MELVIN AND BREN SIMON CANCER CENTER
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POSTER #1

TARGETED INHIBITION OF MAPK AND PI3K SIGNALING PATHWAYS ENHANCES CHEMOTHERAPY RESPONSE IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States. Gemcitabine (Gem) remained the standard of care agent over the past 16 years despite limited clinical benefits. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel, NPT) has shown greater efficacy in combination with gemcitabine against advanced PDAC. Activating K-ras mutations occur at a frequency of up to 90% in this malignancy. As currently no therapeutics exist that can effectively target this oncogene product, alternative strategies therefore focus on inhibition of downstream effectors of KRAS signaling pathways. The RAF-MEK-ERK (MAPK) and the PI3K signaling pathways are well-described mediators of KRAS induced transformation and tumorigenesis. We evaluated combination treatment benefits of nab-paclitaxel plus gemcitabine (NPT+Gem), the current chemotherapeutic standard, with the MEK inhibitor trametinib (Tra) and the AKT inhibitor MK-2206 (MK) to define a novel and more effective therapeutic strategy for PDAC.

In experimental PDAC, nab-paclitaxel has superior antitumor activity compared with gemcitabine or solvent-based taxane docetaxel while a nab-paclitaxel plus gemcitabine combination showed a significant antitumor response. Median animal survival in human intraperitoneal PDAC xenografts in mice revealed that the median survival was 21 days in controls; this was significantly improved by the NPT+Gem combination (35 days, a 67% increase over controls, p=0.0007). Median survival was further increased by addition of MK-2206 or trametinib to NPT+Gem chemotherapy group: NPT+Gem+MK (39 days, a 86% increase over controls, p=0.0003), NPT+Gem+Tra (43 days, a 105% increase over controls, p=0.0003) and NPT+Gem+MK+Tra (48 days, a 129% increase over controls, p=0.002). In human subcutaneous PDAC xenografts, treatment of tumor-bearing mice with NPT+Gem, with and without MK-2206 or trametinib, resulted in significant net tumor growth inhibition. Compared to controls (100±34.8), the percent net local tumor growth in different therapy groups was: NPT+Gem 21.8±5.9 (p=0.003), NPT+Gem+MK 17.2±4.8 (p=0.002), NPT+Gem+Tra 7.1±11.2 (p=0.002) and NPT+Gem+MK+Tra 5.7±9.1 (p=0.001). Mean tumor weight in different therapy groups was as follows: control 0.76±0.07 g, NPT+Gem 0.44±0.06 g, NPT+Gem+MK 0.37±0.07 g, NPT+Gem+Tra 0.3±0.03 g and NPT+Gem+MK+Tra 0.21±0.06 g. These findings suggest that the effects of the current standard chemotherapeutic regimen NPT+Gem can be enhanced through inhibition of MAPK and PI3K signaling pathways, which clinically could relate to improved antitumor results.

Basic Science ***Faculty***

POSTER #2

TARGETING HISTONE DEACETYLASE 6 (HDAC6) DEPRESSES PROLIFERATION AND INDUCES CASPASE-ASSOCIATED CELL DEATH IN GLIOBLASTOMA MULTIFORME (GBM) CELLS AND GBM STEM CELL-LIKE SPHEROIDS

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Glioblastoma multiforme (GBM) comprises the most common and very aggressive form of primary brain tumor with a dismal prognosis and very poor response to the current therapies. HDAC inhibitors (HDACi) are capable of inducing growth arrest and apoptosis in various tumor cell types. HDAC6 is a unique enzyme having two deacetylase domains, and a ubiquitin-binding domain. HDAC6 interacts with a number of proteins in the cytoplasm and is involved in tumorigenesis, cell motility, and metastasis. Significantly, it has been shown that HDAC6 knockout mice are viable. Therefore, in this study, we evaluated HDAC6 as a relevant target for GBM treatment by using a potent HDAC6 inhibitor, CAY 10603. Our data showed thatCAY 10603 targets the established GBM cell line U86MG, M-HBT-161 early primary cultures, and CD133- and SOX2-positive stem cell-like spheroids. CAY 10603 at 1-5 μM triggered significant inhibition of cell survival and induced apoptotic cell death in GBM cells and GBM spheroids. CAY 10603 reduced cell survival in these cells by 50% (IC50) at 1-3 μM treatment for 48 h. Similarly, CAY 10603 induced significant dose-dependent inhibition of spheroid survival in U87MG and M-HBT161 when the spheroids were grown in a defined GBM stem cell (GSC) growth medium on ultra-low attachment plates for four days. Interestingly, when grown in the same medium but on plates which promote attachment, the spheroids were 3-4-fold more sensitive to CAY 10603, indicating that growth conditions affect the sensitivity of GBM stem cell-like spheroids. Moreover, CAY 10603-triggered cell death in U87MG and M-HBT-161 cells as well as CD133/SOX2-expressing spheroids was associated with activation of caspases-3, -6, and -9. Overall, our results show that CAY 10603 robustly inhibits the growth of GBM cells, and is effective in eliminating spheroids that contain GBM cancer stem cells which play a major role in drug resistance and disease recurrence. These results suggest that use of CAY 10603 alone or in combination with other agents may potentially improve the survival of brain tumor patients.

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POSTER #3

INHIBITION OF IL-6 AND IL-11 PREVENTS CACHEXIA IN COLORECTAL CANCER

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Colorectal cancer represents the third leading cause of cancer-related deaths in the United States. A major contributor of morbidity and mortality in colorectal cancer is cachexia, e.g. unintentional body weight loss with skeletal muscle depletion, resulting in 30%-50% of deaths. Cachexia correlates with tumor burden, increased cytokines, higher metabolic rate, poor food intake and reduced response to chemo/radiotherapy. Ultimately, the majority of all colorectal cancer patients showing body and muscle weight loss will succumb to the disease. No therapy is currently available for the treatment of cachexia.

The molecular pathways responsible for this condition are beginning to be unraveled. Available evidence suggests that pro-inflammatory cytokines play a pivotal role. In particular, IL-6 administration is known to cause muscle depletion, while its inhibition ameliorates cachexia. Along this line, we recently reported that the IL-6/STAT3 pathway is up-regulated in the Colon-26 (C26) carcinoma model of cancer cachexia. Particularly, we showed that STAT3 is sufficient and necessary to mediate C26 tumor-associated muscle wasting. However, IL-6 is only one of several ligands that together with specific co-receptors all bind a common signal transducing receptor, gp130, thus inducing STAT3 activation. Among those ligands we showed that IL-11 is also present at very high levels in C26 tumor-bearing mice. Although IL-11 shares gp130 as a signaling mechanism with IL-6, OSM, LIF and CNTF, its biological activities are unique. These include promoting production of hematopoietic progenitor cells, inhibiting adipogenesis, stimulating osteoclast functions and protecting gut mucosa. However, it is still largely unknown whether IL-11 plays a role in cancer-associated muscle wasting.

The aim of this study was to investigate whether IL-11 is responsible for muscle depletion and whether inhibition of IL-11, alone or in association with IL-6, may represent a strategy for the prevention of colon cancer cachexia.

We show that IL-11 is expressed at high levels in several colorectal cancer cell lines, as well as in many human colorectal tumors. Moreover, muscle from patients with colorectal cancer and muscle wasting showed elevated IL-11 protein. Functionally IL-11, like IL-6, induced C2C12 myotube atrophy *in vitro* and increased STAT3 phosphorylation in a time- and dose-dependent manner. *In vivo* AAV-IL-11 produced severe cachexia, with muscle, fat and bone depletion, splenomegaly and reduced muscle force. Administration of anti-IL-11 neutralizing antibody in C26-bearing mice resulted in a partial rescue of skeletal muscle mass compared to the IgG1-treated hosts, while the combined treatment with anti-IL-6 neutralizing antibody completely rescued C26 cachexia.

Thus we identify IL-11 as a new mediator of muscle wasting in cachexia and suggest that neutralization of IL-11 and IL-6 might represent a powerful therapeutic strategy for the prevention of cachexia in colorectal cancer.

Basic Science ***Faculty***

POSTER #4

TGF-BETA PROMOTES ANGIOGENESIS IN AN RB-DEFICIENT, KRAS-DRIVEN MOUSE MODEL OF PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease that is projected to become the 2nd leading cause of cancer deaths by 2030. PDACs are associated with a high frequency of *KRAS* mutations (95%) and overexpression of many pro-angiogenic cytokines and growth factors. Using a genetically engineered mouse model (GEMM) that we established in which oncogenic Kras is combined with loss of RB (KRC), we have determined that PDACs arising in these mice (mPDACs) harbor many endothelial cells (ECs) and sinusoidal-like vessels that have blood flow, as determined by intravital confocal microscopy. Array analysis of pancreatic cancer cells (PCCs) derived from KRC tumors revealed gene expression profiles that correlate with active TGF-b signaling pathways, as determined by gene set enrichment analysis (GSEA), and include increased expression of multiple pro-angiogenic genes. *In silico* analysis indicated that many of these pro-angiogenic genes were TGF-b targets, and inhibition of the type I TGF-b receptor (TbRI) with SB505124 confirmed that TGF-bs drive pro-angiogenic gene expression in KRC PCCs. Moreover, TGF-b increased the levels of pro-angiogenic cytokines in conditioned media (CM) prepared from KRC PCCs, which when added to ECs, activated canonical TGF-b signaling pathways and stimulated EC proliferation and migration. Although SB505124 blocked TGF-b pathway activation in ECs, it failed to suppress CM-enhanced EC proliferation and migration. By contrast, SB505124 attenuated tumor angiogenesis, growth and metastasis in a syngeneic orthotopic mouse model using KRC PCCs. Taken together, these results suggest that TGF-b promotes angiogenesis in an indirect manner, by up-regulating pro-angiogenic factors in PCCs that act on ECs in a paracrine manner. Therefore, targeting TGF-b in PDAC could be a beneficial anti-angiogenic strategy.

