only having an 11% 5-year survival rate. ATG16L1 is a core autophagy protein typically found in complex with ATG5 and ATG12. This complex mediates conjugation of LC3 to the developing autophagophore, which is essential for autophagy. Many core autophagy proteins also serve non-autophagy functions such as regulation of innate immune signaling and control of viral replication. A single nucleotide polymorphism in ATG16L1 encoding a Thr300Ala amino acid substitution has been previously implicated with an increased risk for the development of Crohn's disease (CD). Several studies have shown that the T300A variant does not alter bulk autophagy suggesting that a non-autophagic function of T300A may underlie its association with CD. Previous work in our lab has shown that the ATG16L1 T300A variant is associated with longer overall survival and reduced metastasis in CRC patients.

This study set out to understand the role of ATG16L1 in tumor development and metastasis. Using homologous recombination, our lab generated human CRC cell lines that either lack ATG16L1 ($ATG16L1^{-/-}$) or have the CD-associated T300A mutation (ATG16L1 T300A). We performed xenograft experiments in $RAG1^{-/-}$ mice with $ATG16L1^{+/+}$ cells injected in one flank and either $ATG16L1^{-/-}$ or ATG16L1 T300A cells injected into the other flank. On the day of necropsy, Brdu was administered intraperitoneally 4 hours prior to sacrifice. Within two weeks post xenograft, there were significant size differences between the $ATG16L1^{+/+}$ tumors and either the $ATG16L1^{-/-}$ or ATG16L1T300A tumors. Immunofluorescent staining of Brdu revealed significantly reduced uptake in both $ATG16L1^{-/-}$ and ATG16L1 T300A tumors compared to $ATG16L1^{+/+}$. Further studies in cell culture determined this defect in Brdu uptake was attributed to slower cell cycling rather than cellular senescence. We also performed an in vitro scratch wound assay to determine the migration kinetics for our three cell types. We found no difference in migration kinetics between the $ATG16L1^{+/+}$ and the ATG16L1 T300A variant. We did see a significant reduction in migration in the $ATG16L1^{-/-}$ cells. This indicates that the reduction in CRC metastasis associated with the ATG16L1 T300A variant is not due to a cell intrinsic defect in migration.

In conclusion, our results reveal that the ATG16L1 T300A variant impairs tumor development in vivo. This was evidenced by the significant reduction in tumor size compared to $ATG16L1^{+/+}$ tumor. These cells exhibit decreased proliferation as shown by the decreased uptake of Brdu and this was attributed to the cells having a slower cell cycle compared to $ATG16L1^{+/+}$ cells. We also revealed that the association of the AGT16L1 T300A variant and reduced metastasis in CRC patients is not due to a lack of migration kinetics in these cells.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #71

REGULATION OF NETRIN-1-UNC5A DEPENDENCE RECEPTOR PATHWAY AND ESTROGEN RECEPTOR SIGNALING IN BREAST CANCER

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The dependence receptors (DR) are cell surface receptors that can mediate two different intracellular signals. In the presence of ligands, DR generates a positive signal leading to cell survival, differentiation or migration. In contrast, in the absence of ligands, DR initiates a signal for cell death/apoptosis. Thus, alteration of the DR signaling pathway may play a role in tumorigenesis. In this study, we focus on the function of UNC5A, a DR, and its secreted ligand Netrin-1 (NTN1) in breast cancer and metastasis. Since estrogen (E2) and its receptor (estrogen receptor-alpha; ERa) are major signaling players in ERa-positive breast cancers, we performed all the studies in ERa-positive breast cancer cells. We observed that *UNC5A* and *NTN1* are estrogen-inducible genes depending on cell types. In addition, RNA-seq analyses of vector control and *UNC5A*-knockdown cells treated with or without E2 revealed ~10-fold increase in several E2-regulated genes in *UNC5A*-knockdown cells. Cell proliferation and the anti-apoptotic BCL2 were significantly up-regulated in *UNC5A* knockdown cells. Moreover, *UNC5A* knockdown cells showed elevated up-regulation of *¿Np63*, a TP53 family transcription factor that promotes breast epithelial stem cell maintenance and basal-like breast cancer. Consistent with the known role of TP63 in cancer stem cells, *UNC5A*-knockdown cells displayed cancer stem cell phenotype as evident from ~3-fold increase in the number of $CD44^+/CD24^+$ and $CD44^+/EpCAM^+$ subpopulation compared with control cells. Furthermore, *in vivo* studies in mice determined that implantation of *UNC5A*-knockdown cells can form tumors in the mammary fat pad and are able to colonize multiple organs such as lungs, ovaries and adrenal glands. Thus, knockdown of *UNC5A* resulted in deregulated expression of E2-regulated genes, E2-independent and anti-estrogen-resistant growth in vitro, and E2-indpendent tumor formation in xenograft models. Overall, our results suggest that E2 induces UNC5A expression as a negative regulatory loop to restrict or fine tune ERa:E2 signaling and maintain luminal phenotype. Loss or mutation of UNC5A, as frequently observed in cancer, could lead to unrestricted E2:ERa signaling and anti-estrogen resistant growth while simultaneously enabling ERa-positive luminal breast cancer cells to acquire basal-like and cancer stem cell-like features.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #72

DEVELOPING HSP60/10 CHAPERONIN INHIBITORS FOR COLORECTAL CANCER CHEMOTHERAPY

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Developing HSP60/10 Chaperonin Inhibitors for Colorectal Cancer Chemotherapy

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Colorectal cancer (CRC) is the third most prevalent cancer in both males and females, accounting for ~10% of all cancers and adding nearly one million new cases worldwide annually. We propose that targeting the 60 kDa Heat Shock Protein chaperonin system (HSP60/10) is a viable chemotherapeutic strategy towards treating colorectal cancers. In colorectal and other cancers (e.g. cervical, ovarian, breast, and prostate), the abnormally propagating cells have hijacked HSP60/10 regulation to help circumvent apoptosis. Studies show that HSP60/10 functions are associated with apoptotic, pro-survival, and metastatic pathways (e.g. modulation of p53, Bax, cytochrome c, survivin, IKK/NF-¿B, ß-catenin, a3ß1 integrin, and HIF-1a). While the role of HSP60/10 in oncogenesis is not fully understood, cytosolic accumulation of HSP60 in tumor environments with or without mitochondrial release is likely contributing towards tumor progression. The latter scenario results in naïve HSP60, still bearing its N-terminal mitochondrial import sequence, which is reported to be more stable than mitochondrial HSP60. Naïve HSP60 forms double-ring tetradecameric complexes instead of single rings. We have been developing inhibitors against both naïve and mitochondrial HSP60 to elucidate possible biochemical/functional differences between these forms and their respective role(s) in pathogenesis. High-throughput screening of ~700,000 compounds led to the identification of several hundred potent chaperonin inhibitors, which block the refolding pathway of HSP60/10. Many of these initial hits and first generation analogs inhibit the growth of colon cancer cell lines (HCT-116, HT-29 and DLD-1). We are currently optimizing these HSP60/10 inhibitors for *in vivo* chemotherapeutic efficacy testing.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #73

DYNAMICS OF RADIATION INDUCED H2AX PHOSPHORYLATION IN MEGAKARYOCYTES OF THE BONE MARROW NICHE

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DNA repair and damage responses are employed to maintain genomic integrity following exposure to a variety of exogenous factors. During the radiosensitization of tumor cells, ionizing radiation induces DNA double strand breaks. Failure of DNA repair and damage responses alter hematopoiesis and lead to the development of cancer prone genetic diseases. Within the bone marrow niche, normal hematopoietic stem cells remain within a relatively quiescent state, and megakaryocytes promote their proliferation and differentiation following injurious insult. Cells of the developing megakaryocyte (MK) lineage must remain viable, progress through the cell cycle, and undergo extensive cellular remodeling to maintain mesenchymal stromal and endothelial cells of the bone marrow vasculature. We evaluated bone marrow and MK responses to radiation induced injury in young C57BL/6 mice in a kinetic model for γH2AX induction. In the non-irradiated mice, 5.82% of DRAQ5 live cells were observed to be γH2AX positive. Following 650cGy TBI, phoshorylated H2AX(pS139) recruits DNA repair proteins and cell cycle checkpoint factors to sites of DNA damage. A significant increase in γH2AX expression was observed within 30 minutes (14.52%, $P<0.0001$), peaking at one hour (17.5%, $P<0.0001$) and returning to normal value by 24 hours. CD41+ c-Kit+ MK progenitor cells significantly increased γH2AX expression one hour following radiation (72.53%, $P<0.0001$) and declined to similar levels as that of the non-irradiated control mice within 24 hours. During apoptosis, the nuclear chromatin-associated enzyme, Poly ADP-Ribose Polymerase, is cleaved by caspase-3, arresting its activity and ability to repair DNA damage. Median values of fluorescent intensity for cleaved PARP were significantly increased in γH2AX+CD41+ckit+ MK progenitors at 1h post irradiation ($p<0.0012$) and peaked at 24h post irradiation ($p<0.0001$) when compared to non-irradiated controls. Megakaryocyte progenitors grown in culture for 5 days demonstrated a greater than 4-fold increase in apoptotic cells expressing γH2AX from isolated spleens 1h following irradiation compared to control cultures. Microscopic analysis of cultured cells demonstrated features of early stage apoptosis, shrunken cells with condensed cytoplasm containing fragmented and pyknotic nuclei. In cultured megakaryocyte progenitors from spleens isolated at 8 and 48h post irradiation, shared features of apoptosis and necrosis were observed with cellular membrane blebbing, chromatin and cytoplasm condensation, and cell shrinkage. This study represents the first in kind of γH2AX DNA damage and apoptosis in megakaryocyte lineage cells of the bone marrow niche and may lend to translational applications in the evaluation of drugs as potential radiosensitizers.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #74

ACETYLATION REGULATES THE DNA BINDING PROPERTIES OF REPLICATION PROTEIN A (RPA)

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Genome maintenance is critical for cellular survival and growth. Replication Protein A (RPA), a single-strand DNA (ssDNA) binding protein, is vital for various aspects of genome maintenance such as replication, recombination, repair and checkpoint activation. RPA binding to ssDNA protects it from degradation by cellular nucleases, prevents secondary structure formation and from illegitimate recombination. Within the cell, RPA is subject to many post-translational modifications including phosphorylation, SUMOylation and ribosylation. These modifications regulate the activity of RPA by altering its interactions with DNA and other binding partners. In addition to other forms of modification, acetylation of specific lysine residues has also been identified by mass spectrometry in a global acetylome study. In our current study, we evaluated the effect of lysine acetylation on different enzymatic properties of human recombinant RPA such as ssDNA binding affinity, DNA strand annealing and melting efficiencies.

For the biochemical studies, RPA was *in vitro* modified by the catalytic domain of the acetyltransferase, p300. To study the effect of acetylation on its ssDNA binding function, we made use of electro-mobility gel shift assay (EMSA) and bio-layer interferometry (BLI) technology. Using various length oligonucleotides, we tested the binding property of unmodified and acetylated RPA. Our results showed that acetylation of RPA increased its binding affinity compared to unmodified RPA. Interestingly, the acetylated form of RPA was also able to bind more stably to oligonucleotides of lengths shorter than what was previously reported for optimal binding efficiency. Additionally, acetylated RPA showed more stable binding in the presence of competitor ssDNA compared to the unmodified form. All our results indicate that the acetylation of RPA improves its ssDNA binding function. Using mass spectrometry analysis we have correlated the *in vitro* lysine sites on RPA that were modified to those reported in cells in the acetylome study. The acetylation-based alteration in its DNA binding properties would have significant implications in maintenance of genome fidelity, since improved DNA binding function of RPA will presumably better protect the genome from both endogenous and exogenous stresses.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #75

THE VITAMIN D RECEPTOR PROMOTES HUMAN BREAST CANCER CELL GROWTH VIA A LIGAND INDEPENDENT CYTOPLASMIC FUNCTION

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Previous reports show that vitamin D deficiency promotes human breast cancer growth in bone. While this effect appeared to be mediated indirectly through changes in the bone microenvironment, vitamin D and the vitamin D receptor (VDR) may also exert direct actions on cancer growth in bone. The current study therefore aimed to define whether the VDR has a role in cancer growth.

We found that knock-down of VDR expression in human breast cancer cells reduced cancer cell proliferation and increased apoptosis *in-vitro*, bothin the presence and absence of its cognate ligand, 1,25-dihydroxyvitamin D. Implantation of these VDR knockdown cells into nude mice resulted in reduced tumour growth in both bone and soft tissues, suggesting that the VDR affects cancer cell growth independent of the bone microenvironment. Overexpression of a mutant VDR deficient in the nuclear localization signal (mVDR) in VDR Knockdowncells restored cell growth to the same level as seen in controls. This was associated with cytoplasmic accumulation of mVDR.

We conclude that the cytoplasmic VDR promotes breast cancer growth both in *vitro* and *in vivo*. These effects strongly contrast the well-established anti-proliferative and pro-apoptotic actions of the VDR-Vitamin D ligand complex. This discovery adds to our understanding of VDR signalling in breast cancer and may help to resolve discrepancies regarding the association between vitamin D status and breast cancer prognosis.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #76

STAT3 MEDIATES APC-DRIVEN CHEMOTHERAPEUTIC RESISTANCE IN BREAST CANCER

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Breast tumors frequently develop resistance to treatment with a variety of standard (i.e. cisplatin or doxorubicin) or targeted chemotherapeutic agents, resulting in tumor recurrence and increased patient mortality. The Adenomatous Polyposis Coli (APC) tumor suppressor is lost by hypermethylation or mutation in up to 70% of sporadic breast cancers; however, the downstream effects of APC loss have not been well explored in breast cancer. Heterozygous *Apc* mutation using the $Apc^{Min/+}$ mouse model enhanced tumorigenesis in the mouse mammary tumor virus – Polyoma Middle T transgenic model (MMTV-PyMT). Cells isolated from MMTV-PyMT;$Apc^{Min/+}$ mice are resistant to cisplatin and doxorubicin, express higher levels of multidrug resistance protein 1 (MDR1), and have a greater population of tumor initiating cells (TICs) compared to controls. Here we demonstrate that APC loss-of-function cells have increased activation of signal transducer and activator of transcription 3 (STAT3) and the anti-apoptotic protein Mcl-1. STAT3 over-expression is common in breast cancer, leads to poor prognosis, and up-regulates MDR1 expression leading to chemotherapeutic resistance. To explore the functional role of STAT3 in mediating therapeutic resistance in APC loss-of-function models, we examined the impact of STAT3 inhibition on MDR1 and the TIC population to cause therapeutic resistance in the MMTV-PyMT;$Apc^{Min/+}$ vs MMTV-PyMT;$Apc^{+/+}$ murine breast cancer cells. We have made the novel observation that chemotherapy treatment has a time-dependent effect on the activation of STAT3 and expression of Mcl-1 specific to *Apc*-mutant cells. Finally, we have assessed the use of STAT3 inhibitors in combination with standard chemotherapy to alleviate APC-mediated resistance. Combined these data suggest that loss of APC activates STAT3-driven pathways resulting in the development of chemotherapeutic resistance.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #77

MECHANISMS ASSOCIATED WITH SYSTEMIC EFFECTS OF BREAST CANCER

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Breast cancer is one of the most common malignancies. With its progress, breast cancer causes systemic effects such as functional limitation, sarcopenia, and cachexia. These effects are manifested as muscle weakness, body pain or depletion of skeletal muscle without apparent loss of body weight. Although onset of cachexia in breast cancer is not as rapid and severe as in lung and pancreatic cancer, over a quarter of breast cancer patients experience a precachexia to cachexia syndrome. However, mechanistic studies on systemic effects of breast cancer are limited. In this project, we used myoblast cell line C2C12 and rat embryonic cardiomyocyte cell line H9C2 for in vitro assays and MMTV-Her2/Neu and MMTV-PyMT mammary tumor models for in vivo assays to illuminate key players linked to systemic effects, to identify functional biomarkers, and to develop a rational therapy for systemic complications of breast cancer. In *in vitro* studies, we found treatments with conditioned media from a number of mammary tumor cell lines, including PO1058 (poorly invasive tumor cells from PyMT-WapCre-mGFP+ mice in C57BL6 background), PO1059 (highly invasive cells from PyMT-WapCre-mGFP+ mice), and MMT8 (tumor cells from MMTV-Neu mice in FVB/N background) resulted in a significant decrease of miR486 in C2C12 cells but not in H9C2 cells. miR486 is a muscle-enriched microRNA, which controls differentiation of myoblasts and its deregulation is linked to musculoskeletal defects in muscular dystrophy patients. Interestingly, we also found lower circulating miR-486 in the plasma of patients with pancreatic and bladder but not lung cancer. These data are consistent with our previous report of lower circulating miR486 in plasma of breast cancer patients with metastasis compared with healthy women. In *in vivo* study, we observed deteriorating physical and functional conditions in $PyMT^+$ mice with the progression of mammary tumor. Compared to wildtype mice, $PyMT^+$ mice with mammary tumors showed decreased fat mass and decreased grip strength, both markers of advancing cachexia. In our preliminary drug intervention study, Diaminomethylparthenolide (DMAPT), a NF-¿B inhibitor, partially reduced mammary tumor development in tumor numbers and sizes in $PyMT^+$ mice and several of the systemic effects of cancer. To date, we have not found an effect of mammary tumor on cardiac functions of 12-week old $PyMT^+$ mice. Continuous monitoring of these mice with the progression of their tumors will reveal more detailed mechanistic changes associated with systemic effects of breast cancer. In summary, mammary tumor resulted in changes in body composition and the decrease of grip strength of $PyMT^+$ mice, which may be associated with altered NF-¿B pathway. Additionally, circulating miR-486 may serve as a useful clinical indicator of progression and systemic effects of certain cancers.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #78