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POSTER #5

INVESTIGATING THE ROLE OF BRCA1 IN SENSITIVITY TO IMETELSTAT, A TELOMERASE TEMPLATE ANTAGONIST, IN BREAST CANCER CELLS

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BRCA1 is a tumor suppressor gene with a variety of functions related to safeguarding genomic integrity. Regulation of telomere length is crucial in maintaining genomic stability, with critically short telomeres leading to telomere uncapping, end-to-end fusions, activation of the DNA damage response, and cell cycle arrest. Recent evidence suggests knockdown of BRCA1 in cell lines increases both telomerase activity and average telomere length, but likely results in more unstable telomeres compared to BRCA1 wild-type cell lines. The objective of this study was to determine whether imtelstat (GRN163L), a telomerase template antagonist currently in clinical trials, has enhanced activity in BRCA1 mutant breast or ovarian cancer cell lines compared to paired BRCA1 wild-type breast/ovarian cancer cell lines. In addition, we sought to determine whether BRCA1 cell lines have shorter average telomere lengths than other cancer cell lines, making them more amenable to inhibition of telomerase activity. We utilized a panel of breast and ovarian cancer cell lines harboring both N-terminal and C-terminal BRCA1 mutations, in addition to matched cells expressing exogenous wild-type BRCA1, and assessed the effects of imtelstat or a mismatch oligonucleotide on telomerase activity and telomere length using the TRAP (Telomere Repeat Amplification Protocol) and TeloTAGGG assays, respectively. All cell lines tested exhibited a dose-dependent response to treatment with imtelstat, but not a mismatch control oligonucleotide. In addition, we observed differential sensitivity between BRCA1 mutant and BRCA1 wild-type cell lines tested to clinically relevant concentrations of imtelstat in both TRAP and clonogenic survival assays. Of interest, the majority of the cell lines tested showed gross morphological changes as early as 12 hours following inhibitor treatment. We found UWB1.289 cells have a shorter average telomere length at baseline compared to their BRCA1 wild-type counterpart cells, and saw progressive telomere shortening over a three-week period of treatment with imtelstat, but not with mismatch oligonucleotide. Furthermore, long-term (12 week) treatment with GRN163L preferentially induced complete cell death in the HCC1937 (BRCA1 mutant) breast cancer cell population compared to the HCC1937 cells reconstituted with wild-type BRCA1. Finally, we demonstrate that pretreatment with GRN163L acts synergistically with cis-platin in UWB1.289 and UWB1.289+BRCA1 ovarian cancer cells. In summary, this work provides insight into the role of BRCA1 mutations in cancer cells and their impact on sensitivity to the cellular effects of telomerase antagonism.

Basic Science ***Faculty***

POSTER #6

INDIANA CENTER FOR BIOLOGICAL MICROSCOPY

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The Indiana Center for Biological Microscopy, "ICBM" has been providing imaging capabilities to Indiana University Researchers since 1996. In 2002 the ICBM has become the NIDDK George O\'Brien Center for Advanced Microscopic Analysis, whose objective is to develop methods of intravital multiphoton microscopy for renal research. For the last few years the Center has been providing an intravital microscopy service for Cancer Center investigators through the Optical Microscopy Core for the CEMH.

We are organized around the principle of providing researchers with access to and training on the latest instruments in optical microscopy. We emphasize hands-on access to instruments for individual investigators and unparalleled assistance from on-staff imaging experts. In addition to providing efficient, state-of-the-art biomedical imaging support, the center is also actively involved in biological imaging research and the development of new methods of microscopy and digital image analysis software.

The ICBM imaging facility is located in Research II Building (second floor) of IUPUI campus and is available to anyone at the Indiana University and researchers from outside the institution.

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POSTER #7

ROLE OF PRL2 PHOSPHATASE IN NORMAL AND MALIGNANT T-LYMPHOPOIESIS

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The phosphatase of regenerating liver (PRL) family of phosphatases, consisting of PRL1, PRL2, and PRL3, represents an intriguing group of proteins being validated as biomarkers and therapeutic targets in human cancer. We have been investigating the role of PRL2 in normal / malignant hematopoiesis and found that PRL2 is important for HSC self-renewal (Kobayashi. 2014). The receptor tyrosine kinase KIT can balance quiescence for HSC maintenance and proliferation for progeny supply. The defects seen in the PRL2-deficiency, mainly hematopoietic and testis cells, recapitulate the phenotype of c-Kit mutant mice, suggesting that the SCF/KIT signaling may be impaired in the absence of PRL2 (Kobayashi. 2014; Dong. 2013). Given that KIT also plays critical role in maintaining postnatal T-lymphopoiesis in thymus, we hypothesized that PRL2 is important for T cell development. Here we report a functional requirement for PRL2 in T cell development. PRL2 is highly expressed in early thymic progenitors. PRL2 deficiency resulted in around 50% reduction of splenocyte and thymocyte count. T-cell reconstitution from *Prl2* null HSCs was significantly reduced, showing near 20 times less chimerism in thymus and spleen of post-transplant recipients. Also early T-progenitor (ETP) to DN2 transition was disturbed in recipients repopulated with *Prl2* null HSCs. *Prl2* null HSPCs also show impaired T-cell differentiation *in vitro*. We found that Notch/RBPJ signaling upregulated PRL2 as well as c-Kit expression in early stage T cell progenitors. We further discovered that PRL2 was an important mediator of SCF/c-Kit signaling. PRL2 null ETP leads to failure of sustaining c-Kit and reduce its signal activity. Furthermore, PRL2 null ETP showed reduced Tcf-1 and Bcl-2 those are important for T-cell specification and survival. Thus, we have identified a critical role for PRL2 phosphatase in corporation with Notch and c-Kit signaling in early T lineage progenitors.

As Notch-induced T-cell differentiation was disturbed, we also expected PRL2 was involved in T-lymphoid leukemogenesis. When constitutive active Notch was introduced into *PRL2-WT* or *-null* BM cells, followed by transplantation into recipient mice, T-leukemia development was dramatically impaired in the recipient mice repopulated with *PRL2-null* BM cells. Our data clearly indicates PRL2 is important for both normal and malignant T-lymphopoiesis and inhibition of PRL2 is the potential therapeutic target.

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POSTER #8

GENE EXPRESSION ANALYSIS OF PRE-MALIGNANT/HISTOLOGICALLY NORMAL BREAST EPITHELIUM TO UNMASK EARLY MOLECULAR ALTERATIONS IN BREAST CANCER INITIATION.

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Despite significant advances in diagnosis and treatment, breast cancer remains the leading cause of cancer-related death in women worldwide. The failure in eradicating this disease is largely due to the lack of identification of the molecular mechanisms responsible for cancer initiation and progression. While substantial information is available about the molecular alterations characterizing the tumor, current understanding of events that initiate or predispose to breast cancer is limited.

In this study we focus our analysis on pre-neoplastic, histologically normal breast tissue which presumably contains the early molecular alterations leading to cancer development. These specimens have been labeled as "susceptible normal" breast tissues to distinguish them from the pre-malignant lesions (Atypical hyperplasia and ductal carcinoma in situ) which already show histologic abnormalities. The "susceptible normal" tissues, obtained from the Susan G. Komen Tissue Bank at the IU Simon Cancer Center (KTB), were indeed donated by women diagnosed with breast cancer 1-3 years after their normal tissue donation.

To identify the early molecular lesions and pathway alterations, we will compare the expression profiles of "susceptible normal" and healthy control breast tissues matched for age, race, menopausal status and menstrual phase. As the ductal epithelium is considered the origin of breast cancer, we laser microdissect all specimens to enrich for epithelial RNA and minimize signaling from stroma. Gene expression profiles are obtained using next generation RNA-sequencing.

In addition, given the functional interaction between epithelial and stroma compartments, the microenvironment components of the tissue, stroma and fat are independently microdissected and their gene expression profiles investigated.

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POSTER #9

ACTIVIN RECEPTOR II B BLOCKADE PRESERVES SKELETAL MUSCLE IN A MOUSE MODEL OF ORTHOTOPIC PANCREATIC CANCER INDUCED-CACHEXIA

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Rationale

Pancreatic cancer is the fourth leading cause of cancer death in the US.Cachexia, progressive loss of fat and muscle mass despite adequate nutrition, is a devastating complication of pancreatic cancer associated with increased mortality. The Transforming Growth Factor-B (TGF-B) signaling pathway affects the skeletal muscle anabolic/catabolic balance and includes ligands such as Activin A and myostatin that negatively regulate muscle size by activating Activin Receptor IIB (Acvr2b). Soluble Acvr2b/Fc fusion protein increases muscle mass and strength in normal mice and mice with other types of cancer. Here we tested whether Acvr2b/Fc might influence cancer cachexia and survival in a model of pancreatic cancer.

Materials and Methods

Acvr2b/Fc was purified from CHO conditioned media. KPC32043 cells, a gift of David Tuveson, were derived from pancreatic ductal adenocarcinoma tumors arising in LSL-KrasG12D/+/LSL-Trp53R172H/+;Pdx1-Cre mice. Sixty-six 10-week old C57BL/6J male mice (Jax) were used. Sixty mice were injected with 500,000 KPC cells in 40µL PBS directly into the pancreas and randomly assigned to receive PBS (n=30)or Acvr2b/Fc (10mg/kg i.p.) (n=30) every 5 days. Six mice underwent sham surgery. Body weights, body composition, functional tests (ambulatory activity, hanging wire test), body condition and behavioral scoring were used to assess muscle strength and disease progression.

A subset of mice was euthanized for tissue collection on day 21. Organ and muscle mass and morphometry were measured. QPCR measured gene expression in quadriceps. Results were analyzed with Student's t-test or one-way ANOVA with Tukey's Multiple Comparisons test. The reminder of the mice (n=20 PBS and n=20 Acvr2b/Fc) were assessed for survival.

Results

PBS-treated tumor-bearing mice lost weight while Acvr2b/Fc-treated mice maintained their weight. By day 21, body weight was reduced by ~16% in PBS-treated mice but unchanged in Acvr2b/Fc mice. Skeletal muscle mass and fiber size were decreased by ~30% in PBS-treated mice compared with sham ($p<0.001$). Muscle mass was completely preserved in mice injected with Acvr2b/Fc. The tumor induced expression of TGF-B superfamily members and atrogenes in the quadriceps, the latter of which were inhibited by Acvr2b/Fc. PBS-treated mice lost 60% of their epididymal fat pad compared with sham ($p<0.0001$). Acvr2b/Fc only partially rescued fat loss (-35%). Reduction inambulatoryactivity was more pronounced in PBS-treated mice than in mice injected with Acvr2b/Fc by day 24. Acvr2b/Fc-treated mice performed significantly better at the wire-hang test compared with PBS mice. Body condition and behavioral scoring was higher in Acvr2b/Fc-treated mice than in PBS mice by day 24 indicating better health. Acvr2b/Fc prolonged survival by 4.5 days in tumor-bearing mice ($p<0.05$).

Conclusions

Acvr2b/Fc preserves muscle mass and prolongs function and survival in murine orthotopic pancreatic cancer cachexia. Our study suggests that targeting this pathway might be an effective approach for muscle preservation in pancreatic cancer cachexia.

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POSTER #10

BONE PARAMETERS ARE UNCHANGED BY ACTIVATION OR DELETION OF TGF-B SIGNALING IN MATURE OSTEOCLASTS

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Calcified bone matrix is the most abundant source of transforming growth factor beta (TGF-B) in the body. TGF-B that is released and activated during osteoclast-mediated bone resorption can act locally to influence bone remodeling or enter the circulation to exert systemic effects. In the setting of cancer bone metastasis, high TGF-B levels induced by increased osteolysis play a key role in the feed-forward cycle of tumor progression in bone. In addition to its activity on tumor cells, TGF-B in the microenvironment may also signal directly to bone cells to modulate their differentiation and activity. TGF-B has been shown to increase osteoclast differentiation, but whether TGF-B signals directly to mature osteoclasts to modulate their activity or survival, and thus impact bone mass *in vivo*, is not known.To test the importance of TGF-B signaling in mature osteoclasts, we targeted the pathway using transgenic cathepsin K (CatK)-cre mice to either delete TGFBR2 (CatK-cre; TBR2 flox/flox) or induce the expression of constitutively active TGFBR1-T204D (CatK-cre; TBR1-T204D). We monitored the bone phenotype of both male and female mice ($N \geq 11$ per group) *in vivo* using micro-computed tomography and dual-energy X-ray absorptiometry at regular intervals from 6 weeks to 6 months of age. Neither deletion nor activation of canonical TGF-B signaling in mature CatK-positive osteoclasts had any profound effect on trabecular or cortical bone parameters or bone mineral density *in vivo*. Likewise, *ex vivo* analyses, including bone marrow cell differentiation assays, failed to indicate any differences between control mice and those with altered TGF-B signaling in mature osteoclasts. Therefore, we conclude that any direct effects of TGF-B signaling in the osteoclast lineage likely occur earlier during the differentiation process and that normal bone homeostasis may not rely on TGF-B signaling in mature osteoclasts.

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POSTER #11

INHIBITION OF MDM2 AND AKT SIGNALING NETWORKS SYNERGIZE TO ACTIVATE FORKHEAD BOX O-CLASS TRANSCRIPTION FACTORS AND PROMOTE CELL DEATH IN MUTANT P53 GBM CELLS

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A multi-targeted approach will be necessary to eradicate glioblastoma multiforme (GBM) cells due to the immense genetic heterogeneity associated with GBM. Mouse double minute-2 (MDM2) regulates multiple signaling pathways and is a promising therapeutic target in GBM. In wild type (wt) p53 cells, MDM2 binds to wtp53, ubiquitinates it, and negatively regulates p53-mediated downstream events. In wtp53 and mutant (mt) p53 cells, MDM2 binds to and sequesters p73a, thereby blocking p73a-mediated signaling. Our objective in the present studies was to determine if the p73a-MDM2 axis could be exploited to increase death of mtp53 GBM cells. We utilized MDM2 antagonists nutlin3a or RG7112 to block protein-protein interactions between MDM2-p53 and MDM2-p73a. In a panel of GBM cell lines, TMZ resistance was reduced in both wt53 and mt53 cells in the presence of MDM2 antagonists. In mtp53 cells, siRNA knockdown of p73a indicated that sensitivity to treatment was dependent on p73a levels. Isobologram analysis indicated that while dose-ratios of TMZ to MDM2 antagonists were additive to synergistic in inhibiting growth of wtp53 GBM cells, this was not the case in mtp53 GBM cells (SF118, GBM43, gain-of-function-mtp53 R273H U373 and MHBT32). Analysis of intracellular targets in mtp53 GBM cells exposed to TMZ and MDM2 antagonists indicated that p73a and MDM2 expression increased by 24 hours post-treatment. In addition, AKT activity was increased or sustained in mtp53 GBM cells following treatment with TMZ in the absence or presence of MDM2 antagonists. Since increased AKT activity may render cells resistant to therapy, the AKT inhibitor GDC0068 was evaluated in combination with TMZ and RG7112. As a measure of AKT-downstream target modulation, phosphorylation status of the Forkhead box O-class (FoxO) transcription factors (TFs) was determined. In the non-phosphorylated state, FoxO TFs upregulate expression of proteins involved in cell-death pathways. While phospho-FoxO1/FoxO3a TFs were increased in TMZ/RG7112-treated mtp53 GBM cells compared to controls, it was decreased in GDC0068-, TMZ/GDC0068- and TMZ/RG7112/GDC0068-treated mtp53 GBM cells which is consistent with inactivation of AKT and activation of FoxO TFs. Isobologram analysis of mtp53 GBM cell growth indicated that combination RG7112 and GDC0068 inhibited growth in a synergistic manner even in the absence of TMZ. For *in vivo* studies, an intermittent dosing regimen of TMZ/RG7112/GDC0068 was developed to avoid normal tissue toxicity. GBM43 flank tumor growth was significantly inhibited in mice with tumors treated with RG7112/GDC0068 and inhibited to a larger extent by the triple combination TMZ/RG7112/GDC0068 compared to vehicle and single-agent exposure (n=9-10 mice per group; single agent vs GDC0068/RG7112 or TMZ/RG7112/GDC0068, $p<0.05$). The present data indicate that targeting the p73α-MDM2 and AKT-FoxO signaling networks inhibit mtp53 GBM cell growth and with an appropriate dosing schedule can be utilized *in vivo* with an acceptable toxicity profile.

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POSTER #12

THE PRESENCE OF TELOMERE FUSION IN SPORADIC COLON CANCER INDEPENDENTLY OF TP53/KRAS MUTATION STATUS, MEAN TELOMERE LENGTH, AND TELOMERASE ACTIVITY

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Defects in telomere maintenance can result in telomere fusions that likely play a causative role in carcinogenesis by promoting genomic instability. However, this proposition remains to be fully understood in human colon carcinogenesis. In the present study, the temporal sequence of telomere dysfunction dynamics was delineated by analyzing telomere fusion, telomere length, telomerase activity, and hotspot mutations in *KRAS* or *BRAF*, and *TP53* of tissue samples obtained from 18 colon cancer patients. Our results revealed that both the deficiency of p53 and the shortening of mean telomere length were not dependent upon producing telomere fusions in colon tissue. In a few cases, telomere fusion and/or DNA aneuploidy were observed even in tissue adjacent to cancerous lesions, suggesting that genomic instability is initiated in pathologically non-cancerous lesions. The extent of mean telomere attrition increased with lymph node invasiveness of tumors, implying that mean telomere shortening correlates with colon cancer progression. Telomerase activity was relatively higher in most cancer tissues containing mutation(s) in *KRAS* or *BRAF* and/or *TP53* compared to those without these hotspot mutations, suggesting that telomerase could become fully active at the late stage of colon cancer development. Interestingly, the majority of telomere fusion junctions in colon cancer appeared to be a chromatid-type containing chromosome 7q or 12q. In sum, this meticulous correlative study not only highlights the concept that telomere fusion is present in the early stages of cancer regardless of *TP53/KRAS* mutation status, mean telomere length, and telomerase activity, but also provides additional insights targeting key telomere fusion junctions which may have significant implications for colon cancer diagnoses.

Basic Science ***Faculty***

POSTER #13

MUSCLE WEAKNESS IS ASSOCIATED WITH OSTEOLYSIS IN MULTIPLE MYELOMA

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Osteolytic bone destruction affects greater than 80% of patients with multiple myeloma leading to vertebral compression fractures, pathologic fractures that require surgical repair, pain and hypercalcemia.

Using a model of breast cancer metastatic to bone (MDA-MB-231 cells) we have shown significant muscle dysfunction associated with osteolysis. In this model, an increase in muscle weakness correlates with an increase in bone destruction. Since severe osteolysis is a prominent clinical finding in patients with multiple myeloma we hypothesized that bone destruction due to multiple myeloma could cause skeletal muscle weakness. We tested this in a model of human multiple myeloma (JJN-3 cells), which causes severe osteolysis but does not cause cachexia.

SCID mice were inoculated with JJN-3 cells directly into the left tibia. Four weeks later X-ray analysis showed significant bone destruction in the tumor-inoculated limb whereas there were no visible lesions in the contralateral (right) limb. The myeloma bearing mice do not lose body weight or hindlimb muscle weight compared to saline injected controls. *Ex vivo* contractility of the extensor digitorum longus (EDL) muscle from the unaffected contralateral limb showed a significant reduction in maximum specific force (corrected for muscle size) compared to control saline injected mice ($311.5 kN/m^2 \pm 27.5$ v. $358.3 kN/m^2 \pm 8.6$; $p<0.001$). There was a significant correlation ($p<0.05$) between increased bone destruction and decreased muscle function in the contralateral limb. These data show that systemic muscle weakness occurs in multiple myeloma that is not associated with a decrease in muscle weight and suggests that the associated bone destruction is an important contributing factor.

We have previously found that bone metastases due to breast cancer are associated with oxidative overload and oxidation of muscle proteins (e.g. sarcomeric proteins tropomyosin/myosin and excitation-contraction coupling proteins, including the sarcoplasmic reticulum calcium release channel RyR1). Oxidation of RyR1 causes depletion of the stabilizing subunit calstabin1 resulting in intracellular calcium leak and muscle weakness. In this study, we found that the contralateral EDL from mice with multiple myeloma showed an increase in RyR1 oxidation and reduction in calstabin1 binding. These data suggest that, as in breast cancer metastatic to bone, intracellular calcium leak via oxidized RyR1 channels contributes to muscle weakness in multiple myeloma and that bone osteolysis plays an important role.

Basic Science ***Faculty***

POSTER #14

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 mitotic high-fidelity chromosome segregation. However, the relative contribution of interphase and mitotic events leading to mutagenesis upon loss of FA signaling has not been quantified. Here, we dissect the origins of genomic instability in FA-deficient cells *ex vivo* and *in vivo*. We found increased *in vivo* chromosome segregation errors during human FANCA-/- and murine *Fancc-/-* hematopoiesis. Quantitative micronucleus assays revealed that both interphase DNA damage and mitotic errors significantly contribute to genomic instability in FA-deficient human CD34+ hematopoietic cells and other tissue types. Functional studies of primary FA patient cells coupled with superresolution microscopy showed that FANCA shuttles to the pericentriolar layer during centrosome maturation to regulate spindle assembly during cell division, providing a mechanistic link between loss of FANCA and mitotic chromosome instability. Finally, we show that interphase and mitotic chemotherapeutics challenge distinct FANCA-dependent cell cycle checkpoints. Collectively, our findings lay foundation for precision medicine strategies against cancers driven by FA deficiency.

Basic Science ***Graduate Student***

POSTER #15

INHIBITION OF EZH2 OVERCOMES RESISTANCE TO SUNITINIB IN CLEAR CELL RENAL CELL CARCINOMA MODELS

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Background: Alterations in epigenetic mechanisms including histone modification and hyper-methylation at gene promoter regions have been implicated as mechanisms of drug resistance in cancer. Alternation of epigenetic regulators such histone methyltransferase, EZH2, has been reported in numerous cancer types including advanced renal cell carcinoma (RCC). Previous studies suggest that sunitinib may have a direct anti-tumor effect and that acquired sunitinib resistance may be induced in tumor cells rather than just in endothelial cells. In our study, we investigated the role of EZH2 in sunitinib resistance in clear cell renal cell carcinoma. Methods: Human RCC cell lines 786-0 were treated and exposed to increasing concentrations of sunitinib to develop a resistant cell line, 786-0R. Parental and resistant cell lines were treated with either sunitinib, GSK126 (EZH2 inhibitor) or both. In parallel, EZH2 was knocked down in 786-0 cells and exposed to increasing concentrations of sunitinib. Cell viability was quantitated by absorbance of crystal violet stained cells using a spectrometer at 570nm. In a second set of experiments, control and treated cells were collected for western analysis. Mice bearing human ccRCC patient derived xenograft (PDXs); RP-R-01, RP-R-02 and RP-R-02LM (a metastatic ccRCC model established from RP-R-02) were implanted into SCID mice. When tumors reached an average volume of 50mm3, mice were randomly grouped into 2 arms; control, sunitinib treatment (40mg/kg, 5days/week) or EZH2i compound2 (500mg/kg, 2x/day, 5days/week). Tumors volumes and body weight were assessed once per week. Tumor tissues and lungs were collected for immunohistochemistry analysis. All assessments and quantification were done blindly. Results: Our in vitro and in vivo data showed an increased expression of EZH2 with resistance to sunitinib. Furthermore, inhibition of EZH2 in our in vitro and in vivo studies correlated with a significant increase in the anti-tumor effect of sunitinib in both parental and resistant cell lines. Conclusion: Overall our data suggest the potential role of epigenetic alterations, specifically EZH2 overexpression and its association with resistance to sunitinib.