ROLE OF NOVEL SERINE 316 PHOSPHORYLATION OF THE P65 SUBUNIT OF NF-¿B IN DIFFERENTIAL GENE REGULATION

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Nuclear factor kappa B (NF-¿B) is a central coordinator in immune and inflammatory responses. Constitutive NF-¿B is often found in some types of cancers, contributing to oncogenesis and tumor progression. Therefore, knowing how NF-¿B is regulated is important for its therapeutic control. Post-translational modification of the p65 subunit of NF-¿B is a well-known approach for its regulation. Here, we reported that, in response to interleukin 1b (IL-1ß), the p65 subunit of NF-¿B is phosphorylated on the novel serine 316 (S316). Overexpression of S316A (serine 316-alanine) mutant exhibited significantly reduced ability to activate NF-¿B and decreased cell growth as compared to wtp65 (wild type p65). Moreover, conditioned media from cells expressing the S316A-p65 mutant had a considerably lower ability to induce NF-¿B than that of wtp65. Our data suggested that phosphorylation of p65 on S316 controls the activity and function of NF-¿B. Importantly, we found that phosphorylation at novel S316 site, and other two known phosphorylation sites—S529 and S536—either individually or cooperatively, regulated distinct groups of NF-¿B-dependent genes, suggesting the unique role of each individual phosphorylation site on NF-¿B-dependent gene regulation. Our novel findings provide an important piece of evidence regarding differential regulation of NF-¿B-dependent genes through phosphorylation of different p65 serine residues, thus shedding light on novel mechanisms for the pathway-specific control of NF-¿B. This knowledge is key to develop strategies for prevention and treatment of constitutive NF-¿B-driven inflammatory diseases and cancers.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #79

CORRELATED CHEMO-MECHANICAL PROPERTIES IN OVARIAN CANCER METASTASIS

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Ovarian Cancer has a distinct form of metastasis, wherein tumor cells attach to the mesothelial cells that line the peritoneum wall, where they anchor in the collagen-rich sub-mesothelial matrix and proliferate. Distinct changes are observed in peritoneal tissue associated with aging that correlate with the incidence of ovarian cancer (OvCa). The occurrence of OvCa is strongly correlated with the age of patient, with >85% of cases occurring in women over the age of 45, and >50% occurring between the ages of 45-75. Data suggests that distinctive changes occur to the mesothelial cells and the associated extra-cellular matrix (ECM) with age. We hypothesize that microscopic variation in the biomechanical properties of peritoneal tissues affect the metastatic success of ovarian cancer. To test this hypothesis, we propose instrumentation for correlating the microscopic ECM structure with elastic properties (Young's modulus) of the peritoneum tissue by performing AFM force-distance measurements simultaneously with SHG imaging. To our knowledge, no has measured how the ECM structure correlates to tissue stiffness. The development of technology to assess and correlate the ECM molecular structure with tissue mechanical properties will transform understanding of OvCa metastasis. We have obtained a value for the Young's modulus from very soft samples (E≈200 KPa) like PDMS and tissue in air. The thickness of tissue requires optical access from the top, the same side as the AFM. We are currently working to address challenges associated with experiments in solution. Ultimately, we will combine SHG – Force measurements to assess age-related changes in the peritoneal tissue. Differences have been observed in the SHG imaging of peritoneal tissue. This marker will provide evidence of our ability to correlate force measurements with structural information observed in the SHG measurements.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #80

THE ROLE OF MT1-MMP IN THE REGULATION OF OVARIAN CANCER CELL MIGRATION

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Ovarian cancer presents a unique mechanism of intra-abdominal metastasis that has occurred in 79% of cases. Since ovarian cancer has a distantly spread survival rate of only 27%, it is critical to understand how secondary metastases arise and how to develop better treatments. The detachment of single cells or multicellular aggregates (MCAs) and implantation into the mesothelium of the organs initiate ovarian cancer cell metastasis. The factors and molecular events that regulate the attachment of MCAs to the surface of the peritoneal cavity are generally unknown. Matrix metalloproteinases (MMPs) function by helping to break down the extracellular matrix in typical cell function. MT1-MMP is a transmembrane collagenase that is known to be highly expressed in ovarian cancer tumors. MT1-MMP has been shown to aid in ovarian cancer cell metastasis by promoting cellular detachment and MCA formation. In this study, we investigated the potential role of MT1-MMP in promoting ovarian cancer cell migration. We generated mutant ovarian cancer cell lines expressing wild-type MT1-MMP, phosphomimetic MT1-MMP-T567E mutants which mimic cytoplasmic tail Thr phosphorylation, MT1-MMP-T567A which functions as a phospho-defective mutant, and the catalytically inactive MT1-MMP-E240A mutant. Wound healing assay was performed to evaluate cell migration *in vitro*. Phospho-mimetic mutants of Thr567 exhibit enhanced cell migration, suggests that MT1-MMP phosphorylation plays a role in ovarian cancer cell migration, which facilitates ovarian cancer metastasis.

Basic Science ***Research Scientist***

POSTER #81

IDENTIFICATION OF CANCER-SPECIFIC SIGNALING NETWORKS: WHAT IS "NORMAL" CONTROL?

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Success of precision medicine depends on definitive identification of cancer-specific alterations in signaling pathways. However, identifying cancer-specific signaling networks is challenging because of lack of proper control tissue for differential gene expression analyses. Most studies in breast cancer utilize tumor-adjacent normal tissue or reduction mammoplasty samples as "normal" controls. We recently reported that breast epithelial cells from healthy donors as well as tumor-adjacent normal are in different differentiation states compared with tumor cells and the differences in differentiation status alone could account for major transcriptome variations between normal and tumor. To overcome these limitations, we propagated breast epithelial cells from three healthy donors (healthy-normal), two high-risk patients, two tumor-adjacent normal (HR/AD-normal) and five tumor samples of different molecular subtypes. Phenotypically defined (CD49f+/EpCAM+) luminal progenitor cells were sorted from these cultures and subjected to RNA-seq analyses. Pathway analysis revealed activation of cell-intrinsic pro-inflammatory signaling in HR/AD-normal cells compared with healthy-normal cells. This signaling network was further amplified in tumor cells. The pro-inflammatory chemokine CCL2, which is overexpressed in highly aggressive breast cancer, and the cytokine TNFRSF11B were elevated in HR/AD-normal luminal progenitor cells. Despite using phenotypically defined cells in the transcriptome analyses, cancer-specific signaling network identification was directly influenced by the type of controls used; healthy-normal or HR/AD-normal. While cancer-enriched PI3K and NF-κB activation was observed when compared to any kind of control, SRC kinase activation was noted only when cells from healthy-normal were used as a control. In general, the number of tumor signaling networks identified using healthy-normal as a control was higher than when compared with HR/AD-normal as a control. These results suggest that considerable attention should be placed on the type of tissues used as control for definitive identification of cancer-specific signaling networks and therapies to target such pathways. Additionally, these data show that non-cancer tissues of breast cancer patients acquire a cell intrinsic pro-inflammatory phenotype, which may be prerequisite for cancer development and potentially an early-detection tool.

Basic Science ***Research Technician***

POSTER #82

SKELETAL MUSCLE PROTEOME IN CANCER CACHEXIA VS. CHEMOTHERAPY-INDUCED CACHEXIA: SO CLOSE, YET SO FAR

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Cachexia, usually associated with depletion of muscle and fat, represents one of the major complications of advanced metastatic colorectal cancer. Evidence suggests that chemotherapeutic regimens, such as Folfiri, a combination of 5-fluorouracil, leucovorin and CPT-11, may also be responsible for these derangements. Despite this, it is unclear whether the same molecular mechanisms contribute to the development of cachexia as occurring in the presence of either chemotherapy or cancer.

The purpose of the present study was to investigate the cachexia signature in different conditions associated with severe muscle wasting, namely Folfiri- and Colon-26 (C26)- associated cachexia.

Administration of Folfiri for up to 5 weeks to normal mice caused marked decreases in adipose tissue and skeletal muscle mass, coherent with reduced muscle strength. Moreover, TEM analysis unveiled a marked depletion in muscle mitochondrial content and alterations of the sarcomeric structure consistent with loss of muscle structural proteins in the mice receiving chemotherapy. Similarly, growth of the C26 tumor caused a progressive depletion of muscle and fat tissue, associated with general inflammation and muscle weakness. By taking advantage of a LC/MS quantitative approach we identified 389 proteins significantly modulated in the quadriceps muscle of Folfiri-treated CD2F1 mice, and 273 proteins differentially expressed in the C26 hosts ($p<0.05$, $-1.5 = \text{fold change} = +1.5$). Both experimental conditions reveal modulation of calcium-related proteins, metabolic enzymes, structural proteins, markers of stress response and folding, mediators of proteasomal degradation and proteins associated with cellular transport. However, strong up-regulation of pro-inflammatory acute-phase proteins and a more coordinated modulation of mitochondrial and lipidic metabolisms seem to play a prominent role in the muscle of C26 tumor hosts, differently from the Folfiri treatment.

In the overall, our results suggest that cancer and chemotherapy contribute to muscle loss by activating several common signaling pathways. Regardless, peculiar differences resulting from the effects due to either cancer or chemotherapy are also shown. In an attempt to translate our findings into the clinical setting, our data support the undertaking of combination strategies that aim to both counteract tumor growth and reduce chemotherapy side effects.

Basic Science ***Research Technician***

POSTER #83

UCHL1 EXPRESSION IN HIGH-GRADE SEROUS OVARIAN CANCER CORRELATES WITH POOR PROGNOSIS

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Ovarian cancer is the fifth most leading cause of death in women in the United States with an estimate of ~21,550 new cases and 14, 600 deaths per year. High-grade serous ovarian cancer (HGSOC) is the most prevalent sub-type of ovarian cancer, which accounts for 70-80% of ovarian cancer deaths. Despite significant advances in the understanding of HGSOC at the molecular level, the current ovarian cancer treatments fail due to presence of highly metastatic and drug resistant tumors. Thus, it is critical to understand the molecular mechanisms that regulate HGSOC tumorigenesis. Ubiquitin Carboxyl-Terminal Hydrolase 1 (UCHL1) is a cancer associated deubiquitinating enzyme, its aberrant expression and methylation has been implicated with several cancers. UCHL1 activity is reported be related to cell survival and metastasis under hypoxia as it is a Hypoxia Inducible Factor 1a (HIF1a) deubiquitinating enzyme and prevents (von Hippel-Lindau) VHL-mediated degradation of HIF1a. Recent studies in ovarian cancer have reported UCHL1 promoter methylation in low-grade ovarian cancer. However, its expression and function remains unknown in HGSOC. In the present study, we characterized the expression and role of UCHL1 in HGSOC tumorigenesis and patient prognosis. Employing three different algorithms that integrate gene expression and survival data from different datasets of HGSOC patients, including TCGA data, we observed high UCHL1 levels were significantly associated with poor disease free survival (HR = 2.1 (1.26- 3.65), p = 0.004), overall survival (HR = 1.31 (1.11-1.55), p=0.002), progression free survival (HR = 1.28 (1.09-1.49), p=0.002) and stage III and stage IV survival (HR = 1.52 (1.04 – 2.21), p = 0.03) of HGSOC patients. UCHL1 expression levels (RNA and protein) were significantly high in a panel of HGSOC cell lines (Kuramochi, OVCAR4, COV362, OVCAR8) compared to non-HGSOC cell lines (HeyA8, SKOV3, A2780, OVCAR5). UCHL1 silenced Kuramochi and OVCAR4 HGSOC cells were significantly less proliferative compared to un-silenced controls. UCHL1 silencing also exhibited significantly reduced cell migration and clonogenicity. In addition, treatment with UCHL1 inhibitor (LDN-57444) resulted in reduced overall cell proliferation in OVCAR4 cells ($p<0.005$; n=2). These results were in line with clinical outcome in HGSOC patients. Together, these results suggest that UCHL1 promotes HGSOC tumorigenesis possibly in an indirect manner, by up regulating or promoting pro-oncogenic factors in HGSOC. Therefore, targeting UCHL1 in HGSOC could be a useful anti-cancerous approach.

Basic Science ***Research Technician***

POSTER #84

MOLECULAR ALTERATIONS IN THE BREAST ASSOCIATED WITH EARLY MENARCHE

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One in 8 women in the US will develop invasive breast cancer in her lifetime, and while significant advances in diagnosis and treatment continue to reduce the rate of mortality from the disease, prevention of breast cancer remains the ultimate goal. Prevention strategies are likely to have the greatest effect when initiated early in life and sustained. Our study focuses on the early-life risk factor of early age at menarche, which has been widely shown to be associated with an increase in a woman's risk for developing breast cancer. Each 1-year decrease in age at menarche increases breast cancer risk by 5%, and an age at menarche of less than 12 shows a relative risk (RR) of 1.21 according to the Gail model. The mechanisms behind the relationship between early age at menarche and increased risk of breast cancer are not well understood. We hypothesize that early age at menarche results in permanent molecular alterations in the breast tissue and that those abnormalities may contribute to the tissue's susceptibility to carcinogens and breast cancer development. To test our hypothesis we used the resources available at the Susan G. Komen Tissue Bank at the IU Simon Cancer Center (KTB). From the KTB, young women with early menarche (age = 10 years) and with late menarche (age = 15 years) were selected and matched for age (ranging from 18-30), race, BMI, and menstrual status. Breast tissue biopsies from these women were microdissected using a laser microdissection microscope (Leica LMD 6500) in order to isolate the breast epithelium, here defined as terminal ductal lobular units (TDLU). The TDLU are the basic functional and histopathological units of the breast that give rise to most breast cancers. RNA was isolated from the microdissected TDLU and next generation RNA-sequencing was used to generate a transcriptome profile for each sample. The stromal compartment of each of the samples was also evaluated using immunostaining, as the breast epithelium and stroma are in a state of continuous interaction. Together, this information will give us the opportunity to better understand early age at menarche as a breast cancer risk and advance research for women's health.

Basic Science ***Research Technician***

POSTER #85

DNA REPAIR TARGET CANCER THERAPY: OPTIMIZATION OF TDRL-551 REVERSIBLE RPA INHIBITORS

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DNA repair target cancer therapy: Optimization of TDRL-551 reversible RPA inhibitors

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Chemotherapeutics cisplatin and carboplatin react with DNA to form platinum (Pt)-DNA adducts that block DNA replication, transcription and cell division, culminating in cell death through apoptosis. Repair of Pt-DNA adducts via nucleotide excision repair (NER) or homologous recombination repair (HRR) can substantially reduce the effectiveness of the Pt therapy and is a major contributor to cellular resistance. Inhibition of these repair pathways holds the potential to sensitize cancer cells to Pt treatment and increase clinical efficacy. We have targeted Replication Protein A (RPA) which plays essential roles in both NER and HRR as well as DNA replication and DNA damage checkpoint activation. Each of these functions requires RPA binding to single-stranded DNA (ssDNA) and we have developed small molecule inhibitors that disrupt this protein-DNA interaction. Our lead compound, TDRL-551, displays synergy with Pt in tissue culture models of epithelial ovarian cancer (EOC) and in vivo efficacy as a single agent and in combination with platinum in a non-small cell lung cancer (NSCLC) xenograft model. We have expanded our synthetic scheme and defined structure-activity relationships (SARs) to towards optimization of the TDRL-551 core structure as an anticancer therapeutic for the treatment of lung and ovarian cancer.

Basic Science ***Research Technician***

POSTER #86

TARGETING RPA WITH ISOBORNYL SMALL MOLECULES OF MCI VIA THE ZINC-FINGER MOTIF

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Replication protein A (RPA) is a heterotrimeric protein that contains the subunits p70, p32, and p14 (kDa) and is essential in the processes of DNA replication, recombination, and repair. RPA's role in these pathways is mediated by binding to damaged DNA. The binding enables the recruitment of other proteins and proper stabilization of the damaged single-stranded DNA regions. Targeting RPA with inhibitors of DNA repair poses the possibility of sensitizing current DNA damaging chemotherapeutic regimens and therefore reducing the amount of harmful chemotherapy that would have to be administered to a patient. Previously, our lab identified and analyzed a class of irreversible small molecule inhibitors (SMI); the isobornyl haloesters that interact with full-length RPA and demonstrate inhibitory effects with its binding activity to single-stranded DNA. It was also shown that the isobornyl haloester derivatives, MCI13E and MCI13F, do not show any significant inhibition of the central OB-folds of RPA 70, the RPA-AB-box. Therefore, the purpose of the research within is to further characterize the specificity of these compounds by identifying the exact location of where the isobornyl haloesters are inhibiting the full-length RPA. A zinc-finger motif within the C domain of RPA that is responsible for proper destabilization during the transition from the -8nt to the -30nt binding mode has been mutated; four cysteine residues have been mutated to four alanine residues in the preparation of full-length RPA. The data demonstrates that this class of SMI's specifically targets the zinc-finger motif of RPA by creating cysteine-MCI13E/F covalent adducts.

Basic Science ***Research Technician***

POSTER #87

THE IMPACT OF XPC ON LUNG DISEASE IN A MOUSE MODEL OF CHRONIC CIGARETTE SMOKE

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Background: Lung cancers in smokers exhibit extreme genotypic diversity, containing thousands of mutations, which may be due to defects in DNA repair. Exposures to cigarette smoke (CS) cause DNA damage which includes bulky DNA lesions and oxidative damage to DNA bases. The DNA repair protein Xeroderma Pigmentosum Group C (XPC) plays a key role the repair of both bulky and oxidative DNA damage. We hypothesized that XPC protects against the development of early malignant changes induced by CS and therefore loss of XPC function increases the susceptibility to CS-induced lung injury.

Methods: XPC -/- and WT mice (B6;129 background) were exposed to 3, 6 or 9 months of ambient air control (AC) or chronic CS (5 hours/day, 5 days/week; TE-10 apparatus). Hematoxylin and eosin (H&E) staining of formalin fixed lung tissue was evaluated by a lung pathologist. Pulmonary function testing was performed on anesthetized mice using the flexiVent system (Scireq). Immunohistochemical (IHC) analysis for oxidative DNA lesions was performed with 8-oxoG antibody (Abcam). Quantitative data were compared with Student's t-test or 2-way ANOVA.

Results: Mice exposed to chronic CS showed lung morphologic and significant functional changes in lung compliance consistent with mild emphysema that were CS and genotype specific. Mice exposed to chronic CS showed evidence of CS-induced metaplastic changes in airway epithelial cells, with more marked dysplasia noted in XPC -/- mice (when qualitatively scored, in a blinded fashion). Dysplastic changes in XPC-deficient lungs included increased nuclear size, decreased cytoplasm-to-nuclear volume and nuclear atypia. Measures of autophagy, including LC3B and p62, were altered by Western Blot analysis in lung homogenates by CS exposure and XPC expression.

Conclusions: XPC deficiency may accelerate development of CS-induced lung pre-cancerous lesions and emphysema and may play a role in regulation of autophagy in the lung. Measures to regain XPC function may mitigate the risk of CS-induced lung cancer.

Funding sources: American Cancer Society, Showalter Trust Foundation, Indiana Clinical and Translational Sciences Institute

Basic Science ***Research Technician***

POSTER #88

IMPACT OF XPC ON EMPHYSEMA AND APOPTOSIS IN THE LUNGS OF CHRONIC CIGARETTE SMOKE-EXPOSED MICE

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Chronic cigarette smoking (CS) causes lung diseases, including emphysema, through a number of mechanisms, including alterations in programmed cell death through apoptosis. Xeroderma Pigmentosum group C (XPC) is a key protein in the nucleotide excision repair (NER) pathway, which is essential for removing bulky DNA lesions, including those produced by CS. We hypothesized that a deficiency in XPC would accelerate CS-induced lung changes of emphysema through an increase in cellular apoptosis. For these experiments, XPC knock-out (KO) and wild-type (WT) mice were exposed to 9 months of CS or air control (AC). Lung homogenates were evaluated for apoptosis by caspase-3 activity. Frozen and fixed, paraffin embedded sections of lung tissue were evaluated for apoptosis by terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay and immunohistochemistry (IHC) staining for active caspase-3 respectively. Chronic CS exposure caused emphysematous changes in both WT and KO mice, most apparent in KO mice. An increase in caspase-3 activity was noted in CS-exposed XPC KO mice. Active caspase-3 staining was observed in all groups without a clear genotypic effect. Low levels of TUNEL staining were observed in all groups, with positive and negative control samples staining appropriately. However, we were unable to conclude the impact of CS or genotype on TUNEL staining due to low number of samples. We conclude that XPC deficiency leads to an increase in emphysema; it is not clear if this is due to apoptosis or an alternate mechanism. Further studies will focus on the mechanism of this observed action.

Basic Science ***Undergraduate Student***

POSTER #89

INHIBITION OF ACTIVIN SIGNALING PROTECTS AGAINST PANCREATIC CANCER-INDUCED CACHEXIA

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Background and rationale. Cancer-related cachexia is a multifactorial syndrome defined by an ongoing loss of muscle and fat. It is common in patients with pancreatic cancer and is responsible for at least 20% of mortality. Activin signaling is known to promote cancer progression and exacerbate cachexia. In this study, we investigate how inhibition of activin signaling could limit cancer progression and protect against cachexia in a mouse model for pancreatic cancer.

Methods. Cancer was induced through orthotopic implantation in mouse pancreas of 50,000 cells from the C57BL/6J-derived pancreatic cancer cell line KPC32043.

For systemic inhibition of activin signaling, we purified soluble activin IIB receptor (ActrIIB-Fc) from mammalian cells genetically engineered to secrete ActrIIB-Fc. Following KPC32043 implantation in 3-month old C57BL/6J males, systemic inhibition of activin signaling was achieved through multiple intra-peritoneal injections of ActrIIB-Fc.

For skeletal muscle inhibition of activin signaling, we used male mice from a C57BL/6J congenic strain which carry a transgene expressing a dominant negative activin IIB receptor under the control of a myosin light chain promoter, MLC::dnActrIIB. In transgenic mice, there is a strong expression of dominant negative activin IIB receptor in skeletal muscle allowing tissue specific inhibition of activin signaling. Implantation of KPC32043 was performed in 3-month and 7-month old transgenic and non-transgenic littermates.

In order to monitor the development of cachexia, we recorded for each mouse body weight (daily), whole body composition (weekly), and grip strength (weekly).

Results and conclusion. We found that systemic inhibition of activin signaling is associated with reduction of tumor mass and limited cachexia. We also found that skeletal muscle inhibition of activin signaling in older mice limits cachexia in absence of tumor mass reduction. However, in younger mice, skeletal muscle inhibition of activin signaling did not have any effect on cachexia.

In conclusion, we propose that systemic inhibition of activin signaling limits pancreatic cancer growth *invivo*; in addition, skeletal muscle inhibition of activin signaling protects against cachexia in age-dependent manner.

Basic Science

POSTER #90

RELATIONS OF SELF-EFFICACY TO HEALTH BEHAVIORS IN A WEIGHT LOSS INTERVENTION TRIAL FOR BREAST CANCER SURVIVORS AND THEIR DAUGHTERS

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Introduction: Few trials have aimed to improve diet and exercise among cancer survivors and their family members. The Daughters And MothErS (DAMES) Against Breast Cancer trial found significant effects of two 12-month mailed weight loss interventions on exercise and weight-related outcomes for overweight breast cancer survivors and their overweight adult daughters relative to a control condition. Based on Social Cognitive Theory, the interventions targeted participants' self-efficacy for engaging in health behaviors. This study aimed to examine whether self-efficacy for dietary and exercise behaviors at the intervention mid-point (6 months post-baseline) predicted these behaviors post-intervention (12 months post-baseline).

Methods: Early-stage breast cancer survivors and their adult daughters (*n*=68 dyads, 74% Caucasian, mean age=61 years for mothers and 33 years for daughters) were recruited from medical centers and the community. Participants completed measures of diet quality, caloric intake, exercise, and self-efficacy for diet and exercise at baseline, 6- and 12-months. Data from dyads enrolled in the intervention groups (*n*=50 dyads) were examined. Autoregressive panel models tested the relationships between self-efficacy and behavior change (controlling for baseline behavior).

Results: All models fit the data well ($¿^2$ *p*s>0.05, RMSEA<0.06). Among daughters, higher levels of self-efficacy for diet at 6 months were associated with lower caloric intake at 12 months. In addition, greater self-efficacy for diet at 6 months was associated with improved diet quality at 12 months, but this relationship fell short of significance (*p*=0.06). Self-efficacy for diet did not predict mothers' dietary behaviors, and self-efficacy for exercise at 6 months did not predict mothers' or daughters' minutes of exercise at 12 months.

Conclusions/Implications: Findings suggest that improving self-efficacy for engaging in dietary behaviors via mailed interventions may lead to dietary change among daughters of breast cancer survivors.

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Behavioral ***Graduate Student***

POSTER #91

AFRICAN-AMERICAN WOMEN'S PERSPECTIVES ON DONATING HEALTHY BREAST TISSUE FOR RESEARCH: IMPLICATIONS FOR RECRUITMENT

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African-American women die of breast cancer at a higher rate than any other race or ethnicity. The Komen Tissue Bank (KTB) is a clinical trial that represents a critical tool in efforts to find new approaches for the treatment and prevention of breast cancer; however, African-Americans display reticence toward participation not only in the KTB but in clinical trials and medical research in general. Through the lens of the Integrated Behavioral Model, this study recruited Black women to share their perspectives on donating breast tissue for research purposes. Seventy-one (n=71) eligible self-identified African-American women who were previous tissue donors to the Komen Tissue Bank responded to an email questionnaire with open-ended questions that addressed such issues as their perspectives on donating breast tissue, social support for their decision to donate, and how their race may have affected their decision to donate.

Findings revealed that participants had positive instrumental attitudes toward donating, including a desire to help with breast cancer research, to help Black women specifically, and to honor or support someone who was known to the participant and who had breast cancer. Participants discussed generally positive normative influences on their decision to donate, but also revealed that they felt they were creating norms themselves and leaving a "legacy" for others to follow their example. In considering how race played into their decision to donate and their experience with donating, participants acknowledged the negative history of African-Americans in medical research, but offered their perceptions regarding the importance of Black people involving themselves in medical research. In fact, participants discussed that health communication strategies to recruit black people into research should not shy away from the race issue, but instead embrace it as part of the message. In conclusion, this study revealed important perspectives about how African-American women feel about participating in medical research generally and donating breast tissue specifically. The findings from this study are currently being used in practice to improve recruitment of black women to donate to the KTB. Additionally, the findings from this study have important implications for other health communication scholars who work in applied clinical settings and are interested in addressing racial disparities in medical research through more effective and targeted recruitment messaging.

Behavioral ***Graduate Student***

POSTER #92

HOW DID ADVANCED PANCREATIC CANCER PATIENTS AND CAREGIVERS COMMUNICATE THEIR NEEDS IN THE UNSTRUCTURED MEDICAL ENCOUNTER

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Background

Although it is one of the leading causes of cancer deaths in the United States, little is known about how patients with advanced pancreatic cancer (APC) and their caregivers communicate their needs with healthcare providers. APC patients, caregivers, and healthcare providers face unique communication challenges related to handling high psychological distress and overwhelming information within a short period. Consequently, these communication difficulties may also affect their quality of life and decision making process. This pilot study seeks to explore how APC patients and their caregivers communicate their concerns about the disease, patterns of communication, and psychological reactions with oncologists.

Method

De-identified transcripts of APC patients' audio-recorded office visits were selected from a large randomized controlled trial called the Values and Options in Cancer Care. Among the 37 qualified transcripts, we purposeful selected four transcriptions with different levels of prognosis discussion as prognosis discussion may be a marker for patients' and caregivers' emotional status. Selected transcripts were analyzed in terms of discussion topics, message quality, patients' emotional cues, and oncologists' responses to these emotional cues. Message quality was evaluated based on several patient-centered clinician verbal behaviors purposed by Drs. Epstein and Street. We used Medical Interview Aural Rating Scale to code patients' emotional cues and oncologists' corresponding responses. Emotional cues were coded according to psychological depth. Oncologists' responses was coded into five categories of cue-responding behaviors (name, understand, respect, support, and block).

Results

Twelve participants contributed to four transcripts. Each consultations contained three participants: oncologist, patient and caregiver who contributed to 2806.25, 731 and 463.5 words, respectively. Among the nine categories of consultation topics identified, physical symptoms and signs were the most frequent mentioned topic (n=22) followed by care procedure (n=5), drug (n=4) and lab results (n=4). Oncologists initiated more topics (n=35) than patients (n=8) and caregivers (n=4). Moreover, oncologist-initiated interruptions occurred in all consultations with an average of 5.25 interruptions per consultation.Although neither patient nor oncologist discussed emotion related topics explicitly, patients and caregivers experienced a variety of emotional fluctuation. Overall, 87 patients' (n=54) and caregivers' (n=33) emotional cues were identified. The majority of the cues were level one, implicit emotional cues (n=80). The most frequent oncologists' responding strategy was blocking, including switching focus (i.e., the oncologist switched away from the emotion, but within the context of the patients disclosure) and overt blocking (i.e., the oncologist completely disrupted the conversation by moving away from all the content of and all the cues).

Conclusion

To our knowledge, this is the first study to analyze unstructured conversation to identify APC patients' concerns and how these concerns were addressed in office visits.Our findings provide valuable insight for identifying needs and enhancing end of life care and communication of this population.

Behavioral ***Graduate Student***

POSTER #93

SYMPTOM IMPORTANCE IN METASTATIC BREAST CANCER PATIENTS

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Research on symptoms in cancer patients has largely focused on symptom severity, frequency, and distress. Assessing patients' perceptions of symptom importance—how important it is for them to see improvement in a symptom—would also inform patient-centered treatment approaches, but this has not been examined in patients with cancer. In order to address this gap, this study aimed to identify physical and psychological symptoms considered most important to metastatic breast cancer patients, a population with high symptom burden. Subgroups of patients were derived based on ratings of symptom importance. Eighty metastatic breast cancer patients were recruited from the Indiana University Simon Cancer Center to participate in this cross-sectional telephone interview study. The interview included measures of demographics, quality of life, symptom severity, and the importance of seeing improvement in specific symptoms post-treatment. An exploratory cluster analysis was performed on patient-rated symptom importance for 10 symptoms (i.e., pain, fatigue, anxiety, sadness, numbness/tingling in hands/feet, swelling of arms or legs, nausea, hot flashes, sleep problems, and attention/thinking/memory problems) and revealed four clusters of patients based on these ratings: 1) all symptoms rated highly, 2) thinking, sleep, and fatigue rated highly, 3) pain and fatigue rated moderately, and 4) pain, fatigue, anxiety, sadness, sleep and thinking rated highly important. One-way ANOVAs indicated that the clusters differed on years of education [$F(3, 76) = 4.58$, $p = .005$, ¿$^2 = .15$], quality of life [$F(3, 76) = 8.16$, $p < .001$, ¿$^2 = .24$], and usual symptom severity for anxiety [$F(3, 76) = 6.11$, $p = .001$, ¿$^2 = .19$], sadness [$F(3, 76) = 3.89$, $p = .012$, ¿$^2 = .13$], sleep problems [$F(3, 76) = 3.07$, $p = .033$, ¿$^2 = .11$], and thinking problems [$F(3, 76) = 10.83$, $p < .001$, ¿$^2 = .30$]. Findings suggest that subgroups of metastatic breast cancer patients have different symptom treatment priorities, which highlights the importance of tailoring treatment to these priorities.

Behavioral ***Graduate Student***

POSTER #94

DISTRESS AND AVOIDANT COPING IN BREAST CANCER SURVIVORS

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Introduction: The majority of breast cancer survivors (BCS) demonstrate a great capacity to recover from the negative sequelae of cancer treatment; however, some report persistent and disruptive distress well into disease-free survivorship. More information is needed on the contributing factors to distress in this growing population, including the role of avoidant coping in predicting distress.

Methods: In a large cross-sectional study, breast cancer survivors (n = 1,127) who were 3 to 8 years post diagnosis completed a survey assessing demographic characteristics, avoidant coping, distress (state anxiety, depression, and post-traumatic stress symptoms) and theoretically-related factors, including: fear of recurrence, fatigue, attention function, social support from a partner, social constraints from a partner, and body image. Multiple mediation analyses were conducted to determine if avoidant coping mediated the relationship between distress and all other factors. As all factors were highly correlated, multiple mediation analyses were chosen to account for each factor's unique contribution to distress.

Results: In all 18 mediation models, avoidant coping significantly ($p < .001$) mediated the relationship between each contributing factor (fear of recurrence, fatigue, attention function, social support, social constraints, and body image) and each distress indicator (depression, state anxiety, and impact of event). Effect sizes ranged $k2 = 0.14$ to $k2 = 0.22$, all of which were classified as medium effect sizes.

Conclusion/Implications: Interventions targeting avoidant coping may be effective in reducing distress in long-term breast cancer survivors.