Basic Science ***Graduate Student***

POSTER #16

ANTI-TUMOR AND ANTI-METASTATIC EFFECT OF SUNITINIB IN A PATIENT DERIVED METASTATIC CLEAR CELL RENAL CELL CARCINOMA XENOGRAFT MODEL

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Background: Sunitinib is considered first-line therapy for patient with advanced renal cell carcinoma. The mechanism by which sunitinib harnesses angiogenesis is by targeting receptors of pro-angiogenic growth factors such as VEGF, PDGF and kit. Recently, it has been speculated that sunitinib may have a direct anti-tumor effect. Our previous studies have shown that tumors resistant to sunitinib still present decreased tumor vasculature compared to untreated tumors, suggesting that sunitinib anti-tumor effect may be in part independent from its anti-angiogenic effect. Hence, we wanted to further investigate the anti-tumor effect of sunitinib in human renal cell carcinoma (RCC) cell lines and a patient derived clear cell RCC xenograft model. In addition, we examined the effect of sunitinib on early and late stage metastasis in a spontaneous metastatic human clear cell renal cell carcinoma model. Methods: Human ccRCC PDX RP-R-02 (skin metastasis isolated from patient with hereditary ccRCC; VHL syndrome) was implanted into SCID mice subcutaneously. Parental RP-R-02 was then implanted orthotopically in male SCID mice to develop a spontaneous metastatic ccRCC model, RP-R-02LM. RP-R-02LM tumor bearing mice were treated with sunitinib (40mg/kg; 5days/week). Tumor volume and body weights were assessed weekly. Tumor tissues and lungs were collected for immunohistochemistry analysis. In parallel, human RCC cell lines were treated in vitro with varying concentrations of sunitinib for 24, 48 and 72 hours. Then, cells were stained with crystal violet, fixed and absorbance was read to quantitate viable cells. Results: Human RCC cells lines treated in vitro with sunitinib at pharmacological achievable concentrations showed a decrease in cell proliferation, suggesting a direct anti-tumor effect of sunitinib in RCC. RP-R-02LM but not parental RP-R-02 implanted either subcutaneous or orthotopically in the kidney, spontaneously metastasize to the lungs and closely mimics what is seen in the clinic. PCR results indicated that both xenograft still maintain human Alu sequence. In our in vivo system, RP-R-02LM treated with sunitinib had no anti-tumor effect on tumor at the primary site; however, we observed an inhibition of dissemination to the metastatic lung site as indicated by significantly low numbers of metastasis compared to the controls. In addition, immunohistochemical analysis showed decrease in tumor vasculature with sunitinib treatment compared to the control as indicated by CD31 staining, with increased tumor cell proliferations as indicated by ki67. Conclusion: Our studies suggest that sunitinib has a direct anti-tumor in vitro and eventually tumor cells acquire drug resistance. Also, data from our in vivo studies show that tumors resistant to sunitinib have decreased vessel density as compared to the untreated tumors, suggesting that sunitinib is still a potent anti-angiogenic agent. In addition, we show the anti-metastatic effect of sunitinib in a spontaneous metastatic human clear cell renal cell carcinoma. Overall our data suggest that sunitinib maintains an anti-metastatic effect in sunitinib resistant tumor bearing animals that is independent from its anti-angiogenic effect

Basic Science ***Graduate Student***

POSTER #17

ROLE OF PDGF-D IN GEMCITABINE RESISTANT PDAC

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Pancreatic ductal adenocarcinoma (PDAC) patients have a 5-year survival rate of just 7%, largely due to intrinsic and acquired resistance to gemcitabine, the first line treatment for PDAC. The mechanism of gemcitabine resistance still remains largely unknown, creating a problem in successful treatment of PDAC. Recently, we found that gemcitabine resistant PDAC cell line, G3K derived from the parental MiaPaCa-2 cells, had epigenetic changes due to alteration of Uhrf1 and DNMT1 expression (Mol Pharmacol. 2014 Nov;86(5):561-9). Global profiling analysis of gene methylation revealed 66 genes with methylation change that are consistent with gemcitabine resistance. One of these genes, PDGFD, was validated to have increased expression in the gemcitabine resistant G3K cells compared with the parental MiaPaCa-2 cells and confirmed in another gemcitabine resistant PDAC cell line FgR, derived from the parental Fg cells. PDGF-D is known to activate MAP, Akt, mTOR, NF-KB, Notch, and STAT3 via binding to its receptor, PDGFR-B, a receptor tyrosine kinase. In this study the role of PDGF-D in gemcitabine resistant PDAC was evaluated and we found that PDGF-D overexpression may contribute to acquired gemcitabine resistance by binding to and activating PDGFR-B, leading to STAT3 activation.

Basic Science ***Graduate Student***

POSTER #18

DETERMINING THE ROLE OF TUMOR SUPPRESSOR ETS PROTEINS IN PROSTATE CANCER

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Misregulation of various members of the ETS family of transcription factors has been shown to track with tumor formation and progression in thyroid, pancreatic, liver, prostate, colon, lung, and breast carcinomas. Four members of the ETS family (ERG, ETV1, ETV4, and ETV5) have been shown to undergo chromosomal rearrangements in prostate tissue that lead to their aberrant overexpression and the formation of prostate tumors. These transcription factors activate cellular programs involved in epithelial to mesenchymal transition (EMT), angiogenesis, and cell survival. 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. Three ETS family members (EHF, SPDEF, and ETS2) are normally expressed within prostate tissue, have been implicated as tumor suppressors, and have been shown to regulate genes involved in EMT and cancer stem cells through transcriptomic analyses. The exact mechanism through which these tumor suppressors regulate these pathways is not understood since genomic binding analyses have not been performed for these transcription factors. They could be working in direct opposition to the oncogenic ETS by regulating gene expression in an opposite fashion at the same sites, or they could bind to unique sites and through these sites suppress the formation of tumors. Genomic analyses, functional assays, and bioinformatic analyses along with data freely available from Encode, JASPAR, and TCGA will be combined to gain insight into the similarities and differences between suppressive and oncogenic ETS. By better understanding the mechanisms of how these transcription factors work it might be possible to find ways to inhibit the formation or progression of prostate tumors.

Basic Science ***Graduate Student***

POSTER #19

CRYSTAL STRUCTURES OF THE SETMAR DNA-BINDING DOMAIN BOUND TO DNA

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SETMAR (also called Metnase) arose through the fusion of a *mariner*-family DNA transposase gene, *Hsmar1,* downstreamof a lysine methyltransferase gene, SET, approximately 50 million years ago (1). Although the SETMAR retains the ability to bind the terminal inverted repeat (TIR) DNA, it is no longer a functional transposase. In the human genome, the *SETMAR* gene is localized on chromosome 3p26, which is a region of frequent abnormalities in various cancers, such as leukemia and breast cancer (2). *SETMAR* is expressed in most human tissues (3) but is overexpressed in acute myeloid leukemia (AML) patient samples as compared to healthy individuals (4). Previous studies showed that SETMAR is able to enhance chromosome decatenation in AML by promoting activity of Topo IIα-assisted decatenation. Meanwhile, SETMAR has been shown to contribute resistance to TOPO II inhibition by etoposide, which is widely used in cancer treatment (5). SETMAR has also been reported to facilitate restart of stalled replication forks (6) and integration of foreign DNA into the host chromosome (3, 7). However, little is known about the molecular mechanism of DNA binding activity of SETMAR. Determining how SETMAR interacts with DNA is central to understanding the molecular basis of its biological functions in humans. Here, we present a 2.37 Å crystal structure of the SETMAR DNA-binding domain (DBD) bound to TIR-DNA and a 3.06 Å structure of the DBD bound to non-TIR-DNA. The structures reveal a bipartite interaction of two helix-turn-helix (HTH) motifs with sequence specific major groove recognition joined by a linking region that recognizes the minor groove. Surprisingly, a large number of the interactions with TIR and non-TIR DNA are conserved. The structures along with DNA-binding studies provide a basis for understanding how SETMAR recognizes both its ancestral TIR DNA as well as non-TIR sequences and will guide future studies designed to probe the functional role of SETMAR's DNA-binding activity in both normal and cancer cells.

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Basic Science ***Graduate Student***

POSTER #20

MUTANT P53 ENHANCES HEMATOPOIETIC STEM CELL SELF-RENEWAL THROUGH REGULATING EPIGENETIC PATHWAYS

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Acute myeloid leukemia (AML) is an aggressive blood cancer with poor prognosis. Several studies have demonstrated that leukemia evolves from hematopoietic stem cells (HSCs) that acquire preleukemic mutations, allowing clonal expansion and subsequent acquisition of mutations leading to cancer. The tumor suppressor p53 is a critical regulator of HSC behavior, and we demonstrated that p53 maintains HSC quiescence and regulates HSC response to irradiation. While *TP53* mutations are less common in AML (10%), they are associated with shorter survival rates and drug resistance. Recently, clinical analysis found healthy individual with *TP53* mutation in his blood cells progressed to AML with further acquisition of other lesions, indicating that *TP53* mutations may be preleukemic mutations that play an important role in the evolution of leukemia. Therefore, it is important to investigate the role of mutant p53 in regulating normal HSC function.

To define the role of mutant p53 in the AML, we screened 9 hot-spot p53 mutants identified in AML patients and found that hematopoietic cells expressing $p53^{R248W}$, $p53^{Y220C}$ or $p53^{R273H}$ show enhanced repopulating potential following transplantation. As codon 248 of the p53 is most frequently mutated in AML, we examined HSC behavior in the humanized $p53^{R248W}$ knock-in mice. In p53 knockout mice, there is a dramatic increase of HSCs; however, we found that both wild type and $p53^{R248W}$ mice have similar number of HSCs. While wild type p53 maintains HSC quiescence, expression of $p53^{R248W}$ in HSCs does not affect their quiescent state. Moreover, we found that repopulating ability of $p53^{R248W}$ HSCs was significantly higher than that of the wild type HSCs, demonstrating that the $p53^{R248W}$ enhances HSC self-renewal *in vivo*.

p53 is an important mediator of the genotoxic stress response and we next want to investigate whether HSCs expressing mutant p53 have a competitive advantage under stress conditions. We found that mutant HSCs show significantly higher repopulating ability in a competitive transplantation following 2.5Gy total body irradiation or 5-FU administration demonstrating that mutant p53 protects HSCs from genotoxic stress induced by irradiation or chemotherapy.

To understand how mutant p53 enhances HSC self-renewal, we performed gene expression profiling assays by using HSCs isolated from wild type and $p53^{R248W}$ mice. While we did not observe change in the expression of p53 target genes in $p53^{R248W}$ HSCs, epigenetic pathways that are important for HSC self-renewal and leukemogenesis were altered in HSCs expressing $p53^{R248W}$, demonstrating that $p53^{R248W}$ is a gain-of-function mutant.