Behavioral ***Post-Doctoral/Medical Fellow***

POSTER #95

A SYSTEMATIC REVIEW ON THE USE AND ACCEPTANCE OF CANCER-SPECIFIC PATIENT CENTERED TECHNOLOGIES AMONG UNDERSERVED POPULATIONS

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Introduction: In the US, more than 1.6 million new cases of cancer are estimated to be diagnosed each year. However, the burden of cancer among the US population is not shared equally with racial and ethnic minorities and lower-income populations having a higher cancer burden when compared to their counterparts. A wide range of technologies (e.g., Internet-based [eHealth] technologies and mobile [mHealth] applications) are available to patients that are designed to improve their access to care and empower them to participate actively in their care providing a means to reduce health care disparities; yet, little is known of their use among underserved populations.
Objective: The purpose of this article is to systematically review the current evidence on the use of cancer-specific patient-centered technologies among various underserved populations.
Methods: Computer-based searches were conducted in the following academic databases: 1) PubMed [cancer subset]; 2) MEDLINE, 3) PsycINFO; and 4) CINAHL. We included articles that appeared in peer reviewed journals, were published in the English language, and were conducted in the US. Each study was individually assessed for relevance with any disagreements being reconciled by consensus. We used a 3-step inclusion process in which we examined study titles, abstracts, and full-text articles for assessment of inclusion criteria.
Results: This ongoing review includes 11 articles that use patient-centered technologies targeting African-American (n=6), Hispanic (n=3), and low-income (n=2) underserved populations. A majority of studies used mHealth technologies (n=9) while 2 studies used eHealth applications. Seven studies assessed text messaging and found that participants reported overall favorable responses to receiving health information via text; however, challenges were experienced with respect to a lack of knowledge of how to text among some participants. More complex mobile technologies (i.e., a risk assessment tool, and tablet-based intervention) were also found to be easy and favorable to use, as well as new and innovative which peaked an interest with participants who desired to keep up with technology. These more complex technologies also resulted in more significant barriers. More specifically, participants expressed concerns regarding security and unfamiliarity with the technology. With respect to eHealth applications, African-Americans were found to be more likely to use eHealth for information and health management. Separately, while Hispanics were receptive to eHealth, several usability issues were identified related to content comprehension, navigation issues, and cultural appropriateness.
Conclusion: Despite the potential of patient-centered technologies to reduce disparities, their use among underserved populations has received little attention. Preliminary results of this review reveal that while underserved populations are receptive to these technologies, challenges still exist. We conclude by discussing an agenda for future research which should continue to explore effective design strategies and culturally appropriate tailoring of these technologies to underserved populations.

Behavioral ***Post-Doctoral/Medical Fellow***

POSTER #96

THE AMPATH-ONCOLOGY INSTITUTE: LONGITUDINAL ANALYSIS OF HPV AND CERVICAL CANCER IN WOMEN WITH HIV/AIDS

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Background. Cervical cancer is the most common malignancy in women living Kenya. While HIV infection accounts for much of the high incidence and mortality of cervical cancer in Kenyan women, other modifiable cofactors are likely to be important. The AMPATH-Oncology Institute (AOI) is a part of AMPATH, a multidisciplinary collaboration of North American universities, led by Indiana University, with Moi University and Moi Teaching and Referral Hospital in Eldoret, Kenya. One goal of the AOI is to study the influence of HIV infection on the natural history of cancers, including cervical cancer.

Methods. The goals of this five-year project are to enhance the research workforce in Kenya and to study the natural history of HPV infections and cervical cancer in HIV-infected women. Two projects to be conducted are 1) defining modifiable factors predicting persistence of oncogenic HPV and cervical dysplasia in HIV-infected women, and 2) evaluating the impact of VIA screening and treatment with cryotherapy or LEEP in HIV-infected women with cervical intraepithelial neoplasia (CIN).

Results and Conclusions. All work will be performed in western Kenya. To date, more than 140 women have enrolled in the study, approximately one-third of the total number of participants needed. Specimens have been collected for HPV testing, and personnel in the Kenya Medical Research Institute in Kisumu have received training in performing the HPV assay used for the project. The direct outcome of these studies will be a better understanding of the natural history of various HPV types in women with and without HIV infection, the modifiable risk factors for cervical cancer in these women, and the implications of local therapies for women with CIN lesions. Further studies are being developed including mentored pilot projects related to the main studies.

Population Science/Epidemiology ***Faculty***

POSTER #97

PREVALENCE OF ADVANCED COLORECTAL NEOPLASIA IN VETERANS: EFFECTS OF AGE, SEX, AND RACE

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Background: Understanding the effects of demographic features on the risks of colorectal cancer (CRC) and advanced neoplasia (AN) has been difficult because previous studies have had relatively small sample sizes and/or uncertain generalizability. While age and male sex are clear risk factors for colorectal cancer (CRC) and AN, the effect of race has been less consistent.

Objective: To quantify the effects of age, sex, and race on prevalence of AN and CRC among Veterans.

Methods: Through remote electronic data extraction, we identified a cohort of veterans who underwent a first diagnostic or screening colonoscopy between 2002 and 2009 at one of 14 VA Medical Centers. Colonoscopies done for an indication of inflammatory bowel disease or neoplasia surveillance were excluded. We used natural language processing of colonoscopy and pathology reports to identify the most advanced colorectal findings and location within the colorectum as proximal (splenic flexure) or distal. AN was defined as advanced adenoma (tubular adenoma >1 cm, villous histology, or high-grade dysplasia), sessile serrated polyp > 1 cm, or adenocarcinoma (CRC).

Results: A total of 90,691 eligible colonoscopies were identified. Mean (SD) patient age was 61.6 (9.4) years; 5.1% (n=4679) were women. Among 72,527 (80%) patients whose race was known, 55,180 (76.1%) were Caucasian, 14,155 (19.5%) were Black, 2,498 (3.4%) were Hispanic, and 734 (1.0%) were other. Overall prevalence of AN was 8.9% (n=8082), of which 14.5% (n=1171) was CRC. AN increased from 4.0% for those < 50 years to 12.1% for those >= 75 years (P<0.0001), and was present in 9.2% of men and 3.9% of women (adjusted OR=2.06%; 95% CI, 1.77-2.39). While age-related increases in AN prevalence were present overall for both sexes, AN prevalence in women < 65 years was 3.2% to 3.9% (P=0.87). There were no differences in AN prevalence between Blacks (8.96%), Caucasians (9.43%), Hispanics (8.41%), and Other (8.99%) (P=0.14). When CRC prevalence was considered, similar patterns for age, sex, and race were observed. Among 1096 CRCs where location within the colorectum was ascertained, women had more proximal CRC (63%), while men had more distal CRC (60%) (P=0.024). Within the subgroup of 30,446 (33.6%) screening colonoscopies, the prevalence of CRC and AN were lower; however, the effects of age, sex, and race were similar to the overall sample.

Conclusions: Older age and male sex were strongly associated with prevalence of both AN and CRC, while race had no effect on either outcome. Among women aged 50-64 years, the prevalence of AN and CRC was low and remained constant. A risk-based model based on age, sex, and perhaps other features may be useful for both resource allocation and tailoring CRC screening in Veterans.

Population Science/Epidemiology ***Faculty***

POSTER #98

CADMIUM EXPOSURE AND RISK OF LUNG CANCER: A META-ANALYSIS OF COHORT AND CASE–CONTROL STUDIES AMONG GENERAL AND OCCUPATIONAL POPULATIONS

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The association between cadmium exposure and risk of lung cancer is still unclear. We quantitatively reviewed the observational studies that investigated the association between cadmium exposure and lung cancer risk in both general and occupational populations published through April 2015. The final data set is comprised of three cohort studies in the general population totaling 22,551 participants (354 events) with a mean follow-up of 15 years, five occupational cohort studies including 4205 individuals (180 events) with an average follow-up of 31 years, and three occupational case–control studies including 4740 cases and 6268 controls. Comparing the highest to the lowest category of cadmium exposure, the weighted relative risk and 95% confidence interval of lung cancer in the general population was 1.42 (95% CI (0.91, 2.23)); the weighted risk estimates (95% CIs) of lung cancer in three occupational cohort studies and three case–control studies were 0.68 (95% CI (0.33, 1.41)) and 1.61 (95% CI (0.94, 2.75)), respectively. No linear association was found. When comparing participants exposed to cadmium with non-exposed based on available data, the association became statistically significant. According to findings from this meta-analysis, the possibility that cadmium exposure may increase risk of lung cancer cannot be completely ruled out in either general or occupational population.

Population Science/Epidemiology ***Graduate Student***

POSTER #99

DIFFERENCES IN CERVICAL CANCER SCREENING KNOWLEDGE, PRACTICES, AND BELIEFS: A LOCAL SURVEY OF MINORITY WOMEN

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Background: There exist concerns that HPV vaccination could result in decreased cervical cancer screening rates. This study examines the relationship between cervical cancer screening rates and HPV vaccination in a local sample of predominately minority women.

Methods: In July 2015 we surveyed women between the ages of 21 and 35 (i.e., born between 1980 and 1994) who attended a minority health fair. We excluded women who reported a history of hysterectomy, as well as those who had received the HPV vaccine less than 3 years prior to the survey. Outcomes assessed included: receiving a Papanicolaou (Pap) test within the last three years, awareness and comfort with current cervical screening recommendations, and knowledge regarding the purpose of a Pap test.

Results: Responses from 291 women were included in the analysis. The mean age was 28.5 years and 62% were non-Hispanic Black. Most (84%) received a Pap test in the last three years and one-third (33%) received at least one HPV vaccine. Logistic regression showed that vaccinated women did not have lower odds of having a Pap test in the past three years (OR=1.32; 95% CI=0.66-2.65). In fact, in an adjusted multivariable logistic regression that controlled for age and race revealed vaccinated women were three times more likely to have had a Pap test (AOR=3.06; 95% CI=1.37-6.83). Two-thirds (64%) of respondents thought average-risk women should get a Pap test every year. Only 26% of women knew the purpose of a Pap test, and the proportion who correctly answered this question varied by race. Participants who answered incorrectly were over four times as likely to be non-Hispanic Black as compared to those who were White (OR=4.20; 95% CI=2.00-8.81; $p<0.001$).

Conclusions: Women who have been vaccinated for HPV are more likely to have been screened for cervical cancer, alleviating concerns among healthcare providers regarding whether would decrease cervical cancer screening due to HPV vaccination. However, many women are unaware of the purpose of a Pap test and current screening recommendations, suggesting the need for better health education.

Population Science/Epidemiology ***Graduate Student***

POSTER #100

RACIAL DIFFERENCES IN DIETARY CHANGES AND QUALITY OF LIFE AFTER A COLORECTAL CANCER DIAGNOSIS: A FOLLOW-UP OF THE STUDY OF OUTCOMES IN COLORECTAL CANCER SURVIVORS (SOCCS) COHORT

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BACKGROUND: Significant racial disparities exist in colorectal cancer diagnosis and survival.

OBJECTIVE: Exploratory studyto assess the racial differences in dietary change in relation to quality of life (QoL), recurrence and survival after receiving a colorectal cancer (CRC) diagnosis.

DESIGN: Four hundred fifty-three stage-II CRC patients were enrolled into the cohort study via the North Carolina Central Cancer Registry. Self-reported diet, physical activity, treatment, comorbidities, demographic characteristics and QoL were collected at diagnosis, 12, and 24 months post-diagnosis. QoL was assessed with the FACT-C and SF-12 inventories. An overall dietary index score was calculated. Generalized estimating equations and logistic regression models were utilized to explore potential associations.

RESULTS: African Americans (AAs) (n=81) were more likely to increase intakes of reduced fat milk, vegetables, and fruit ($p<0.05$) compared to Caucasians (n=184) 24 months following diagnosis. AAs also decreased their intakes of regular cheese, red meat, fried food, fast food, and fat ($p<0.05$), comparably. The least-square means (standard errors) for changes in dietary index were 6.05 (0.40) and 4.07 (0.27) for AAs and Caucasians, respectively ($P<0.001$). AAs exhibited higher scores on portions of the FACT-C (CCS: ß=1.04, 95% CI: 0.29, 1.82) and the SF-12 (PCS: ß=2.49, 95%CI: 0.51, 4.48). While there was no significant difference in survival and recurrence between racial sub-groups, those who improved their dietary quality over 24 months had lower risk of recurrence and mortality overall (OR: 0.42; 95% CI: 0.25, 0.72).

CONCLUSIONS: AAs made more healthful changes in diet and had a higher quality of life compared to Caucasians in this study using self-reported dietary data. No racial differences in recurrence or survival were evident, even though improvements in dietary quality did reveal survival benefits overall. More prospective research on racial disparities in health behavior changes post-diagnosis are desperately needed.

Population Science/Epidemiology ***Post-Doctoral/Medical Fellow***

POSTER #101

IDENTIFICATION AND IMAGING OF NOVEL METABOLIC HUBS OF BREAST CANCER WITH PROGNOSTIC VALUE

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The complex yet interrelated connections between cancer metabolism, gene expression, and oncogenic driver genes have the potential to identify novel biomarkers and drug targets with prognostic and therapeutic value. The goal of this study was to generate global metabolic profiles of breast tumors isolated from multiple transgenic mouse models and to compare these profiles with gene expression data to develop a network analysis, and to use that network to identify critical metabolic pathways in breast cancer. Using GC-MS, LC-MS/MS, and capillary zone electrophoresis (CZE)-MS platforms, we quantified and compared the levels of 374 metabolites in breast tumor tissue from normal tissue and transgenic mouse breast cancer models overexpressing a panel of oncogenes (PyMT, PyMT-DB, Wnt1, , and C3-TAg). We developed a correlation-based network analysis that captures the interactions between metabolic profiling and gene expression data. Our network analysis identified 35 metabolite and 33 gene "hubs" that had the most network correlations. These hubs have prognostic value and likely are integral to tumor metabolism and breast cancer. They are candidate breast cancer metabolites ('oncometabolites') and genes that promote breast cancer. We show in this study that gene hubs can affect cancer progression. In addition, we detected metabolite localization patterns and concentration gradients across tumor tissue by MALDI imaging. Metabolites were detected in both cancer cells as well as within the tumor microenvironment with distinct spatial resolution, suggesting regulated metabolic heterogeneity across tumor tissue.

Translational/Clincal Research ***Faculty***

POSTER #102

NOVEL MICROSOFT EXCEL PROGRAM TO PARSE VARIAN ECLIPSE DATA

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Introduction: Cancer patients are regularly treated with radiotherapy. The departments of radiation oncology at Indiana University, Methodist Hospital, and the Richard L. Roudebush VA Medical Center create radiation plans using software called Eclipse, a product of the Varian company. In order to avoid toxicities by overdosing critical structures, Eclipse produces a graph called a dose-volume histogram (DVH) that allows radiation oncologists to evaluate the dose delivered to a certain organ or the cancer target volumes. Rather than visually estimate these values, a novel Microsoft Excel program extracts exported data and calculates the desired dosimetric qualifiers.

Methods: This program was specifically designed for IRB# 1412029028 "Stereotactic Radiotherapy (SBRT) of the Lung for Primary Tumors" but can be modified for any patient data set. Normal organ data of interest included esophagus, lungs, proximal bronchial tree, chest wall, spinal cord, brachial plexus, heart, aorta, skin, and stomach. The gross tumor volume (GTV), internal target volume (ITV), and the planning target volume (PTV) were also evaluated. Examples of meaningful DVH data included the mean and max doses to certain structures, the dose being received by 1 or 5 ccs (D1cc or D5cc), the volume receiving 20 Gy (V20), and the percent PTV covered by the prescription isodose line.

Results: Exporting the DVH in a tabular format produces a single column of data. This column was imported into the Excel program. For each organ or target volume, multiple rows of data are produced in increments of 0.1 cGy. Each row includes in a character string: the dose in cGy, the relative dose (%) and the volume in ccs which receive the aforementioned dose. If an organ received a maximum of 6000cGy, then 60,000 lines of code were produced. The strings were transformed into their numerical correlates, and calculations were performed to display the desired data.

Conclusion: An easily modifiable Microscoft Excel spreadsheet was created to handle large amounts of DVH data and display the results in an organized format.

Translational/Clincal Research ***Faculty***

POSTER #103

LETROZOLE AND OVARIECTOMY CAUSE BONE LOSS, MUSCLE WEAKNESS, AND INCREASED BREAST CANCER BONE METASTASES IN MICE

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Adjuvant endocrine therapy using an aromatase inhibitor (AI) is a standard treatment for postmenopausal women with estrogen receptor (ER)-positive breast cancer. Unfortunately, 50% of women treated with an AI develop musculoskeletal complications. Previous studies in our laboratory have demonstrated bone loss and muscle weakness in ovariectomized (OVX) mice. We therefore hypothesized that complete estrogen deprivation using the AI letrozole would cause more profound muscle weakness and bone loss than OVX alone, and that this high bone turnover state could accelerate the progression of breast cancer bone metastases and negatively impact muscle function.