Collectively, we demonstrated that mutant p53 enhances HSC self-renewal through regulation of epigenetic pathways. Furthermore, we discovered that HSCs expressing mutant p53 harbor a competitive advantage both in steady state and under stress conditions compared to normal HSCs, implicating *TP53* mutations may be early events in the evolution of leukemia.

Basic Science ***Graduate Student***

POSTER #21

TACKLING ANTI-ANGIOGENIC THERAPY FAILURE IN PANCREATIC DUCTAL ADENOCARCINOMA

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Angiogenesis, the sprouting of new blood vessels from existing vessels, is a process exploited by many cancers for tumor growth. Pancreatic ductal adenocarcinoma (PDAC), the 4th leading cause of cancer death in the US, is known to overexpress a number of angiogenic growth factors and receptors, and anti-angiogenic agents result in reductions in tumor volume, tumor spread, and microvessel density, and lead to improvements in survival in subcutaneous and orthotopic nude mouse models of human PDAC (hPDAC). However, clinical trials using anti-angiogenic therapy have been overwhelmingly unsuccessful. We hypothesized that this could be due to incorrect patient selection and/or inappropriate pre-clinical models.

To identify patients that might selectively benefit from anti-angiogenic therapy, we quantified endothelial cells (ECs) from over 50 hPDAC samples to determine if vascularity correlated with other tumor properties like differentiation status or stromal abundance. When no such trend was found, we hypothesized that gene expression data could inform PDAC angiogenic status instead. Accordingly, we analyzed RNA-Seq data from The Cancer Genome Atlas (TCGA) and determined that only 12% (16/85) of PDAC patients express a strong pro-angiogenic gene signature, raising the possibility that targeted anti-angiogenic therapy should be limited to this subset of patients. Microarray analysis on tumors from the **KRC** PDAC mouse model, which has oncogenic **K**ras and deleted **R**b1 in the pancreas due to **C**re-mediated recombination, and the **KPC** PDAC mouse model, which has oncogenic **K**ras and mutated Tr**p**53 in the pancreas due to **C**re-mediated recombination, revealed that KRC mice have superior enrichment and differential expression of this pro-angiogenic gene signature.

To further elucidate the mechanism by which KRC cells might promote angiogenesis, conditioned media (CM) from KRC cells was found to enhance the proliferation of ECs and a multiplex ELISA of the CM identified six cytokines known to activate Stat3. Accordingly, blockade of Stat3 in ECs led to abrogation of the ability of KRC CM to enhance EC proliferation. To translate the importance of this finding *in vivo*, treatment of KRC and KPC mice with ruxolitinib, a Jak1/2 inhibitor, resulted in suppression of murine PDAC (mPDAC) progression and improved survival in KRC mice while KPC mice showed no slowing of mPDAC progression. Therefore, angiogenic heterogeneity exists both in hPDAC and GEMMs of PDAC, and we have demonstrated that KRC mice are an ideal GEMM for investigating angiogenic mechanisms and anti-angiogenic therapies directed at PDAC patients with the strong pro-angiogenic gene signature.

Basic Science ***Graduate Student***

POSTER #22

IDENTIFICATION AND BIOSTATISTICAL ANALYSIS OF CONSERVED METABOLIC PROFILES IN BREAST TUMORS FROM TRANSGENIC MOUSE MODELS

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Novel breast biomarkers with prognostic and therapeutic value include metabolites that are differentially expressed in breast tumors. The goal of this study is to identify global metabolic profiles of breast tumors derived from multiple established transgenic mouse models to gain insight into breast cancers of differing origin. We compared breast tumor samples to normal tissue and between cancer models from transgenic mouse breast cancer models overexpressing the following oncogenes: PyMT, PyMT-DB, Wnt1, Neu, and C3-TAg transgenic mice. Our breast tissue samples were analyzed on GC-MS and LC-MS/MS platforms, and analysis included 374 biochemical compounds of known identity.

Comparison of global metabolic profiles between the different mouse breast cancer and normal tissue revealed many metabolic differences between genetic models. The vast majority of metabolomic profiles demonstrated differential levels of biochemicals between normal and tumor tissues consistent with changes in metabolism that support rapid growth. Breast tumors had increased glucose metabolism, amino acid metabolism, catabolism, and levels of the TCA cycle intermediates, consistent with increased energy production and anaplerotic contributions from amino acid catabolism. We also saw increased phospholipid metabolism, cholesterol uptake, and nucleotide metabolites.

Combined with published gene expression data for these mouse models, we were able to conduct biostatistical analysis to search for correlation between gene expression and metabolite levels. We identified novel key metabolites and genes that correlate with the most other metabolites/genes; the list of these "hub" metabolites/genes could prove to be novel sites of regulation for breast cancer progression.

We identified novel metabolic profiles of breast cancer that will be foundational in identifying metabolites with clinical prognostic value. Currently we are using both 3D culture and preclinical animal models to examine the role of the differentially expressed metabolites in breast cancer progression and treatment.

Basic Science ***Graduate Student***

POSTER #23

THE ROLE OF BRADYKININ SIGNALING IN THE MODULATION OF NEURONAL ACTIVITY INDUCED BY PACLITAXEL IN SENSORY NEURONS

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Paclitaxel is a microtubule stabilizing agent that is used in the treatment of solid tumor cancers such as breast, ovarian and lung cancer. Despite the efficacy of paclitaxel in cancer therapy regimens, patients are frequently forced to discontinue treatment due to the side effect of peripheral neuropathy. Peripheral neuropathy is defined as the dysfunction of peripheral nerve fiber activity that results in pain and sensory abnormalities. It affects approximately 30-50% of patients with varying degrees of severity. The severity of neuropathic pain experienced is largely dependent on the single and cumulative dose, concurrent treatment with other chemotherapeutic drugs and prior existing conditions. Clinically, peripheral neuropathy is characterized by burning pain, tingling, numbness, and hypersensitivity to cold and mechanical stimuli. These symptoms, which present in the hands and feet in a "glove and stocking" distribution, typically lasts months to years following treatment. Currently, there are no prophylactic or therapeutic treatments available for peripheral neuropathy. This is, in large part, due to the lack of understanding regarding the basic mechanism of action by which paclitaxel alters neuronal activity.

Our data shows that paclitaxel augments neuronal activity through the activation of the bradykinin signaling pathway. Bradykinin is an endogenous peptide that mediates acute and inflammatory pain via activation of it's cognate GPCR's, B1 and B2. These receptors are both coupled to the activation of $G_{\alpha q}$ and are involved in calcium mobilization and phosphoinositide metabolism. Bradykinin also directly activates sensory neurons via depolarization of the cell membrane to elicit action potentials and the subsequent release of neuropeptides in cultured embryonic dorsal root ganglion (DRG) neurons. Given the role of bradykinin in nociceptive signaling, we studied the signaling mechanism of bradykinin in the presence and absence of paclitaxel in neuronal cultures derived from DRG. We used the release of the nociceptive neuropeptide, calcitonin gene related peptide (CGRP), as an indicator of neuronal activity. In the absence of paclitaxel, we found that bradykinin augments peptide release through both the bradykinin B2 receptor and protein kinase C (PKC). Inhibition of the bradykinin B1 receptor had no effect on bradykinin-stimulated peptide release. Furthermore, in the presence of paclitaxel, we found that paclitaxel (1μM, 48hrs) augments bradykinin-stimulated peptide release through the bradykinin B2 receptor. This suggests that the bradykinin B2 receptor mediates paclitaxel-induced changes in neuronal activity. Surprisingly, neither of the downstream secondary messengers of the classical bradykinin signaling pathway, phospholipase C (PLC) nor PKC mediated the effects of paclitaxel. Further work is necessary to determine the signaling cascade downstream of the bradykinin B2 receptor that is responsible for mediating paclitaxel-induced changes in neuronal activity.

Basic Science ***Graduate Student***

POSTER #150

ZNF217 INTERACTS WITH THE TUMORIGENIC ISOFORM OF PYRUVATE KINASE PKM2

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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 this protein. To identify additional proteins that interact with and may regulate ZNF217, we used ZNF217 as bait in a two-hybrid screen and identified a panel of interacting proteins including pyruvate kinase isoform M2 (PKM2). PKM2 is the embryonic and tumorigenic form of pyruvate kinase. In tumors, it is one of the main drivers of the Warburg effect, the observed accumulation of lactate in tumors. The metabolically active PKM2 is a tetramer found in the cytoplasm, but in recent years, the PKM2 dimer has also been shown to also function as a histone kinase in the nucleus, highlighting a possible role for PKM2 in gene regulation. In this study, we investigate the interaction between and localization of ZNF217 and PKM2. Determining the cellular localization of the interaction between ZNF217 and PKM2 will help to uncover the significance of their interaction.

As determined by both immunohistochemistry and western analysis, we find that ZNF217 protein is predominantly nuclear but can be cytoplasmic in some breast cancer patient tumors and cell lines. Human tumor samples also express smaller isoforms of ZNF217 more than the full length. Interestingly, overexpressing p300, a histone acetyltransferase, increases the expression of the smaller ZNF217 isoforms. Our current efforts focus on further elucidating the underlying mechanisms of the interaction of ZNF217, PKM2, and p300 and in understanding the role of alternative ZNF217 isoforms in breast cancer progression. In future studies, we will investigate the effects on chromatin remodeling caused by this interaction. These mechanisms of regulation may be the basis of a biomarker assay used in patients for personalized treatment strategies. Identifying the mechanism and regulation of ZNF217 may bring about novel drug targets for tumors that overexpress ZNF217 and cause poor prognosis in patients.

Basic Science ***Graduate Student***

POSTER #24

MISMATCH REPAIR PROTEINS RECRUIT DNA METHYLTRANSFERASE 1 TO SITES OF OXIDATIVE DNA DAMAGE

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Epithelial cells at sites of inflammation are exposed to high levels of reactive oxygen species, resulting in oxidative damage to proteins, lipids and DNA. At sites of chronic inflammation epithelial cells contain DNA methylation changes that are similar to those that occur in cancer, suggesting that inflammation may initiate epigenetic alterations that then potentially participate in carcinogenesis. Recently, we demonstrated that oxidative damage causes epigenetic silencing proteins including the DNA methyltransferases (DNMTs), the histone deacetylase SIRT1, and the polycomb group protein EZH2 to become part of a large complex that is localized to GC-rich regions of the genome, including promoter CpG islands that become epigenetically silenced in cancer. However, it was unclear if these proteins are recruited directly to damaged DNA or during the DNA repair process. Here we demonstrate that after oxidative damage the recruitment of DNMT1 to damaged chromatin is not dependent on PCNA unlike at double strand breaks, but rather PCNA recruitment is dependent on DNMT1. Instead we find that the mismatch repair (MMR) protein heterodimer MSH2-MSH6 plays a role in the oxidative damage-induced increase in tightness of binding of DNMT1 and SIRT1 to chromatin. Hydrogen peroxide treatment induces the interaction of MSH2 and MSH6 with DNMT1 suggesting that the recruitment is through a protein-protein interaction. Furthermore, we demonstrate that knockdown of either MSH6 or DNMT1 abrogates the oxidative damage-induced reduction in transcription of genes with CpG island-containing promoters providing evidence that the role of DNMT1 at sites of oxidative damage is to reduce transcription so that it potentially does not interfere with the repair process. While further work needs to be performed to determine if this process can result in heritable transcriptional repression as well as how hydrogen peroxide induce the among MSH2 and MSH6 with DNMT1, this study uniquely brings together several factors that are known to contribute to colon cancer namely inflammation, MMR proteins, and epigenetic silencing proteins.