To test this, four-week female athymic nude mice underwent OVX or sham surgery and were treated daily with vehicle or AI (10μg/day; n=20/group). Two weeks after surgery and onset of treatment, bone mineral density was reduced in OVX-AI mice relative to vehicle-shams ($p<0.01$) as assessed by dual energy X-ray absorptiometry. Using bone micro-computed tomography (SCANCO viva40CT), trabecular bone volume fraction (BV/TV) of the proximal tibia was reduced by 53% is OVX-vehicle mice ($p<0.001$) and by 67% in OVX-AI mice ($p<0.001$) relative to vehicle-sham.

After confirming bone loss, the same animals were inoculated with ER-negative MDA-MB-231 human breast cancer cells into the left cardiac ventricle and were followed for osteolytic lesion formation (n=10-15/group). Since MDA-MB-231 is ER-negative, effects of complete estrogen deprivation should be indirect. Five weeks after inoculation, osteolytic lesion area was larger in OVX-AI mice relative to sham-vehicle ($p=0.0215$), while OVX or AI alone did not alter lytic lesion area. Skeletal muscle function was assessed by ex vivo measurement of maximal contractile specific-force of the extensor digitorum longus muscle. At 200Hz maximal contractile force in sham-letrozole and OVX-vehicle mice was reduced by 7% ($p<0.05$) and reduced by 12% in OVX-AI mice ($p<0.001$) relative to sham-vehicle.

Our murine studies confirm that AI treatment induces bone loss and skeletal muscle weakness, recapitulating effects in cancer patients. As hypothesized, the severe bone loss resulting from AI-induced estrogen depletion may prime the bone microenvironment for the development of breast cancer metastases to bone and potentiate muscle weakness. This model serves as an excellent tool to study the mechanisms of underlying musculoskeletal defects in cancer patients and assess potential therapeutics.

Translational/Clincal Research ***Faculty***

Conclusion

We conclude that the biomarker panel measured at diagnosis or day +100 post-HCT may allow patient stratification according to risk of cGVHD.

Translational/Clincal Research Faculty

POSTER #105

USE OF TRANSCRIPTION ACTIVATOR LIKE EFFECTOR-TRANSCRIPTION FACTORS (TALE-TFS) TO INDUCE CYP1A2 GENE EXPRESSION AND VALIDATE MIRNA PREDICTIONS

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BACKGROUND: miRNAs are predicted to regulate the expression of several CYP genes, but these predictions need to be validated in cells that express the endogenous CYP genes. We used TALE-TFs to induce CYP1A2 expression in HeLa and HepG2 cells and validate the miRNA predictions.

METHODS: Four TALE-TFs that target the CYP1A2 promoter were designed and transfected into HeLa and HepG2 cells. TALE-TFs were then cotransfected into HeLa and HepG2 cells with either miRNAs predicted to target CYP1A2, inhibitors of those miRNAs, or C. elegans miRNA negative control. CYP1A2 mRNA expression was measured using quantitative PCR that was normalized to GAPDH.

RESULTS: The transfection of all four TALE-TFs in HeLa and HepG2 cells increased CYP1A2 mRNA levels 164- and 189-fold, respectively, compared to the empty vector control. In HeLa cells increased CYP1A2 expression was observed when the inhibitors of the four miRNAs were tested: hsa-miR-431-5p (2.6-fold, p-value=0.026), 668-3p (3.5-fold, 0.026), 143-3p (2.1-fold, 0.025), 320b (2.4-fold, 0.005). None of the four miRNAs reduced CYP1A2 mRNA expression. No changes where observed in HepG2 cells.

CONCLUSION: TALE-TFs can be used to induce CYP1A2 expression. The increased expression of CYP1A2 mRNA in cells transfected with miRNA inhibitors suggests that miRNAs regulate CYP1A2 expression either directly or indirectly. The differential effect of the miRNA inhibitors in HeLa vs HepG2 cells further suggests that the miRNA effects on CYP1A2 are cell type specific.

Translational/Clincal Research ***Graduate Student***

POSTER #106

NEXT GENERATION SEQUENCING OF CIRCULATING TUMOR DNA TO PREDICT RECURRENCE IN TRIPLE-NEGATIVE BREAST CANCER PATIENTS WITH RESIDUAL DISEASE AFTER NEOADJUVANT CHEMOTHERAPY

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PURPOSE: Next-generation sequencing to detect cell-free tumor DNA (ctDNA) is a minimally invasive method for tumor genotyping and for monitoring therapeutic response. The majority of studies so far have focused on detecting ctDNA from patients with metastatic disease. Herein, we tested whether ctDNA could be used as a biomarker to predict relapse in triple-negative breast cancer (TNBC) patients with residual disease after neoadjuvant chemotherapy.

PATIENTS AND METHODS: BRE09-146 was a Phase II clinical trial that randomized TNBC patients with residual disease after neoadjuvant chemotherapy to Cisplatin or Cisplatin+Rucaparib. From the combination arm, 1ml of plasma was collected at four time points post-surgery. In total, 38 patients with matched tumor, blood, and plasma were analyzed. Extracted DNA underwent library preparation and amplification using the Oncomine Research Panel consisting of 134 cancer genes, followed by high-coverage sequencing and bioinformatics.

RESULTS: We first detected high-quality somatic mutations in primary tumors. TP53 mutations were the most prevalent (79%) followed by PIK3CA (16%). We then analyzed the plasma-sequencing data to detect the same mutations in the circulation. Out of 38 patients, 13 patients had a clinical relapse (median trial follow-up for disease free survival = 24 months). Of the 13 patients, we detected somatic ctDNA in 4 patients (3 TP53 mutations, 1 AKT mutation). Notably, all 4 patients had a rapid recurrence (0.3, 4.0, 5.3 and 8.9 months). Overall, patients whose ctDNA was detected had inferior disease free survivial ($p<0.0001$, median DFS: 4.6mos. vs. NR; HR=12.6, 95% C.I.:3.06-52.2).

CONCLUSION: Next-generation ctDNA-sequencing of TNBC patients with residual diease after neoadjuvant chemotherapy can predict recurrence with high specificity, but moderate sensitivity. For those patients where ctDNA is detected, recurrence is rapid.

Translational/Clincal Research ***Graduate Student***

POSTER #107

AN ANGIOGENESIS GENE SIGNATURE POINTS TO ACTIVE TGF-BETA/JAK SIGNALING PATHWAYS IN A SUBSET OF HUMAN PANCREATIC DUCTAL ADENOCARCINOMA CANCER PATIENTS THAT ARE DISTINCT FROM PATHWAYS IN PANCREATIC NEUROENDOCRINE TUMORS

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Pancreatic Ductal Adenocarcinoma (PDAC), which comprises 85% of pancreatic cancers, is the 4th leading cause of cancer death in the United States with a 5-year survival of 8%. While human PDACs (hPDACs) are hypovascular, they also overexpress a number of angiogenic growth factors and receptors. Additionally, the use of anti-angiogenic agents in murine models of PDAC leads to reduced tumor volume, tumor spread, and microvessel density, and improved survival. Nonetheless, clinical trials using anti-angiogenic therapy have been overwhelmingly unsuccessful in hPDAC. On the other hand, pancreatic neuroendocrine tumors (PNETs) account for only 5% of pancreatic tumors, yet they are very vascular and classically angiogenic, respond to anti-angiogenic therapy, and confer a better prognosis than PDAC even in the metastatic setting.

By analyzing the recently expanded TCGA (The Cancer Genome Atlas) dataset, we report here that an angiogenesis gene signature is present in ~35% of PDACs and is mostly distinct from an angiogenesis signature present in PNETs. Additionally, principal component analysis (PCA) of the entire or angiogenic PDAC and PNET transcriptomes from TCGA indicates that there are large differences in gene expression between these two tumor types. For example, PDACs exhibit a transcriptome that reflects active TGF-beta signaling, and up-regulation of several pro-inflammatory genes, including members of JAK signaling pathways. Functionally, targeting the TGF-beta type I receptor kinase with SB505124 and JAK1/2 with ruxolitinib blocks proliferative crosstalk between human pancreatic cancer cells and human endothelial cells. Tumors from the **KR**C (oncogenic **K**ras, deleted **R**b1) PDAC mouse model show superior enrichment and differential expression of the angiogenic gene signature compared to tumors from the **KP**C (oncogenic **K**ras, mutated Tr**p**53) PDAC mouse model. Moreover, treatment of KRC and KPC mice with ruxolitinib suppresses murine PDAC progression in KRC mice but not in KPC mice. These findings suggest that targeting both TGF-beta and JAK signaling in the 35% of PDAC patients whose cancers exhibit an angiogenesis gene signature should be explored in the clinic and that this could lead to improved responses to anti-angiogenic therapy in PDAC.

Translational/Clincal Research ***Graduate Student***

POSTER #108

DIFFERENTIAL RESPONSE TO A DUAL PI3K/MTOR INHIBITOR IN PI3KCA MUTANT UROTHELIAL CANCER PATIENT DERIVED XENOGRAFTS

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Background: PI3K pathway has been reported to be deregulated in up to 30% of bladder cancer patients. Our patient derived xenografts (PDX) models carry unique helical domain mutations in the PI3KCA gene that are known to be highly deregulated in bladder cancer. This sets our models as unique tools for understanding the molecular background that would dictate an optimal response to targeting the PI3K pathway in vivo as well as the potential mechanisms of resistance. **Methods**: RNA-seq was carried out to understand the molecular make up of our PDX models (RP-B-01 and RP-B-02) and to identify clinically relevant drug targets. In-vivo drug testing was done using a dual PI3K-mTOR inhibitor to test the response to this class of drugs. Cell lines derived from the PDX models were used for in-vitro studies to validate our in-vivo data and to calculate the IC-50 for this class of agents in our cell lines. Western blot was used to assess the molecular effects of these agents using both in-vivo specimens as well as cell lines that were treated in vitro. **Results:** Our RNA-seq data have shown that our models (RP-B-01 and RP-B-02) are molecularly distinct where RP-B-01 has a basal-like phenotype while RP-B-02 is has a luminal like phenotype. Additionally; they carry two unique helical domain mutations in the PI3KCA gene (E542K and E545K mutations respectively). Despite the molecular similarity between these two mutations, the two models responded differently to a dual PI3K-mTOR inhibiter in vivo; where RP-B-02 tumor model significantly responded to the drug compared to vehicle treated mice (P Value = 0.03) while the RP-B-01 model was resistant. Cell lines derived from these models were able to recapitulate the same phenotype in-vitro and IC-50 for each cell line was significantly different (IC50 for RP-B-01 cells = 204.1 nM; IC50 for RP-B-02 cells = 73.21 nM). Western blot analysis has shown that despite the ability of the dual PI3K-mTOR inhibitor to hit the target in RP-B-02 PDX, it was not able to do the same in the other model (RP-B-01). Interestingly, supplementing media with insulin and nutrients switched the response phenotype and rendered cells derived from the RP-B-02 model more resistant to the PI3K-mTOR inhibitor. **Conclusion:** Response to PI3K pathway inhibitors in bladder cancer is not only dictated by the presence of targetable mutations but more importantly by the molecular make up of individual tumors which should be taken into account when using these agents in the clinic.

Translational/Clincal Research ***Graduate Student***

POSTER #109

MECHANISTIC PBPK MODEL TO PREDICT TISSUE SPECIFIC CONCENTRATIONS OF IMATINIB

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The efficacy and safety of drugs are typically linked to the drug's pharmacokinetic properties. Clinically, drug concentrations are usually determined in plasma. However, distribution of drug can exhibit a high inter-tissue variability, and target site drug concentration can significantly vary from plasma concentration. Knowledge of drug concentration in target tissues is fundamental in the determination of clinical drug activity. Physiologically based pharmacokinetic (PBPK) modeling is a useful tool to predict tissue concentration of drugs. In this study, we develop a PBPK model to predict tissue concentrations of the tyrosine kinase inhibitor imatinib. The PBPK model was defined by a set of ordinary differential equations was coded in R using the *lsoda* package. Physiologic and drug specific parameters were obtained from the literature. Tissue specific concentration-time profiles for brain, liver, and kidney of mice receiving imatinib were obtained from the literature. As imatinib tissue-specific partition coefficients not available, these were predicted from the physiochemical properties of the drug and by fitting select parameters. Acceptability criteria include predicted Cmax and AUC within 2-fold of observed values. An interspecies extrapolation was performed utilizing tissue partition coefficients determined in mice to predict the tissue concentration of imatinib in humans. The mouse model was able to estimate plasma AUC within 1.5-fold of the observed AUC for an orally administered dose of imatinib. Brain exposure was predicted to be within <10% of the observed values. After extrapolation to humans, the model was able to produce a predicted plasma exposure within 30% of the observed data. The PBPK model for imatinib is able to accurately predict tissue concentrations in mice can be used to predict tissue concentrations of imatinib in humans.

Translational/Clincal Research ***Graduate Student***

POSTER #110

A PBPK MODEL OF VINCRISTINE THAT INCORPORATES CYP3A METABOLISM

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Background: Vincristine (VCR) is widely used for treatment of a variety of neoplastic malignancies in adults and children including acute lymphoblastic leukemia and non-Hodgkin's lymphoma. A dose-limiting side effect of VCR is peripheral neuropathy, which presents as paresthesia in fingers and toes and in severe cases can lead to paralysis. These adverse effects may be related to drug exposure. VCR is predominantly metabolized by cytochrome P450 3A (CYP3A) enzymes. We have observed that neuropathy is more severe in patients with polymorphisms in CYP3A5 that lead to reduced clearance of VCR. Here we utilize *in vitro* metabolism data to develop a PBPK model for VCR and to explore the effect of CYP3A5 genotype on VCR exposure.

Methods: Simcyp v. 15 was used to develop a physiologically based pharmacokinetic (PBPK) model for VCR. Volume of distribution was predicted using the Rodgers and Rowland method. Hepatic metabolism by CYP3A4 and CYP3A5 were extrapolated from *in vitro* data from human liver microsomes (HLM) and recombinant CYP3A4 and CYP3A5. Biliary clearance was determined from clinical reports. The robustness of the model was determined by comparing simulated concentration-time profiles with published VCR pharmacokinetic data. The model was deemed acceptable if pharmacokinetic parameters calculated were within 2-fold of observed parameters.

Results: For the adult cancer patient, this PBPK model was able to adequately predict VCR plasma concentration. The predicted systemic clearance (CL) of 10.7 L/h (95% CI 11.5 – 14.9 L/h) was within the range of reported values (7.4 -35 L/h). The predicted volume of distribution (V) of 13.5 L was also consistent with observed V. The estimated median CL of VCR in CYP3A5 expressers was nearly 4-fold higher than in nonexpressers (39.6 L/h vs 9.9 L/h, $p<0.05$).

Conclusions: A PBPK model accounting for the CYP3A4 and CYP3A5-mediated clearance of VCR simulated plasma concentrations reported in adult cancer patients. This model indicates that individuals who express CYP3A5 exhibit a higher exposure to VCR than nonexpressers, which may lead to increased risk of neurotoxicity.

Translational/Clincal Research ***Graduate Student***

POSTER #111

TARGETING NON-CODING RNA HOTAIR WITH TUMOR SPECIFIC PEPTIDES REVERSES CHEMOTHERAPY RESISTANCE IN OVARIAN CANCER

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ıe recently discovered long noncoding RNAs (lncRNAs) are emerging as key contributors to cancer biology. O cRNA that has attracted significant attention is the Hox transcript antisense intergenic RNA termed HOTAI pressed from the developmental HOXC locus located on chromosome 12q13.13 9. By shuttling the polycor ɔressive complex (PRC2) to specific gene targets, HOTAIR represses gene expression through trimethylation stone H3 on lysine K27 (H3K27me3) and uniquely exerts epigenetic repression in *trans* on dista romosomes. Although a vital role for HOTAIR in ovarian cancer (OC), the fifth leading cause of cancer death S. women and the deadliest form of all gynecological cancers was recently suggested, its biological role remai ɹsive.Our group has recently shown that HOTAIR contributes to cisplatin (CDDP) resistance by activating NF-¿ ring DNA damage leading to increased MMP-9 expression. Furthermore, treatment with high dose of CDDP le NF-¿B dependent cellular senescence commonly associated with increased IL-6 secretion and CDI sistance (Ozes et al. *Oncogene*. In press). Moreover, studies on functional roles of HOTAIR in cancer indica at targeting HOTAIR may have therapeutic importance. In this study, we introduce an “anti-lncRNA” delive ent using sequence-specific peptide nucleic acids (PNAs) that bind to previously validated single strand gions of HOTAIR and inhibit its interaction with the PRC2. We show the candidate PNAs to be non-toxic a ve minimal effect on cell proliferation. We further show PNAs have specific effects in OC cells with elevat vels of HOTAIR and can inhibit invasion, chemotherapy resistance, NF-kB activation and IL-6 secretic portantly, conjugation of PNAs to pH-low insertion peptide (pHLIP) produced a novel construct that can livered in vivo to the acidic microenvironment (pH approximately 6) of solid tumors. In mouse xenograft mode e anti-lnc suppressed HOTAIR oncogenic activity, significantly inhibited formation of high-grade serous ovari nors, and increased survival. Collectively, these results are the first to demonstrate an inhibitor targeting cogenic lincRNA and validate the use of anti-lncs as new therapeutic tools to reverse drug resistant OC a ely other cancers.