Basic Science ***Graduate Student***

POSTER #25

S6K1 IS A REGULATOR OF SELF-RENEWAL OF HEMATOPOIETIC STEM CELLS AND LEUKEMIA INITIATING CELLS

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Regulation of hematopoietic stem cell (HSC) function(s) and leukemia initiating cells (LIC) via the mechanistic target of rapamycin complex1 (mTORC1) and its upstream regulators including PI3K and Akt has been described before. To this end, we and others have shown that hyperactivation and deficiency of the PI3K-mTORC1 pathway results in altered development, maintenance and function(s) of HSCs. Furthermore, pharmacological targeting of the components this pathway has been shown to inhibit the growth of acute myeloid leukemia (AML) cells. However, the role of downstream effector of mTORC1, S6K1, in HSC development and functions and LIC is unknown.

We studied the role of S6K1 in HSCs and LICs using a genetic model of S6K1 knockout mice (*S6K1-/-*). S6K1 deficiency in bone marrow hematopoietic cells resulted in decrease of absolute number of bone marrow hematopoietic mononuclear cells as well as HSCs were significantly reduced relative to controls. S6K1 deficiency results in increased cycling of HSCs following 5-FU treatment and *S6K1-/-* mice are more susceptible to repeated myeloablative treatment. When transplanted into lethally irradiated primary and secondary recipients, S6K1 deficient HSCs show significantly reduced engraftment relative to controls. As previous studies have shown that hyperactivation of mTORC1 pathway affects the engraftment and self-renewal of HSCs too, we next studied the effect of S6K1 overexpression in HSCs. Interestingly, overexpression of S6K1 in wild type HSCs also resulted in reduced engraftment of HSCs in primary and secondary recipients, suggesting that S6K1 overexpression in HSCs leads to decreased self-renewal. As S6K1 plays a role in regulating the self-renewal of hematopoietic stem cells, we studied whether S6K1 has any role in self-renewal of LICs or not. We used a well-defined leukemia model of murine AML using mixed lineage leukemia (MLL) fusion gene (MLL-AF9). WT and *S6K1-/-* HSCs were transduced with MLL-AF9 fusion oncogene and transplanted into irradiated recipients. S6K1 deficiency does not affect the initiation of AML in primary recipients. Leuke Deficiency of S6K1 results in increased survival of secondary recipients suggesting that S6K1 regulates self-renewal of LICs.

In summary, our study identifies S6K1 as a critical regulator of hematopoietic stem cell development and functions both under steady-state conditions and following genotoxic stress. Using both gain of function and loss of function approaches, we also demonstrate that the level of expression and activation of S6K1 in HSCs plays a critical role in the maintenance of HSC self-renewal and engraftment. Furthermore, as S6K1 is also a regulator of self-renewal of LICs, it could be used as a therapeutic target to treat AML patients with minimal residual disease.

Basic Science ***Graduate Student***

POSTER #26

CONTRIBUTION OF GRP78 TO CHEMORESISTANCE 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 has a five-year survival rate of 5%. This poor prognosis is associated with late detection, aggressive tumor biology, and poor response to available therapies. Neoadjuvant chemotherapy before attempts at surgical resection of PDAC are routinely used to downstage tumors in an effort to improve long-term survival. However, only approximately one-third of neoadjuvant patients will show a significant response to gemcitabine, the chemotherapeutic standard of care for PDAC patients. The remaining patients show no benefit and the survival rate beyond 18 months is extremely low. In late stage diagnosis scenarios, treatment with gemcitabine yields a median survival rate of only 6 months. These statistics are alarming and, therefore, demonstrate a critical need for the identification of the molecular mechanisms responsible for chemoresistance in PDAC. GRP78 is an endoplasmic reticulum (ER) chaperone protein that primarily resides in the lumen of the ER. It is the master regulator of the unfolded protein response (UPR) and functions to both facilitate the folding of proteins into their correct conformational form and target misfolded proteins for proteasome degradation. In the early stage activation of the UPR, pro-survival pathways are activated. During this time, GRP78 can translocate to the membrane, co-localize with PI3K, and act as a membrane receptor capable of transmitting signals that promote tumor proliferation, anti-apoptosis, survival, and resistance to routinely utilized therapeutic routines. Preliminary histological data has shown that GRP78 is highly upregulated in chemoresistant tumors in PDAC. Further, in typically sensitive tumors, GRP78 becomes upregulated after exposure to gemcitabine. Currently, we have been investigating the critical role of GRP78 in acquired chemoresistance. Overexpression of GRP78 in typically chemosensitive pancreatic cancer cells, via thapsigargin induction, have demonstrated acquired chemoresistance to gemcitabine treatment in vitro. Further, siRNA knockdown of GRP78 in typically chemoresistant pancreatic cancer cells have exhibited acquired sensitivity to gemcitabine treatment in vitro. Further investigation of GRP78 mediated chemoresistance in PDAC will be extended in vivo by generating a KRAS mutant, PTEN, GRP78 knockout mouse model.

Basic Science ***Graduate Student***

POSTER #27

INTEGRATIVE REVIEW: PAIN AND QUALITY OF LIFE IN POST-CRANIOTOMY BRAIN TUMOR PATIENTS

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BACKGROUND: Patients with brain tumors undergo craniotomies, which are believed to be less painful than other surgical procedures due to the brain's lack of innervation. Pain is associated with decreased quality of life. Understanding the experience of post-craniotomy pain will help guide patient care, timely interventions, future research and policy development.

PURPOSE: This integrative review examined prevalence, influencing factors, associated symptom clusters, and consequences of post-craniotomy, post-brain tumor pain.

THEORETICAL FRAMEWORK: The Theory of Unpleasant Symptoms guided this study.

METHODS: A literature search was conducted utilizing Medline, OVID, PubMed and CINAHL using key words traumatic brain injury, pain, post-operative, brain injuries, postoperative pain, craniotomy, decompressive craniectomy, and trephining. The Theory of Unpleasant Symptoms (TOUS) was used as a guide for abstracting information from each article, including: influencing factors, associated symptom clusters, and consequences of post-craniotomy, post-brain tumor pain. Inclusion criteria were indexed, peer-reviewed, full-length, English-language articles.

RESULTS: The search yielded 115 articles, with 27 meeting inclusion criteria. Most studies reviewed (88%) were randomized, controlled trials conducted outside of the United States, and tested pharmacological pain therapies. Although all articles documented the existence of post-craniotomy, post-brain tumor pain, only 11 discussed influencing factors, 12 discussed associated symptom clusters and 19 reviewed patient performance, while four included information on all four aspects.

CONCLUSION: Post-craniotomy, post-brain tumor pain exists and is associated with multiple co-related symptoms. This pain impacts patient performance, decreases quality of life, and may contribute to the development of persistent pain. Further research is needed to improve understanding and management of post-craniotomy, post-brain tumor pain, thereby improving patients' quality of life.

Keywords: brain tumor, craniotomy, pain, integrative review, Theory of Unpleasant Symptoms

Basic Science ***Graduate Student***

POSTER #28

PAIN, ANXIETY AND QUALITY OF LIFE IN BREAST CANCER SURVIVORS

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BACKGROUND: Pain and anxiety are symptoms that frequently occur as co-related groups termed clusters. While pain may be associated with decreased quality of life (QOL), less is understood about the effects of anxiety on breast cancer survivors (BCS).

PURPOSE: The purpose of this study was to: 1) determine levels of pain and anxiety in breast cancer survivors and 2) examine the relationship between anxiety and pain on quality of life in breast cancer survivors (BCS) controlling for age and time post-treatment.

THEORETICAL FRAMEWORK: Ferrell's Quality of Life Model guided this study.

METHODS: A cross-sectional, descriptive design was used. BCS enrolled in a longitudinal cognitive behavioral study completed self-report questionnaires including the Medical Outcome Scale-Short Form, Pain Subscale, Spielberger State-Trait Anxiety Instrument (STAI) State Sub-scale to measure anxiety, and Ferrell's QOL Instrument which measures Physical, Psychological, Social and Spiritual Well-being (higher scores indicating higher quality of life). All data were collected at a baseline assessment before randomization in the cognitive intervention study. Data were evaluated using descriptive statistics and general linear regression, controlling for covariates of age and time post-treatment.

RESULTS: 88 female BCS averaging 57 years old (range 40-74; SD=8.54), primarily Caucasian (88.6%), college-educated (88%), approximately 5.3 (SD=4.0) years post-treatment participated. Cancer-related pain (4 lymphedema, 4 neuropathy) was specified by 8 participants. BCS reported low-moderate pain ($\bar{x}$ = 66.0114; *sd* = 22.348) and moderate anxiety ($\bar{x}$ = 35.0227; *sd*= 8.7749). Clinically significant levels of anxiety ($\geq$ 39 on the STAI-S where higher scores indicated higher anxiety) were reported in 24% of BCS. Age, anxiety, and pain significantly accounted for 45% of the total variance on QOL in the regression model (R = 0.45, F = 18.61; p = <0.000), controlling for age and time post-treatment.

CONCLUSIONS: This study provides support for the fact that both pain and anxiety are negatively associated with QOL. Anxiety is a significant long-term symptom for a sub-set of BCS which negatively impacts all dimensions of BCS quality of life. Findings indicate the need for comprehensive assessment of symptoms of anxiety in long-term BCS and the development of evidenced-based interventions to alleviate anxiety and improve QOL.

Basic Science ***Graduate Student***

POSTER #29

DECIPHERING CELL DEATH MECHANISMS IN EXTRACELLULAR MATRIX-DETACHED EPITHELIAL CELLS

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When epithelial cells detach from the extracellular matrix (ECM), they induce apoptotic cell death termed anoikis. Cancer cells must overcome anoikis in order to survive during the metastatic cascade, where they will be exposed to limited and variable matrix conditions. However, anoikis is not the only mechanism by which ECM-detached cells can be eliminated. Our previous studies have demonstrated that ECM-detachment induced metabolic changes can compromise the survival of detached cells through a caspase-independent mechanism. Therefore, cancer cells need to overcome both anoikis and caspase-independent cell death in order to survive during metastasis. The data presented here suggest that these cells are being killed by regulated necrosis (RN), a genetically programmed type of necrosis that is morphologically indistinguishable from classical necrosis. In general, the understanding of molecular mechanisms involved in RN are poorly understood and our studies have uncovered a novel and distinct pathway that causes RN during ECM-detachment. Previous studies suggest that receptor-interacting protein kinase 1 (RIP1) activation can be a critical effector of RN in certain contexts so we began by investigating the expression and activation of RIP1 following ECM-detachment. Interestingly, ECM-detachment leads to a significant increase in total RIP1 protein and in RIP1 activation. To investigate the role of ECM-detachment induced RIP1 in the viability of ECM-detached cells, we inhibited its kinase activity using necrostatin-1 (Nec-1). Nec-1 treatment led to a substantive increase in cell viability and to the filling of acinar structures in 3-dimensional cell culture models, suggesting that RIP1 kinase activity is necessary for the ECM-detachment-induced RN. To confirm these findings, we decreased RIP1 levels via lentiviral transduction of shRNA and found that reduction of RIP1 protein also leads to enhanced viability of ECM-detached cells. Reports on RN in other model systems have identified receptor-interacting protein kinase 3 (RIP3) and mixed lineage kinase domain-like protein (MLKL) as executioners of RN following activation of RIP1. Intriguingly, our studies using lentiviral transduction of shRNA targeting RIP3 or MLKL suggest that ECM-detached cells undergo RN independently of either RIP3 or MLKL. To assess if RIP1 overexpression (which happens as a result of ECM-detachment) is sufficient to induce RN, we overexpressed RIP1 in attached MCF10A cells expressing the anti-apoptotic protein Bcl-2. Strikingly, we found that this increase in RIP1 does cause cell death independently of caspase activation. Taken together, our data suggest a novel mechanism of RN that eliminates ECM-detached cells that is dependent on RIP1 kinase activity but independent of both RIP3 and MLKL. Therefore, metastatic cancer cells must find ways to antagonize RIP1 overexpression and activation and a better understanding of the mechanisms employed by these cells to antagonize RN may be utilized to develop novel therapeutics aimed at inducing RN in metastasizing cancer cells.