Translational/Clincal Research ***Graduate Student***

POSTER #112

NOVEL COMBINATION THERAPY OF DNA METHYLTRANSFERASE INHIBITOR GUADECITABINE (SGI-110) AND PARP INHIBITOR TALAZOPARIB (BMN-673) FOR BRCA-PROFICIENT HIGH-GRADE SEROUS OVARIAN CANCER

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Ovarian cancer recurrence has been shown to be associated with increased DNA damage response (DDR) mediated by poly-(ADP)-ribose polymerase 1/2 (PARP1/2), which can be therapeutically targeted by PARP inhibitors (PARPi). PARPi are indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer, but most ovarian cancer patients have BRCA-proficient disease. Based on our previous studies supporting a role for DNA methylation in chemoresistant ovarian cancer, mediated by the enzyme DNA methyltransferase 1 (DNMT1), and reports on a functional role for DNMT1 in DNA damage repair, we hypothesize that combining a DNMTi and PARPi will impair BRCA-mediated DDR, resulting in cytotoxicity in ovarian cancer cells. A panel of ovarian cancer cell lines (A2780, platinum sensitive, BRCA1/2-wild type; A2780-cp and HeyC2, platinum resistant, BRCA1/2-wild type; high-grade serous Kuramochi, platinum resistant, BRCA2 mutant) was examined for cell growth using colony formation assays after treatment with DNMTi guadecitabine (low dose, 20-100nm) and PARPi talazoparib (1-10nm), alone or in combination. In all ovarian cancer cell lines, while high doses (10nm) of talazoparib alone reduced ($P<0.05$) colony formation, combining guadecitabine with talazoparib resulted in decreased ($P<0.05$) survival at all doses examined. To focus more specifically on BRCA status, we utilized two high-grade serous ovarian cancer cell lines ("PEO") derived from the same patient but harboring a mutant (PEO1) or wild type (PEO4) BRCA2 gene (Langdon et al, 1988; Sakai et al, 2009). Treatment with low-dose guadecitabine (20 nm, 3 days) increased ($P<0.05$) PARP levels (western blot analysis) as well as enzymatic activity ($P<0.05$; ELISA analysis), while talazoparib treatment alone increased ($P<0.05$) DNMT1 levels and decreased ($P<0.05$) PARP enzymatic activity. Treatment with guadecitabine or talazoparib alone had no effect on cell proliferation; however, combining the two drugs inhibited ($P<0.05$) PEO1 and PEO4 proliferation and increased ($P<0.05$) apoptosis (caspase 3 cleavage) in both cell lines. In summary, combining a PARP inhibitor with a hypomethylating agent (HMA) results in enhanced cytotoxicity in high-grade serous ovarian cell lines harboring either wild type- or mutant-BRCA, indicating that the talazoparib-guadecitabine drug combination impairs BRCA-mediated DDR and may represent an effective treatment regimen for BRCA-related cancers.

Translational/Clincal Research ***Graduate Student***

POSTER #113

PLATINUM INDUCES IL-6-SIGNALING MEDIATED ACTIVATION OF ALDH1A1 AND ENRICHES THE CANCER STEM CELL POPULATION IN HIGH GRADE SEROUS OVARIAN CANCER

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While chemotherapy initially eliminates the majority of tumor cells in high-grade serous ovarian cancer (OC), residual tumors are enriched in ovarian cancer stem cells (OCSCs) that drive chemoresistance and tumor relapse. An emerging model indicates that non-OCSCs may dedifferentiate under certain circumstances, and a critical role for the IL-6 signaling pathway in converting non-CSCs to CSC has been suggested. We investigated the mechanism contributing to OCSC enrichment after platinum treatment, hypothesizing that platinum-induced IL-6 secretion activates IL6 signaling-mediated expression of the CSC marker ALDH1A1, resulting in CSC enrichment. In addition, based on our previous study on targeting OCSCs by hypomethylating agent guadecitabine (SGI-110), we investigated therapeutic intervention combining IL-6 neutralizing antibody (IL-6Nab) with SGI-110 as maintenance strategy to eradicate OCSCs in tumor residuals after chemotherapy. We demonstrated that cisplatin (CDDP, respective IC_{50} dose) induced ($P<0.05$) IL-6 secretion by OCs (Kuramochi and A2780) up to 48h post-treatment, normal omental fibroblasts (NOFs) and co-cultured OC cells with NOFs up to 96h post-treatment, suggesting a role of the tumor microenvironment in platinum-induced IL-6 secretion. By assaying FACS sorted ALDH (+/-) cells, we determined that ALDH (+) cells expressed increased ($P<0.05$) IL-6 receptor levels and secreted higher ($P<0.05$) levels of IL-6 into the media compared to ALDH (-) cells. Treatment of OC cells with IL-6 (100ng/ml)/CDDP enriched the percentage of ALDH (+), and this enrichment was inhibited ($P<0.05$) by IL-6-Nab (400ng/ml) plus SGI-110 (100nM for 3 days). Luciferase assay results revealed that IL-6 transactivated ($p<0.05$) ALDH1A1 reporter gene expression in ALDH (-) cells, which was blocked by IL-6-Nab, and that IL-6- or CDDP increased ($P<0.05$) ALDH1A1, pSTAT3 or pERK protein expression in OC cells, which was inhibited ($P<0.05$) by SGI-110 plus IL6Nab combination treatemnt. Moreover, our *in vivo* study demonstrated that SGI-110 combined with IL-6-Nab as "maintenance therapy" significantly reduced ALDH (+) population in platinum-treated tumor residuals and delayed tumor relapse. Our data indicate that IL-6 is a potent regulator of ALDH1A1 expression and the OCSC phenotype. We suggest that a combination approach of IL-6 Nab with SGI-110 could represent a novel maintenance strategy after chemotherapy for eradicating OCSCs and preventing tumor recurrence.

Translational/Clincal Research ***Graduate Student***

POSTER #114

SYSTEMATIC COMPARISON OF MRNA AND PHOSPHOPROTEINS EXPRESSION IN BREAST CANCER SUBTYPE FROM CELL LINES TO TISSUE

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Background:

Whether the observed variation in transcriptome can predict the variation in proteome has not been fully investigated in subtypes of breast cancer. In addition, it is highly critical to study whether transcriptome/proteome correlations can be preserved in the cell line model when it is derived from primary tumor. Here, we attempt to address the transcriptome/proteome associations in a candidate set of gene/proteins using breast cancer tumor tissues and cell lines.

Method: Reverse-phase protein arrays (RPPA) were used to measure 313 protein expressions and 46 protein phosphorylations among 421 primary breast tumors from Cancer Genome Atlas (TCGA) and 33 cell lines of breast cancer. Correlation analysis between mRNA and RPPA was conducted and compared between primary tissues and cell lines in different breast cancer subtypes. Highly concordant genes/proteins are further analyzed using pathway analysis.

Results: In the breast cancer luminal A/B subtype, high mRNA/RPPA correlations were consistently observed for ER-alpha and PR in both cell line (0.71,0.677), and primary tissue, (0.90, 0.809). In the HER2 subtype, high mRNA/RPPA correlations in EGFR and HER2 were observed in the primary tissue (0.85, 0.78), and cell (0.73, 0.65). In the basal like subtype, consistently correlated mRNA/RPPA expressions were observed in Cyclin E1, GATA3, and AR, which are (0.8, 0.83, 0.7) in the primary tumor tissues and (0.85, 0.59, 0.7) in the breast cancer cell lines. The overall correlation of mRNA/RPPA correlation between cell line and primary tissue is 0.71. We observed that protein expression is affected by different gene isoforms. In addition, mTOR/PI3K pathway is identified to have strong function in basal like subtype of breast cancer.

Conclusion: Strong mRNA/RPPA associations were observed in each breast cancer subtype, ER-alpha and PR in the luminal A/B, HER2 and EGFR in Her2+, and Cyclin E1, GATA3, and AR in the basal like. These strong mRNA/RPPA associations are highly concordant between cell lines and primary tissues. Since most of these genes are well known drug targets, the highly concordance gene/RPPA associations not only confirm the gene expression biomarkers can served as surrogates for their protein products in the drug and target selections, but also imply that breast cancer cell lines shall serve as good models for primary tumor tissues.

Keywords: Breast Cancer, Reverse-phase protein array, mRNA, Cell lines, Primary tumor tissue.

Translational/Clincal Research ***Graduate Student***

POSTER #115

VIBRATIONAL PHOTOACOUSTIC TOMOGRAPHY IMAGING SYSTEM TO IMPROVE THE CLINICAL CARE OF THE HIGHEST INCIDENT CANCERS IN MEN AND WOMEN

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VibrationalPhotoacoustic tomography (VPAT) is a hybrid imaging modality that is showing promise to improve clinical practice. This method utilizes a pulsed laser to excite the overtone transition of target molecular bonds inside of tissue, resulting in a local temperature rise and in turn detectable acoustic signals. Such laser-induced acoustic signal can be detected by ultrasound (US) transducers, which are extensively used in clinics, and then used to reconstruct a molecular map of the tissue. VPAT can provide molecular information at imaging depth beyond conventional optical imaging methods, since the detected signal is an acoustic wave rather than photons, which encounter strong scattering in tissue.

We have developed a mobile VPAT imaging system to be applied to clinical practice. Our system is comprised of a Nd:YAG laser-pumped barium nitrite Raman laser to output 1197 nm laser, which excites the second overtone of the C-H bond stretch. 128-element US transducer arrays are used to detect the photoacoustic signal and standard US signal. We have demonstrated an imaging depth of greater than or equal to 5 mm with 100 μm imaging resolution of lipid detection. Currently, we are applying this VPAT imaging system to the two forms of cancer with the highest incidence: breast cancer and prostate cancer.

In regards to breast cancer, the necessary lag-time of traditional histopathological analysis to confirm complete removal of breast tumor in the breast conserving surgery, or lumpectomy, leads to an average re-operation rate of 25% in clinical practice. We apply our VPAT imaging system to image the lipid that surrounds the breast tumor to determine the success of the lumpectomy, which is defined as a minimum of 2 mm of normal tissue surrounding the tumor. VPAT in this application has the advantage to be performed within minutes to guide the surgeon to remove additional tissue to reduce the re-operation rate. Our preliminary data on 40 breast tissue samples procured from a tissue bank procedures suggests a sensitivity of 93% and specificity of 90% in margin assessment.

In current prostate cancer diagnosis, transrectal ultrasound-guided biopsy is used to confirm and grade prostate cancer. Due to the non-targeted nature of this procedure, a high false negative rate is present and repeat biopsies may be needed to identify a prostate cancer tumor. We apply our VPAT imaging system to identify the location and size of prostate cancer tumors by utilizing the recently discovered contrast of cholesteryl ester accumulation which correlates specifically with increasing prostate tumor aggressiveness.

Translational/Clincal Research ***MD/PhD student***

POSTER #116

CLINICAL UTILITY OF ALTERNATIVE SPLICING EVENTS ACROSS TUMOR TYPES

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The application of Next-Generation Sequencing (NGS) technologies and biological data repositories, such as The Cancer Genome Atlas (TCGA), allow researchers unmatched access to genomic, transcriptomic, epigenomic, and proteomic data. One area which remains unexplored, however, is alternative splicing, an important genetic regulatory mechanism allowing for a limited number of genes to generate widely diverse gene products, and its potential clinical utility. In this study, we developed a protocol to determine the effect of alternative splicing events on the prognosis of cancer survival. We retrospectively predicted patient survival using molecular data from 4,131 samples across 15 cancer types from TCGA. Incorporating matched clinical data to the molecular samples, we used a univariate Cox proportional hazards model to determine significant alternative splicing events. We then randomly split the patient data into training and test sets and built a predictive model using the Supervised PCA method. We identified multiple splicing events associated with patient overall survival in 5 cancer types. Subsequent unsupervised hierarchical clustering on the alternative splicing events identified 3 novel molecular subgroups in triple-negative breast cancer (TNBC) with varying patient survival outcome ($P = 0.03$). Similar patterns were identified in both head and neck squamous cell carcinoma (HNSC) ($P = 0.04$) and breast invasive carcinoma (BRCA) ($P = 0.02$). We further explored the upstream regulator of the 94 splicing events that are associated with overall survival in BRCA and found 33 of the alternative splicing events regulated by the same motif corresponding to the *HNRNPF* (heterogeneous nuclear ribonucleoprotein F) RNA binding protein. Furthermore, overlap of the 33 alternative splicing events with known proto-oncogenes and tumor suppressor genes showed high similarity (74%). Our study provides the first pipeline to determine clinical survival based on alternative splicing events. Our protocol not only incorporates molecular data and clinical utility, but also enables us to understand the molecular mechanism behind transformative medicine.

Translational/Clincal Research ***Medical Student***

POSTER #117

VIRTUAL SURGICAL PLANNING FOR IMPLANT SUPPORTED AURICULAR PROSTHETICS: THREE SCANNING TECHNIQUES

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Objective: To determine the most efficient, accurate method for auricular scanning in implant-supported auricular prosthetics. Implant-supported auricular prosthetics are ideally suited for virtual surgical planning. Since most patients have an intact contralateral ear, it can be used to virtually model the ear that is missing due to cancer, trauma, or congenital defect. Imaging data may be acquired from the intact ear by a variety of 3-dimensional image capture methods including photogrammetry and laser scanning. Once data is captured from the intact ear, it can be copied, mirrored, and placed in the appropriate position for virtual surgical implant planning and surgical guide design. At Indiana University School of Dentistry, the Department of Orthodontics collaborates with the Department of Prosthodontics to help scan facial prosthetic patients. Scanners include 3DMD Face extraoral photogrammetry, CareStream intraoral scanner, and a desktop laser scanner from OrthoInsight. Each technology has strengths and weaknesses when used for scanning facial features. This poster discusses those strengths and weaknesses in the context of an on-going clinical case from the Department of Prosthodontics. Conclusion: 3DMD Face photogrammetry is the recommended facial scanning technology due to the ease of use, speed of image acquisition, and acceptable anatomic detail registration. Although other options capture fine anatomic detail better, the significant advantages of 3DMD Face make this the preferred option for facial scanning in virtual surgical planning implant supported auricular prosthetics.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #118

REPEAT STEREOTACTIC BODY RADIATION THERAPY FOR LIVER TUMORS

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Purpose: Stereotactic body radiation therapy (SBRT) for liver tumors has high rates of local control (LC) and acceptable toxicity. Some patients develop recurrent hepatic disease and additional SBRT can be considered; however, outcomes after repeat SBRT are not well described.

Materials and Methods: 383 patients treated with liver SBRT at a single institution from 2006-2016 were reviewed; 16 patients underwent multiple SBRT courses. 7 patients were re-treated for hepatocellular carcinoma (HCC), 1 for cholangiocarcinoma, and 8 for metastases (LM). 2 patients with HCC were excluded; 1 for incomplete radiation plans and 1 who had routine liver transplant after 1 fraction of repeat SBRT without toxicity. 2 patients received a 3rd course of SBRT.

Results: Median dose for patients with primary liver tumors (PLT) was 48 Gray (Gy)/ 3 fractions for the 1st SBRT and 40 Gy/ 5 fractions for 2nd SBRT, compared to 54 Gy/ 3 fractions and 50 Gy/ 5 fractions for LM for the 1st and 2nd SBRT, respectively. Median follow up was 18.2 months in living patients. Crude LC for the 1st and 2nd treatment was 78.6% and 85.7%, respectively. For the whole cohort, mean progression free survival (PFS) and overall survival (OS) from the 2nd SBRT were 11.9 and 28.1 months, respectively. PFS was significantly shorter in patients with LM compared to PLTs with median values of 4.3 vs 18.4 months, respectively (*p=0.01*), but there was no difference in OS between the two groups (median 20.7 vs. 26.6 months, *p=0.18*). Change in liver volume between the 1st and 2nd SBRT courses was predictive of PFS and OS (*p=0.05 and p=0.02,* respectively). Median OS in patients with liver volume loss between SBRT courses was 13.1 vs 42.5 months in patients without volume loss (*p=0.01,* HR 5.17 [0.83-32.37]). 2nd SBRT was well tolerated, but severe liver decompensation was seen in both patients receiving a 3rd SBRT course.