Basic Science ***Graduate Student***

POSTER #30

JAK/STAT CAN EAT SOME FAT: THE JAK/STAT PATHWAY CONTROLS LIPID ANTIGEN PRESENTATION BY CD1D

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CD1d is a cell surface glycoprotein that presents endogenous and exogenous lipid antigens (Ags) to innate immune lymphocytes called natural killer T (NKT) cells. We have previously demonstrated the regulation of CD1d-mediated Ag presentation by numerous cell signaling pathways, such as those involved in cell proliferation and in cellular stress responses. The importance of JAK/STAT signaling in the development and function of adaptive immune responses is well established. However, it is unknown whether this central signaling pathway in the immune system can impact the host's innate immune function-namely, CD1d-mediated lipid Ag presentation to NKT cells. Aberrant JAK/STAT signaling has been demonstrated in hematological malignancies, solid tumors and other human diseases. Because NKT cells have also been implicated in controlling the majority of these diseases, we hypothesized the JAK/STAT signaling is a negative regulator of CD1d-mediated Ag presentation. Surprisingly and contrary to this original idea, via pharmacological inhibition and genetic approaches, we found a positive regulatory role for JAK/STAT signaling in endogenous lipid Ag presentation by CD1d. Ag presenting cells (APCs) in which the pathway components were inhibited had reduced CD1d recycling and endogenous lipid antigen presentation with no impact on its intracellular localization. Furthermore, overexpression of JAK/STAT pathway components in deficient APCs rescued CD1d function. In this study, we have identified a novel role for JAK/STAT signaling the positive regulation of CD1d-mediated lipid Ag presentation to NKT cells. These findings significantly contribute to our current knowledge of the functional importance of the JAK/STAT pathway in innate immunity and suggest that constitutive JAK/STAT signaling could be a possible explanation for the observed dysfunction in CD1d-NKT axis due to enhanced lipid Ag presentation by CD1d in disease settings. This would consequently lead to NKT cell overt activation and ultimately, anergy.

Basic Science ***Graduate Student***

POSTER #31

SPINNER: SOFTWARE TO RANK AND COMPARE PHENOTYPE-SPECIFIC GENES IN MOLECULAR NETWORKS

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With the rapid accumulation of molecular interaction data, there is a surging interest in characterizing molecular functions in the new context of biomolecular interaction networks such as protein-protein interaction networks. Previous work has focused primarily on 1) assigning functions through immediate network neighbors, or "guilt-by-associations" of a protein's interaction partners; 2) assigning functions through global biomolecular interaction networks to assess the significance and functions of the protein of interest. In this work, we developed an automated software tool **S**eeded **P**rotein **I**nteraction **N**etwork **N**eighborhood **E**xpansion and **R**anking, which can rank and compare genes or proteins from constructed phenotype-specific biomolecular interaction networks. Given the user input of a list of phenotype-specific genes, our tool can query the STRING or HAPPI protein-protein interaction database automatically to retrieve protein-protein interactions among the input genes with user-specified network expansion levels to construct a phenotype-specific network. All the sub-networks are ranked and evaluated statistically to obtain a P-value for its index of aggregation before subsequent analysis. To compare the significant contribution of each protein, we consider its node degree of connectivity, protein interaction quality for its surrounding interacting partners (including both direct and indirect connected partners through iterations), the protein's significance in both unfiltered global network and phenotype-specific network, and other network characteristics. Our tool also provides the gene/protein's PubMed reference citation count for the specific phenotype to help users evaluate the ranked proteins. A family-wise adjusted P-value of all significant ranks against randomized topology-preserving networks are also provided to help assess the rank. We applied the tool to the characterization of a breast cancer case study. The breast cancer gene set consists of 225 "seed" genes forming an expanded 784-gene network consisting of 67 sub-networks with a size range from 2 to 574 (or a relative size range from 0.25% to 74%). The largest sub-network consist of the 574 out of 784 genes are determined as statistically significant for its index of aggregation (P-value=8.7E-08). The adjusted rank revealed that top 40 genes are all statistically significant at P-value<=0.05 when comparing their ranks against a random model. We show that these ranked genes are both statistically stable and biologically significant. Our results indicate that this tool can enable ongoing disease-specific biological studies.

Basic Science ***Graduate Student***

POSTER #32

HIGHLY SPECIFIC PLASMONIC BIOSENSORS FOR ULTRASENSITIVE MICRORNA DETECTION IN PLASMA FROM PANCREATIC CANCER PATIENTS

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MicroRNAs (miRs) are noncoding, short (20 -22 nucleic acid) RNAs that have many functional roles in cellular activities in plant and animal tissues, such as gene regulation. MicroRNAs have been classified as important diagnostic biomarkers for diseases and have been targeted for biosensor extensions. For the first time, a regenerative, solid-state localized surface plasmon resonance (LSPR) sensor, based on highly sensitive nanostructures (gold nanoprisms), was designed that did not use labeling nor needed the amplification of miRs. The designed sensor was applied for the detection of miR-21 and miR-10b in different physiological media such as a buffer, bovine plasma, and human plasma. The direct hybridization-based sensor displayed the ability to detect sub-femtomolar concentration of miRs in all physiological media with high selectivity. The sensor was further successfully applied for the detection of these miRs in human plasma samples collected from pancreatic cancer patients and the results were coordinated with the qRT-PCR results. The sensor eliminates the drawback of current techniques, which are unable to detect the miRs in their native environment due to the lack of a high degree of sensitivity and selectivity. These results promise a highly sensitive and selective detection approach of miRs in their native environment, and can be applied to other types of cancers. This will allow a rational approach to test several disease markers for early diagnosis and will individualize therapy.

Basic Science ***Graduate Student***

POSTER #33

ONCOGENIC MECHANISMS OF ETS TRANSCRIPTION FACTORS 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. We have shown that over-expression of these four oncogenic ETS genes, but not other ETS factors, in a normal prostate epithelial cell line, activates a specific transcriptional program that drives cell migration. Objectives of the present study are to identify therapeutically targetable signaling events and co-factors that cooperate with oncogenic ETS to drive prostate cell migration. Mass spectrometry analysis of ERG identified two phosphorylation sites. Phospho-null mutants of ERG failed to induce cell migration suggesting that signaling events are critical for oncogenic ERG function. To identify putative co-activators, purified ERG was used to pull down co-activators from nuclear extracts. ERG-bound proteins were identified using mass spectrometry. We are currently testing the effect of depletion of several of these candidate co-activators on cell migration in cancer cell lines expressing ERG and oncogenic ETVs. Collectively our data will provide rationale for new therapeutic targets for ETS-positive prostate cancer.

Basic Science ***Graduate Student***

POSTER #34

EFFECTS OF LYSOPHOSPHATIDIC ACID ON OVARIAN CANCER CELL AND MULTI-CELLULAR AGGREGATE DYNAMICS AND PERITONEAL ADHESION

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Epithelial ovarian cancer (EOC) metastasis occurs via direct extension of cancer cells from the primary tumor into the peritoneal cavity, where they survive and travel as single cells and multi-cellular aggregates (MCAs) with the peritoneal fluid flow, subsequently adhering to peritoneal tissues, migrating into sub-mesothelial matrix and forming secondary lesions. This process is often accompanied by accumulation of malignant ascites rich in lysophosphatidic acid (LPA), a bioactive lipid molecule which activates a subfamily of G-protein coupled receptors and is linked to aberrant cell proliferation, oncogenesis and metastasis. It has been previously shown by our lab that LPA disrupts junctional integrity and epithelial cohesion of ovarian cancer cells which may facilitate tumor cell exfoliation from the primary carcinoma (Liu *et al*, 2012). The objective of the current study is to address the effects of LPA on the morphology and behavior of free-floating ovarian cancer single cells and MCAs and their peritoneal adhesivity. Here, we demonstrated that LPA treatment of OvCa429, SKOV3ip and DOV13 2-dimensional adherent monolayers (in TC dishes) and 3-dimentional clusters (in hanging drop suspensions) resulted in shedding of cell surface protrusions into the conditional media as observed by light and scanning electron microscopy. LPA-exposed cells with exfoliated protrusions failed to form large cohesive MCAs but completely restored both original surface morphologic phenotype and cluster cohesivity upon subsequent LPA deprivation. To further investigate the influence of LPA on EOC cell and MCA secondary attachment, we performed an *ex vivo* assay. Single cells seeded on top of mouse peritoneal explants exhibited lower adhesion level upon LPA addition. LPA-exposed MCAs demonstrated poor aggregation and therefore, higher number of disseminated smaller clusters compared to untreated control

MCAs. These data suggest that LPA alters cell and MCA intra-peritoneal dynamics and peritoneal dissemination success. More experiments accessing the effect of LPA on peritoneal tissue properties are currently underway.