Conclusion: A 2nd course of liver SBRT is safe and associated with high LC; however, PFS differs between patients with PLT and LM. Patients with liver volume loss appear to have worse outcomes. Significant toxicity occurred in both patients undergoing a 3rd SBRT. Weaknesses of this study include its retrospective nature and low patient numbers.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #119

THE RELATIONSHIP BETWEEN ENDOMETRIAL CANCER SENTINEL LYMPH NODE MICRO AND MACRO METASTASES AND UTERINE PATHOLOGY FEATURES

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Objectives: The practice of selective lymphadenectomy based on uterine pathology risk factors has been popularized after risk factors for lymph node metastases were identified in large observational single institution and cooperative group studies. Sentinel Lymph Node (SLN) biopsy is an alternative staging technique proposed to overcome the limitations of selective algorithms. The clinical validity of low-volume metastases identified in SLN specimens that have been ultrastaged has been questioned. The objective of this analysis is to identify whether patients with positive SLNs demonstrate the previously identified risk factors for lymph node metastases.

Methods: The Fluorescence Imaging for Robotic Endometrial cancer Sentinel node mapping (FIRES) trial is a multi-institution, prospective cohort study measuring the accuracy of SLN mapping in clinical stage I endometrial cancer (all histologies) in identifying metastatic disease. All patients received a standardized SLN mapping technique with cervical indocyanine green (ICG) injection and robotic fluorescence imaging, followed by hysterectomy with pelvic and para-aortic lymphadenectomy. All H&E-negative SLN specimens were ultrastaged with immunohistochemistry (IHC) to cytokeratin. Pathologic results of the SLNs (including volume of disease: macro metastases versus micro metastases [<2 mm and isolated tumor cells]) were evaluated along with uterine tumor risk factors. Fisher's exact test was used to compare dichotomous variables between groups.

Results: Among 308 patients, 37 (12%) had nodal metastases, 30 of whom mapped at least 1 SLN (81%). Twelve patients (32%) with nodal metastases were detected only with IHC (=2 mm). Compared with patients with macro metastases, micro metastases were less likely to be associated with high grade (P=0.04) or nonendometrioid histology (P = 0.03), para-aortic metastases (P = 0.04), and lymphovascular space invasion (LVSI) (P= 0.001). All but 1 node-positive patient (97%) (including all patients with SLN micro metastases) demonstrated at least 1 previously described uterine pathology risk factor for lymphatic spread (grade 3 histology, outer half myometrial invasion or tumor size >2 cm).

Conclusions: Micro metastases within SLNs appear to be associated with known uterine risk factors for nodal metastases. This supports the validity of metastatic disease identified in SLNs with ultrastaging techniques. The disease-specific outcomes of patients with low-volume disease have not yet been established.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #120

CHANGES IN BODY COMPOSITION CORRELATES WITH SURVIVAL IN PATIENTS WITH PANCREATIC ADENOCARCINOMA ON FOLFIRINOX THERAPY

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Introduction: Patients with malignancy often develop loss of skeletal muscle and/or fat, a process known as cachexia. Development of cachexia has been associated with decreased response to therapy, decreased survival, and worse overall outcomes. Presence of cachexia is common in patients with Pancreatic Adenocarcinoma (PDAC) with up to 80% developing cachexia. This study evaluated the changes in body composition in patients with non-resectable PDAC on FOLFIRINOX therapy and the correlations of these changes with survival.

Methods: This is a retrospective study of patients with PDAC being treated with FOLFIRINOX therapy at Indiana University Hospital between July 1, 2010 and August 31, 2015. Demographic and clinical data were collected on all patients including age, sex, disease extent, best response to therapy, progression free survival, and overall survival. Lumbar skeletal muscle and fat masses were measured on serial computed tomography (CT) scans. Based on these measurements patients were stratified into 3 groups; muscle/fat gainers, muscle and fat losers, and fat only losers.

Results: Fifty-five (55) patients were identified with available CT scans and survival data. Twenty-six (47.3%) presented with locally advanced (LA) disease compared to 29 (53.7%) presenting with metastatic (Met) disease. Twenty-nine (53.7%) of patients presented with sarcopenia compared to 26 (47.3%) who did not have sarcopenia at diagnosis. There was no increased odds of sarcopenia present at diagnosis between LA and Met groups (OR=1.46; 95% CI=0.5033-4.2415). Survival analysis showed no significant difference between patients who presented with sarcopenia and those who did not with median survival being 12.94 months vs. 17.77 months (p=0.375)(HR=1.303; 95% CI= 0.727-2.369). Forty-nine (89.1%) had multiple scans and were divided into 3 groups; 25 (51.0%) were muscle and fat losers, 14 (28.6%) were fat only losers, and 10 (20.4%) were muscle/fat gainers. Median survival for muscle and fat losers was 12.94 months, for fat only losers was 14.48 months, and for muscle/fat gainers was 28.53 months. Comparison of survival for each group showed significant difference in overall survival between muscle and fat losers and muscle/fat gainers (p=0.018)(HR=2.98; 95% CI=1.22-6.12) and between fat only losers and muscle/fat gainers (p=0.006) (HR=3.62; 95% CI=1.62-11.31).

Conclusion: Loss of skeletal muscle and/or fat in patients undergoing FOLFIRINOX therapy for non-resectable PDAC is associated with shorter median overall survival and associated with a significant increased risk of all-cause mortality. Preventing loss of skeletal muscle and fat in patients with PDAC may confer a survival advantage. Further studies in which skeletal muscle and fat loss is prevented are needed to further understand the role of cachexia on mortality in PDAC.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #121

SEGMENTAL CHANGES IN LIVER VOLUME AFTER SBRT

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Purpose/Objectives:

Stereotactic body radiation therapy (SBRT) is used as an ablative therapy for liver tumors and is thought to cause atrophy of liver tissue in the treated segment. The hypothesis is that the liver volume outside of the area treated by SBRT will grow to compensate.

Materials/Methods:

This retrospective analysis included 8 patients from one institution with hepatocellular carcinoma (HCC) who underwent SBRT and liver transplantation between 2007-2012 and had follow-up CT scans at 3 and 6 months. Four patients had Child-Pugh Class (CPC) A cirrhosis and 4 had CPC B cirrhosis. The sum of the maximum tumor diameter ranged from 1.0-5.1 cm (1 patient had 2 lesions). Two patients received 4800 cGy in 3 fractions and 6 received 4000 cGy in 5 fractions. At least 700 cm^3 of normal liver received <1500 cGy in all patients. Elapsed time between completion of SBRT to liver transplantion was 186-1220 days. The Phillips IntelliSpace Liver Analysis application was used for Couinaud segmentation of the pre-SBRT and 3- and 6-month post-SBRT CT scans.

Results:

Pre-SBRT liver volume ranged from 1389 cm^3-2430 cm^3. The mean 3- and 6-month changes in total liver volume were -8.3 +/- 11.1% (range -26.0% to +11.3%) and -13.0 +/- 12.2% (range -35.8% to +4.04%). The mean 3- and 6-month changes in liver volume outside the treated segment were -6.4 +/- 10.5% (range -23.3% to +7.4%) and -8.6 +/- 11.7% (range -30.4% to +3.8%), respectively. The mean 3- and 6-month change in liver volume in the treated segment was -8.0 +/- 23.2% (range -26.5% to +40.2%) and -25.5 +/- 17.2% (range -43.6% to +0.8%), respectively. Pathologic analysis did corroborate ablation of the treated segment. Six of 8 patients (75%) had a complete response and 2 had stable disease.

The planning target volume (PTV) was >100 cm^3 in 3 patients, all of whom received 4000 cGy in 5 fractions. The 2 patients with the largest changes in volume outside of the treated segment at 6 months (-20.1% and -30.4%) had the largest PTVs (207.5 cm^3 and 169.0 cm^3, respectively) and had CPC B cirrhosis. Another patient with CPC A cirrhosis had a PTV of 169.0 cm^3 and experienced a -4.6% decrease in liver volume outside the treated segment. The patient who had a -20.1% change in his liver volume had decompensated from a CPC B to CPC C at the time of liver transplantation and was the only patient who had a decline in CPC pre-transplant.

Conclusions:

Post-SBRT changes in liver volume are highly variable. In this cohort of HCC patients treated with SBRT who underwent subsequent liver transplantation, liver volume outside of the treated segment did not increase significantly at 6 months but rather was stable to decreased. The use of functional imaging to guide planning for SBRT may help protect normal liver, allowing regeneration and/or avoiding deterioration of liver function, especially in patients with advanced cirrhosis. Further studies are needed to confirm our findings.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #122

ROLE OF STEREOTACTIC BODY RADIOTHERAPY AS A BRIDGE TO ORTHOTOPIC LIVER TRANSPLANTATION: EVALUATION OF PATHOLOGIC RESPONSE AND OUTCOMES

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Introduction

Stereotactic body radiotherapy (SBRT) is an emerging radiation treatment technology that utilizes high doses per fraction to deliver an ablative therapy to tumors. The technology is evolving with advances in treatment planning, image guidance and patient immobilization. The technique was applied to early stage, localized hepatocellular carcinoma with our institutional Phase I-II trial showing acceptable toxicities and exceptional rates of local control. The role of SBRT as a bridge to definitive orthotopic liver transplantation (OLT) was analyzed herein.

Materials & Methods

The subjects of this retrospective report are 38 patients with a diagnosis of hepatocellular carcinoma who received stereotactic body radiotherapy per our institutional Phase I-II eligibility criteria, en route to definitive OLT. Demographic and treatment variables of patients with Child-Pugh class (CPC) A-5 to B-7 cirrhosis were analyzed against local control (complete response + partial response + stable disease), response rate (complete + partial response), overall survival (OS) and disease free survival (DFS) calculated from OLT. Pre-OLT radiographs were compared with pathologic gold standard using several accepted radiographic scoring criteria for concordance, sensitivity, specificity and predictive values. An analysis of treatment failures and deaths was undertaken.

Results

With a median follow-up of 4.8 years from OLT, 9/38 patients (24%) developed recurrent disease while 10/38 patients (26%) died. Kaplan-Meier estimates of 3-year OS and DFS are 77 and 74%, respectively. No survival differences were appreciated by CPC or pre-SBRT T stage. The sum longest dimension of tumors was significantly associated with DFS (HR 1.93, p=0.026). Most common dose-fractionation schedules were 16Gy x3 in CPC-A patients and 8Gy x5 in CPC-B patients. With a mean time from SBRT to OLT of 10.7 months, pathologic local control was 100% in 44 evaluable lesions while pathologic response rate was 68%. At the time of transplant, mean 41% reduction in tumor size by CT or MRI was observed as was a mean 3-fold reduction in AFP level. Radiographic scoring criteria performed poorly, with mRECIST producing highest pathologic concordance (kappa=0.428), sensitivity (90%), positive predictive value (74%) and negative predictive value (40%). Explants revealed viable tumor in 79% of patients. 63% of patients maintained their CPC and 50% were assigned pathologic T stage concordant with clinical T stage. Treatment failures had statistically larger sum longest dimension of tumors (4.0 cm vs 2.8 cm, p=0.014) and a trend towards higher percentage of lymphovascular space invasion (44% vs. 17%, p=0.078).

Conclusions

SBRT as a bridge to OLT is a well-tolerated treatment providing 100% pathologic local control rates without a survival benefit, as all patients underwent definitive OLT, 79% having viable tumor. Radiographic response criteria poorly approximate pathology. Factors worthy of investigation for further adjuvant therapy include sum longest tumor dimension and pathologic presence of lymphovascular space invasion.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #123

ENDOBRONCHIAL ULTRASOUND-GUIDED TRANSBRONCHIAL NEEDLE ASPIRATION USE FOR THE SUBCLASSIFICATION AND GENOTYPING OF NON-SMALL CELL LUNG CANCER

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Rationale: The current management algorithm for locally advanced or metastatic non-small cell lung cancer (NSCLC) incorporates testing for multiple genetic alterations, which in turn is used to guide treatment decisions. Emerging targeted agents include kinase inhibitors of epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), ROS1, BRAF, RET, MET, and the human epidermal receptor (HER2neu).

Objectives: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is increasingly used for the diagnosis and staging of lung cancer. The purpose of this study is to examine the yield of EBUS-TBNA in the subclassification and genotyping of NSCLC.

Materials and Methods: Sixty-nine patients at Indiana University Hospital with suspected or confirmed lung adenocarcinoma underwent EBUS-TBNA of lung masses or lymph nodes using a 21-gauge Olympus™ needle. Samples were first reviewed by a pathologist, and if suspicious for NSCLC, were sent for different types of molecular testing based on the clinical scenario. At least 6 extra passes were placed in cell block. For Paradigm testing, 10 passes were sent. EGFR and KRAS testing were performed using the FDA approved Therascreen RGQ PCR Kit. Testing for ALK rearrangement was done using fluorescent in situ hybridization. In some cases, testing for these mutations in addition to ROS1, BRAF, and HER2 was done using the Paradigm Cancer Diagnostics test.

Results: Sixty-nine samples from patients with NSCLC obtained by EBUS-TBNA were sent for molecular testing for EGFR. Results were obtained in all patients (yield = 100%). Mutations were found in 3 patients (4.3%) vs. 66 wild-type (95.7%). 60 samples were sent for molecular testing for KRAS (yield = 100%), of which 10 had mutations (16.7%) vs. 50 wild-type (83.3%). 51 samples were sent for ROS1 testing [0 mutant, 48 (94.1%) wild-type]. Tissue samples were inadequate for testing in 3 patients (yield=94.1%). 64 samples were sent for ALK testing (3 (4.7%) mutant, 55 (85.9%) wild-type, yield = 90.6%). Ten samples were sent for BRAF testing and two samples were sent for HER2 testing, all of which were negative for mutations (yield = 100%).

Conclusion: Tissue sampling obtained from EBUS-TBNA in routine practice is appropriate for molecular mutation analysis and subtyping of NSCLC.

Clinical Implications: EBUS-TBNA with a 21-gauge needle is a potentially useful tool for the subclassification and genotyping of NSCLC. Improving the yield of this technique is important as we start testing for a greater number of mutations.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #124

TARGETING REF-1/APE1 PATHWAY INHIBITION IN PANCREATIC CANCER USING APX3330 FOR CLINICAL TRIALS

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related mortality in the US. Most patients present with advanced disease and ~95% die within five years, with most surviving less than six months. Targeted therapies including Gemcitabine (Gemzar™), FOLFIRINOX (5-FU/leucovorin/irinotecan/oxaliplatin), and sustained release, nab-paclitaxel (Abraxane™) offer modest improvement in survival, albeit at an increase in side effects and unwanted toxicities. Data is presented on redox factor-1 (Ref-1) and specific Ref-1 inhibitor APX3330.

Ref-1 regulates multiple transcription factors involved in pancreatic cancer cell survival signaling due to its redox-coactivator activity, such as HIF-1a, NFkB, NRF2 and STAT3. High expression levels of Ref-1 indicate decreased survival in PDAC as well as other cancers. APX3330 has been shown in multiple *in vitro* and *in vivo* pancreatic cancer models to be effective in reducing tumor growth and metastases as a single agent. The mechanism of action has been extensively investigated and characterized for its specific activity on Ref-1, as well as its preclinical PK/PD, ADME. The safety and dose administration of APX3330 have been established by Eisai pharmaceutical company through a previous development program including toxicology, phase I, and phase II clinical evaluation in non-cancer patients in Japan. We have partnered with ApeX Therapeutics to develop APX3330 for cancer treatment (phase I trial anticipated start date mid-2016).

While developing APX3330 for single agent use, we studied interactions of Ref-1, APX3330, convergent pathways; i.e. HIF-1a and STAT3, and downstream targets like CAIX. Initially, we performed *in vivo* studies demonstrating single and combination effects of APX3330 with Gemcitabine (Gem) showing significantly decreased tumor volume in the APX3330 and Gem combination treatments compared to the single-agents alone. We also tested single and combination studies of APX3330 in an *ex vivo* 3-D tumor-stroma model system using patient derived tumor cells along with patient derived cancer-associated fibroblasts (CAFs). We used the CAIX inhibitor SLC-0111 and JAK2 inhibitor, Ruxolitinib; both agents in clinical trials. In our *ex vivo* 3D co-culture system, APX3330 decreases the tumor area and intensity in a dose-dependent manner. The combination of APX3330 with Gem demonstrated an additive enhancement effect in the tumor. Blocking both Ref-1 redox-signaling activity with APX3330 and CAIX activity via SLC-0111 demonstrated enhanced tumor killing in our models. APX3330 along with Ruxolitinib also demonstrated enhanced tumor killing.

These data demonstrate APX3330 single agent efficacy in our 3D patient PDAC model and enhanced tumor killing when pathways regulated by Ref-1, HIF-1 and STAT3 are blocked.

Additional drug combinations focused on pathways that are dependent on Ref-1 signaling will also be presented.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #125

UNDERSTANDING AND OVERCOMING ZNF217 INDUCED BREAST CANCER CHEMORESISTANCE

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Despite most breast cancer patients being diagnosed with local or regional stage disease, most patient deaths result from metastasis as a result of recurrent disease after the development of therapeutic resistance. Developing a better understanding of the molecular mechanisms of therapeutic resistance is critical to help identify novel therapeutic strategies that overcome metastasis, resistance, and death.

We previously identified the transcription factor ZNF217 (human) / Zfp217 (mouse) as a prognostic indicator for breast cancer patients. ZNF217 is overexpressed in breast cancer and this overexpression promotes reduced survival, increased metastasis, and reduced response to therapy. We found that Zfp217 overexpression promotes an increase in self-renewal capacity, invasion, and metastasis as well as expansion of a progenitor cell population during both normal mammary development as well as during breast cancer progression.

We next determined if Zfp217 overexpression in vivo contributed to chemotherapy resistance. We treated mice overexpressing Zfp217 with a combination therapy of microtubule inhibitor epothilone B, Adriamycin, and cyclophosphamide (EAC). Mice overexpressing Zfp217 that were treated with EAC developed a significant increase in tumor volume over control mice within 21 days of EAC treatment. In addition, we find that chemotherapy resistance after Zfp217 overexpression causes an accumulation of a mammary gland progenitor cell population, which we hypothesize is the cell population resistant to standard treatments.

To overcome breast cancer chemoresistance caused by ZNF217 overexpression, we identified triciribine, a nucleoside analog and AKT inhibitor, as a drug that kills cells that overexpress ZNF217. We found that triciribine treatment inhibited tumor burden in vivo in tumors that overexpressed Zfp217. Triciribine also had synergy with doxorubicin in cell death assays and in xenografts using human cell lines. Using our preclinical animal models of Zfp217 overexpression, we elucidated the appropriate dosing for combination therapy of triciribine and the microtubule inhibitor paclitaxel to treat breast cancer and found that the order of treatment impacts the efficacy of therapy. We also tested the therapeutic efficacy in patient-derived tumor xenografts (PDX) of human tumors with high versus low levels of ZNF217 expression and similarly saw that treatment orders matters for efficacy. Our preclinical studies have influenced the design of Phase II clinical trials using triciribine to treat metastatic breast cancer.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #126

LOW SERUM ALBUMIN LEVELS PRIOR TO PEDIATRIC ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION ARE ASSOCIATED WITH INCREASED CRITICAL CARE NEEDS AND 6-MONTH MORTALITY

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Introduction:

Poor nutritional status in hematopoietic cell transplant (HCT) patients is a negative prognostic factor. However, there are no pediatric studies evaluating albumin levels prior to HCT and need for critical care interventions. We aimed to determine if there is a relationship between serum albumin levels in the pre-HCT period, routinely measured 30 days (± 10 days) prior to transplant, and need for critical care interventions. We hypothesized that pediatric patients with low albumin levels prior to undergoing allogeneic HCT will have a higher critical care needs in the post-transplant period.

Methods:

This is a 5-year retrospective study of pediatric patients who underwent allogeneic HCT for any reason. Patients were excluded if they received an autologous HCT, if missing lab data prior to transplant, and any data from subsequent transplants. Primary outcome was critical care needs. Secondary outcome was 6-month mortality. Critical care needs were defined as: admission to the PICU, requiring noninvasive ventilation (CPAP or BiPAP), intubation requiring mechanical ventilation, and need for vasoactive therapy. Patients were categorized based on albumin levels. Low albumin was defined as <3.1 g/dL. Continuous variables were compared using a Wilcoxon rank sum test. Categorical variables are expressed as percentages. Univariate analysis was completed with logistic regression.

Results:

A total of 73 pediatric HCT patients were included in the study with a median age of 7.4 years (IQR 3.3, 13.2). Patients with low albumin, defined as <3.1 g/dL, had a higher need for critical care interventions including: PICU admission (67% vs 22%, p=0.01), noninvasive ventilation (44% vs 8%, p=0.01), mechanical ventilation (67% vs 17%, p<0.01), and vasoactive therapy (56% vs 16%, p=0.01). These patients also had a higher 6-month mortality of 56% vs 17%, p=0.02.

Conclusions:

Children who are undergoing allogeneic HCT and have low serum albumin levels in the pre-transplant period are more likely to need critical care interventions and have increased 6-month mortality. These findings may identify an at-risk population in which nutritional improvements may be instituted prior to HCT in hopes of improving outcomes.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #127

OVARIAN CANCER VULNERABILITY TO HDAC INHIBITORS IS DEPENDENT UPON BRCA MUTATIONAL STATUS

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Background: Histone acetylation regulates gene expression in cancer and can be exploited for therapeutic targeting. Histone deacetylase inhibitors (HDACi) increase histone acetylation, promoting transcription of genes involved in growth arrest and apoptosis and have been used as anti-cancer agents. BRCA mutations are the best characterized genetic alteration associated with ovarian cancer and can be targeted therapeutically by recently approved PARP inhibitors. We hypothesized that the epigenetic landscape of ovarian cancer is altered by the presence of BRCA mutations and can be manipulated by HDACi for therapeutic benefit.

Materials and Methods: We utilized isogenic cell lines carrying mutated/functional BRCA 1 (UWB1.289; UWB1.289-BRCA), and respectively BRCA 2 genes (PE01/PEO4). We measured HDAC expression and activity by western blotting and an enzymatic assay and characterized globally chromatin marked by H3K9ac (active chromatin) and H3K27ac (poised chromatin) by using ChIP sequencing. To determine whether HDAC inhibitors increase susceptibility to PARP inhibitors, cell proliferation was measured in ovarian cancer cells expressing mutated and wild type (WT) BRCA.

Results: HDAC1 expression and activity were decreased in cell lines carrying defective BRCA proteins. ChIP sequencing identified chromatin regions differentially marked by H3K9ac (178 promoters in BRCA1-null compared to BRCA WT cells) and H3K27ac (131 promoters in BRCA WT compared to BRCA null cells) suggesting differences in gene transcription dependent on histone acetylation in BRCA mutated vs BRCA functional cancer cells. A subset of genes differentially marked by H3K9ac were validated at mRNA level by RT PCR confirming BRCA dependent regulation (*TGM2, Wnt7a, DKK1, MET, FLI1, AXL, TG2, PMEPA1, TWIST2* upregulated) and (E2F2, NID2-downregulated) in BRCA1-null ovarian cancer cells. HDAC1 inhibition with trichostatin A (TSA) and entinostat (MS-275) increased H3K9ac and was associated with an increase in TGM2, FLI1, DKK1 and E2F2, and a decrease in TWIST2 gene expression in both BRCA1-wt and –null cell lines confirming epigenetic regulation of this subset of genes. Functionally, HDACi synergized with PARP inhibitors in BRCA null ovarian cancer cells (combination index 0.63), consistent with the altered gene expression changes induced by differences in chromatin marks.

Conclusions: In conclusion, the chromatin landscape is altered in BRCA null ovarian cancer cells and can be targeted by HDAC inhibitors. Identifying pathways and specific genes altered by histone acetylation may enable future rational targeted combination therapies.

Translational/Clincal Research ***Post-Doctoral/Medical Fellow***

POSTER #128

ROLE OF DICER HAPLOINSUFFICIENCY IN AGGRESSIVE ENDOMETRIAL CANCER IN MICE AND WOMEN

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Endometrial cancer is the most common gynecologic malignancy, affecting 1 out of every 38 women in the United States. Activation of the PI3K pathway through loss or mutation of the tumor suppressor PTEN accounts for 80% of these tumors. Late-stage and recurrent disease carries poor 5-year survival. Decreased DICER expression is associated with poor prognosis endometrial cancers. DICER is the RNAse responsible for processing the precursor miRNA hairpin to generate 2 single-stranded mature miRNA forms, the complementary miRNA-5p and miRNA-3p forms. Published *in vitro* studies have shown that *DICER*+/- cells have a switch in production from the abundant miRNA-5p form to the miRNA-3p form. Based on our interests in DICER processing and miRNA function, we mined TCGA (The Cancer Genome Atlas) endometrial cancer datasets to discover that 16% of *PTEN* mutant (*PTEN*mut) endometrial cancers also contained heterozygous mutations in *DICER* (*DICER*+/-). Translationally important, gene signatures associated with *PTENmutDICER+/-* tumors showed significantly worse survival compared to *PTENmut* only (P=0.02). This is the first human tumor with *DICER* haploinsufficiency to show worse prognosis. Our analysis shows 164 mature miRNAs differentially expressed (fold change 1.25, P<0.05) between *PTENmutDICER+/- and PTENmut* genotype TCGA tumors. Examination of let-7a, a miRNA known to be dysregulated in *DICER+/-* cells *in vitro*, showed a specific dysregulation of let-7a-3p but not let-7a-5p. Integration with *PTENmutDICER+/-* gene signature showed enrichment of let-7a-3p targets, potentially targeting 181 genes out of 2441 genes in our *PTENmutDICER+/-* gene signature (Fisher's exact test, P=0.0002). To investigate the molecular mechanism of *DICER* haploinsufficiency in endometrial cancer, we created both *in vivo* (mouse) and *in vitro* (human endometrial cancer cell line) model systems of *DICER* haploinsufficiency. On a *Pten* deleted background, Kaplan-Meier survival analysis showed protection from loss of 2 alleles of *Dicer* and more aggressive endometrial cancer from loss of 1 allele of *Dicer* (P=0.005) compared to *Pten* cKO. Median survival for *Dicer* haploinsufficient mice was 150 days, 240 days for *Pten* cKO, and 315 days for double cKO. By 12 weeks, invasion through the myometrium occurred in 85% of cases for *Dicer* haploinsufficient tumors compared to 30% for *Pten* cKO. Additionally, *Dicer* haploinsufficient tumors showed an increase in let-7a-3p expression. This is the first in vivo evidence in both mice and women that the miRNA-5p to miRNA-3p miRNA processing switch occurs with *DICER* haploinsufficiency, leading to significant changes in gene expression and more aggressive tumors. *In vitro*, heterozygous deletion of *DICER* resulted in a 65% increase in cellular proliferation. In summary, our model systems will allow us to understand why loss of 1 allele of *DICER* leads to biologically aggressive endometrial tumors and discover novel therapeutic targets for aggressive endometrial cancers or other cancers that are *DICER+/-.*

Translational/Clincal Research ***Research Associate***

POSTER #129

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF IMATINIB USING SIMCYP

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Imatinib mesylate (Gleevec) was the first marketed drug to treat Bcr/Abl – expressing chronic myeloid leukemia. Recently it was also approved to treat c-Kit – expressing gastrointestinal stromal tumors. Imatinib is extensively metabolized by CYP3A and CYP2C19 in the GI tract and liver, and the main metabolite, N-desmethyl imatinib, has comparable activity as its parent molecule. With consideration of brain metastasis of stromal tumors, the brain concentration of imatinib should be estimated in relevance to its therapeutic efficacy. In addition, the drug-drug interaction should be also considered if co-medications are CYP inhibitor or inducers. Thus predication of imatinib concentrations in tissues is important to estimate the dose of imatinib in order to achieve its best therapeutic efficacy and ensure the safety profile. In this study, we developed a physiologically based pharmacokinetic model (PBPK) model of imatinib to explore tissue distribution and CYP450-mediate drug-drug interactions. We collected relevant imatinib pharmacological data from literature and utilized the SIMCYP simulator (Certara) to predict imatinib concentrations in selected tissues.

Initial tissue-to-plasma partition coefficients were predicted using the Rodgers and Rowland method. Parameter estimation algorithms within SIMCYP were then utilized to adjust brain and liver partition coefficients for mouse to fit published data. These fitted values were then extrapolated to predict the pharmacokinetics of imatinib in human plasma and tissues. Human plasma concentration-time profiles from our prediction were comparable to literature reported observed values. Using the available CYP3A interacting drug models available in SIMCYP, we have also predicted the effect of CYP3A inhibitors and inducers on the pharmacokinetics of imatinib. PBPK modeling is a useful tool to investigate effects of drug-drug interactions on plasma and tissue exposure of imatinib.

Translational/Clincal Research ***Research Technician***

POSTER #130

MICRORNA-10B EXPRESSION IN A READILY ACCESSIBLE COMMON HEPATIC ARTERY LYMPH NODE CORRELATES WITH PANCREATIC CANCER RECURRENCE POST RESECTION

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Pancreatic carcinomas (PC) account for more than 50,000 cancer-related deaths in the United States annually, with a 5-year survival of 7%-20% depending on the stage and tumor type. To date, the extent of peripancreatic lymph node involvement is the best prognostic factor for PC. However, detecting lymph node metastases is often limited to patients who are eligible for resection. While pancreatoduodenectomy (PD) remains the treatment of choice for potential cure, the majority of these cancers are non-resectable, due to advanced disease that is locally invasive and/or metastatic at presentation. Moreover, early diagnosis of these carcinomas is hampered by the absence of non-invasive, sensitive, and specific biomarkers. We previously characterized microRNA (miR) expression in pancreatic ductal adenocarcinoma (PDAC) and demonstrated that high miR-10b levels in pancreatic cancer cells are associated with an attenuated response to multimodality neoadjuvant therapy. We also showed that high miR-10b levels in the plasma of PDAC patients, determined by quantitative PCR (q-PCR), differentiated PDAC patients from normal controls. Thus, miR-10b may serve as a prognostic marker in PC. We therefore assessed miR-10b levels in a common hepatic artery lymph node (station 8), which is readily accessible during PD and can be biopsied during diagnostic procedures. We also assessed plasma for several additional miRs that together with miR-10b comprise a panel that differentiates between PC and normal controls. Relative expression levels were used to assess for correlations with disease recurrence, recurrence-free survival (RFS) and overall survival (OS). High miR-10b levels were observed in 14/30 patients with PC, and in 10 of these 14 patients, cancer recurred during the study period. By contrast, other miRs assessed from the panel were not significantly different when comparing patients with recurrent and non-recurrent disease. Furthermore, high miR-10b was associated with shorter RFS, but not OS. Elevated miR-10b levels in station 8 lymph nodes correlates with increased risk of cancer recurrence and could be used preoperatively to assess risk for early disease progression in patients with pancreatic tumors.

Translational/Clincal Research ***Research Technician***

POSTER #131

CAMKK2 INHIBITION AS A "DUAL-HIT" STRATEGY AGAINST ADT-INDUCED OSTEOPOROSIS AND BONE-METASTATIC PROSTATE CANCER

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Approximately 180,000 men are diagnosed with prostate cancer (PCa) each year in the United States, making it the most common cancer among men second only to myeloma. Whereas, patients with localized PCa have a 5-year survival rate of 100%, the survival rate drops to 31% in patients with distant metastases. In the initial stages, PCa cells are heavily reliant on androgens for growth. Hence, androgen deprivation therapy (ADT) is the first line of defense against the disease. However, ADT contributes to weaker bone and predisposes patients to an increased risk of fragility fractures resulting in significant medical costs, loss of productivity and loss of patient's quality of life. Prevention of such fractures will alleviate a great deal of suffering by the patient and his family. Bone remodeling is characterized by the coupling of osteoclast-mediated bone resorption and osteoblast-mediated new bone formation. Imbalances in this process, wherein resorption outpaces synthesis, result in pathological conditions such as osteoporosis. Established anti-resorptive therapies slow the loss of bone, but do nothing to promote bone growth. Anabolic therapies are vastly underdeveloped and form the greatest clinical need in treatment of ADT-associated osteoporosis. We recently identified Ca^{2+}/calmodulin (CaM)-dependent protein kinase kinase 2 (CaMKK2) to have roles in the anabolic and catabolic pathways of bone remodeling, such that inhibition of CaMKK2 positively affects osteoblasts and negatively affects osteoclasts. Pharmacological inhibition of CaMKK2 using its selective cell-permeable inhibitor STO-609 protects from ovariectomy (OVX)-induced osteoporosis and reverses age-associated bone loss in mice. While CaMKK2 is not expressed in normal prostate tissue, it is highly over-expressed in PCa. CaMKK2 is regulated by the androgen receptor (AR) and its inhibition suppresses the growth and migration of PCa cells in vitro. However, the exact downstream mechanism by which CaMKK2 regulates PCa growth and/or migration remains unknown. Based on these studies, we hypothesized that inhibiting CaMKK2 will prevent ADT-induced bone loss and alleviate bone metastatic tumor burden.To test this hypothesis, we performed either bilateral orchiectomy (ORX) or sham surgery on 4-week old mice, and injected approximately 200,000 C4-2B cells into the proximal right tibia of all mice two weeks following surgery. The mice were divided into 4 groups: (a) sham/saline (n=15), (b) sham/STO-609 (n=15), (c) ORX/saline (n=15) and (d) ORX/STO-609 from day 7 (n=15). Tri-weekly intraperitoneal (i.p.) injections of saline or STO-609 (10 µmol/kg mouse body weight) began one week prior to the surgeries and continued for the duration of the study (12 weeks). Tumor progression was monitored through biweekly radiographic analysis. Right and left proximal tibia were analyzed by micro computed tomography, histology and histomorphometry. Preliminary results indicate that pharmacological inhibition of CaMKK2 was able to prevent ORX-induced bone loss and lessen bone metastatic tumor burden in nude mice.

Translational/Clincal Research ***Research Technician***

POSTER #132

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