Basic Science ***Graduate Student***

POSTER #35

PATHOPHYSIOLOGICAL ROLE OF MICRORNA-29 IN PANCREATIC CANCER STROMA

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Dense fibrotic stroma associated with pancreatic ductaladenocarcinoma (PDAC) has been a major obstacle for drug delivery to the tumor bed and may impede attempts to slow down PDAC progression and metastasis. However, current anti-stromal drugs have not improved tumor response to chemotherapy or patient survival. Thus, a better understanding of the molecular mechanisms associated with tumor-stromal interactions is desperately needed to develop novel anti-stromal therapeutic approaches. MicroRNAs (miRNAs) are an abundant class of highly conserved, small non-coding RNAs that function as key regulators of eukaryotic gene expression and cellular homeostasis. miR-29 is known to play a paramount role in the fibrotic process of several organs by providing crucial functions downstream of pro-fibrotic signaling pathways such as TGF-B1 and regulating the expression of extracellular matrix (ECM) proteins, a major component in the PDAC stroma. Upregulation of TGF-B1 is commonly associated with PDAC pathogenesis and is known to activate stromal cells. Furthermore, vascular endothelial growth factor (VEGF), which stimulates tumor angiogenesis, is a predicted target of miR-29. We hypothesize that miR-29 may be misregulated in TGF-B1 activated PDAC stromal cells and leads to excessive accumulation of ECM proteins and VEGF. Restored expression of miR-29 could be therapeutically beneficial to modulate tumor-stromal interactions. To understand the role of miR-29 in PDAC stroma, we examined miR-29 expression patterns in TGF-B1 activated stromal cells using qPCR/northern blot analysis and determined ECM and VEGF protein expression. In activated stromal cells, we observed loss of miR-29 in correlation with a significant increase in ECM and VEGF protein expression. In addition, in both murine and human PDAC samples, loss of miR-29 expression is associated with an increase in stromal percentage estimated by Sirius Red stain. To evaluate the physiological role of miR-29 in stroma, we performed gain and loss-of-function studies by transfectingstromal cells with synthetic miR-29 mimics or locked nucleic acid, a miR-29 inhibitor. Overexpression of miR-29 in stromal cells suppressed matrix and VEGF protein expression, and conversely, depletion of miR-29 lead to their significant increase. Finally, to evaluate the effect of miR-29 overexpression in stromal cells on cancer colony growth, we directly co-cultured miR-29 transfected stromal cells with pancreatic cancer cells for 10 days, and subsequently, cancer colony number and stromal deposition was determined by crystal violet and Sirius Red stains respectively. We observed a significant decrease in the number of cancer colonies and stromal accumulation in co-cultures. In conclusion, our results provide insight into the mechanistic role of miR-29 in PDAC stroma and its potential use as a novel anti-stromal therapeutic agent.

Basic Science ***Graduate Student***

POSTER #36

TISSUE TRANSGLUTAMINASE MEDIATED TUMOR-STROMA INTERACTION PROMOTES PANCREATIC CANCER PROGRESSION

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Purpose: Aggressive pancreatic cancer is commonly associated with a dense desmoplastic stroma, which forms a protective niche for cancer cells. The objective of the study was to determine the functions of tissue transglutaminase (TG2), a Ca^{2+}-dependent enzyme which crosslinks proteins through transamidation and is abundantly expressed by pancreatic cancer cells in the pancreatic stroma.

Experimental Design: Orthotopic pancreatic xenografts and co-culture systems tested the mechanisms by which the enzyme modulates tumor-stroma interactions.

Results: We show that TG2 secreted by cancer cells effectively molds the stroma by crosslinking collagen, which in turn activates fibroblasts and stimulates their proliferation. The stiff fibrotic stromal reaction conveys mechanical cues to cancer cells leading to activation of the YAP/TAZ transcription factors, promoting cell proliferation and tumor growth. Stable knockdown of TG2 in pancreatic cancer cells led to decreased size of pancreatic xenografts.

Conclusions: Taken together, our results demonstrate that TG2 secreted in the tumor microenvironment orchestrates the crosstalk between cancer cells and stroma fundamentally impacting tumor growth. Our study supports TG2 inhibition in the pancreatic stroma as a novel strategy to block pancreatic cancer progression.

Basic Science ***Graduate Student***

POSTER #37

ADENOMATOUS POLYPOSIS COLI REGULATES EPITHELIAL MORPHOGENESIS AND MIGRATION THROUGH FAK/SRC SIGNALING

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Adenomatous Polyposis Coli (APC) is a multi-functional protein that is lost or mutated in many epithelial cancers including breast, colorectal, and pancreatic cancer. Although APC is well known as a negative regulator of the Wnt/B-catenin signaling pathway, it also binds to the cytoskeleton, microtubules and polarity proteins, such as Dlg and Scribble, suggesting functions in regulation of epithelial polarity and cell migration. Our lab has previously determined that the mammary glands of $Apc^{Min/+}$ mice demonstrate mis-regulation of epithelial polarity, exhibit early neoplastic changes, and develop more aggressive mammary tumors when crossed to the MMTV-PyMT model of breast cancer. Cells isolated from these tumors displayed activated FAK/Src signaling. Our lab has also shown that APC knockdown in the Madin-Darby Canine Kidney (MDCK) model altered epithelial morphogenesis, resulted in inverted polarity in 3D culture, and up-regulated gene expression of epithelial membrane protein 2 (EMP2). While restoration of the middle b-catenin binding domain was unable to rescue the phenotype, introduction of either full-length or a c-terminal fragment of APC partially restored these phenotypes. The current studies investigate the Wnt-independent mechanisms by which APC regulates these processes using the MDCK model and primary mammary epithelial cells (MECs) isolated from *Apc* mutant mice. We hypothesize that the c-terminal fragment of APC mediates FAK/Src signaling to regulate 3D morphogenesis, polarity, and migration. Treatment of APC knockdown MDCK cells with PP2, a Src kinase inhibitor, or AIIB2, a B1-integrin inhibitor, eliminated the drastic cyst size changes produced by APC knockdown. Furthermore, inhibition of Src partially restored the polarity phenotype in cysts with APC loss. In addition, shAPC-MDCK cells and MECs isolated from $Apc^{Min/+}$mice exhibited increased cell migration compared to control cells indicating a role for APC in cell motility. Preliminary data demonstrates that treatment of shAPC-MDCK cells with PP2, AIIB2, and a FAK inhibitor decreases migration suggesting FAK/Src signaling as a possible mechanism by which APC mediates cell migration. Interestingly, EMP2 has been shown to bind integrin to activate FAK signaling suggesting an interaction in these pathways, and preliminary studies show EMP2 expression is increased in shAPC-MDCK cells during migration. Future studies will aim to dissect the role of the c-terminal fragment and further devise the mechanism by which FAK/Src signaling and EMP2 play a role in APC regulating gene expression, cell migration, and polarity and 3D morphogenesis in MDCK cells and MECs isolated from *Apc* mutant mice. Investigating the interactions of APC with several targets such as those in the FAK/Src signaling pathway and EMP2 will help identify key players in the role of APC in Wnt-independent tumor development.

Basic Science ***Graduate Student***

POSTER #38

TARGETING APE-1/REF-1 RESULTS IN INHIBITION OF HYPOXIA SIGNALING GENES

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer related mortality in the United States. Most patients present with advanced disease, and most patients die due to their disease. Treatment with chemotherapy has not changed the natural course of this disease, and just recently, with combination of chemotherapeutic agents, the median survival reached a year. Several mechanisms are proposed to play a role in the aggressive, treatment-resistant phenotype of PDAC, including adaptation to hypoxia, which leads to increased potential for metastasis and impairs the efficacy of chemotherapy and radiotherapy. One of the main sensors of oxygen in cells is Hypoxia-Inducible Factor-1α (HIF-1α), a transcription factor that is rapidly degraded under normoxic conditions but upregulates a number of genes under hypoxic conditions that contribute to survival, metastasis, and angiogenic signaling in the tumor microenvironment. One of the most notable HIF targets is Carbonic Anhydrase IX (CA9), which promotes tumor cell survival and metastasis by maintaining a steady intracellular pH while acidifying the microenvironment, thereby encouraging epithelial-mesenchymal transition and contributing to extracellular matrix degradation.

Apurinic/Apyrimidinic Endonuclease1/ Redox Effector Factor 1 (APE1/Ref-1) is a dual function protein that possesses a DNA repair function in base excision repair as well as the ability to reduce transcription factors and enable them to bind to their DNA target sequences. APE1/Ref-1 regulates several transcription factors involved in preventing apoptosis, survival mechanisms, and hypoxia signaling, including HIF-1α. **Therefore, we hypothesized that APE-1/Ref-1 inhibition interferes with HIF-1α-mediated signaling, leading to decreased survival and invasion of tumor cells exposed to hypoxic conditions.**

Methods: We performed co-immunoprecipitation (co-IP) studies to look at the interaction of APE1/Ref-1 with transcriptional targets, HIF-1α, STAT3, and NFkB along with RT-PCR and Western blotting to confirm expression of hypoxia signaling genes. Luciferase reporter assays were used to quantitate transcriptional activation under hypoxia. Boyden chamber was used to look at migration and invasion as well as proliferation based assays following manipulation of APE1/Ref-1 and hypoxia.

Results: HIF-1α and STAT3, but not NFkB associate with APE1/Ref-1 under hypoxia. Moreover, we found that knockdown of APE1/Ref-1 protein diminishes HIF-mediated transcription. Next, we showed that APE1/Ref-1 inhibition diminishes HIF-1a-induced downstream targets including CA9 and ANGPTL4 indicating that APE-1/Ref-1 redox activity is regulating HIF signaling. Importantly, we found that APE-1/Ref-1 knockdown no longer affected hypoxia-induced CA9 mRNA levels in HIF-deficient MEFs, indicating that the effects of APE1/Ref-1 on CA9 expression is mediated by HIF-1α. A blockade of both CA9 activity and CA9 transcription via APE1/Ref-1 leads to decreased PDAC cell proliferation under hypoxia.

Taken together, these data indicate that APE-1/Ref-1 inhibition interferes with hypoxia-mediated signaling

and can further sensitize PDAC cells to CA9/12 inhibition even under the conditions of extreme oxygen deprivation. Ongoing experiments will determine the role APE1/Ref-1 plays in the survival and invasion of tumor cells exposed to hypoxic conditions.

Basic Science ***Graduate Student***

POSTER #39

THE ROLE OF AGING IN OVARIAN CANCER METASTASIS TO THE PERITONEUM

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Ovarian cancer is the most fatal gynecological cancer. Epithelial ovarian cancer (OvCa) is the most common subtype and often goes undetected until metastatic and often fatal stages of the disease. OvCa follows a unique form of metastasis, spreading through the peritoneal cavity and forming metastatic sites on the peritoneum. The peritoneum, a vast, serous membrane lining the abdominal cavity and organs, consists of a single layer of mesothelial cells (MCs) supported by a collagen-rich extracellular matrix (ECM). OvCa metastasis initiates when tumor cells or multicellular aggregates (MCAs) are shed from the primary tumor into the peritoneal cavity. OvCa cells or MCAs adhere to peritoneal surfaces, causing the MCs to retract. Metastasis progresses when OvCa cells penetrate through the mesothelium into the submesothelial ECM, where they anchor and proliferate. The vast majority of OvCa cases occur in women over 40 and the median age at diagnosis is 63. Despite age being a significant risk factor for the development of OvCa, very few studies have examined the role of aging in OvCa metastasis. Furthermore, there is a dearth of information on the aging peritoneum. Our preliminary data suggest that both the mesothelial and submesothelial compartments of the peritoneum accumulate changes as a function of age. Our data also suggests that the age of the host influences OvCa metastasis. Using a C57Bl/6 mouse model of aging and OvCa metastasis, we are testing the hypothesis that aging leads to alterations in peritoneal tissues and that the aging peritoneum impacts metastatic success of ovarian cancer.We found that the mesothelium of middle-aged mice (10-14 months) has a greater density of microvilli on the apical surface than does the mesothelium of young mice (3-6 months). The submesothelial collagen also appears to undergo age-related change. Using a peritoneal explant adhesion assay, we found that ovarian cancer cells attach more efficiently to the peritoneum of young mice than to the peritoneum of middle-aged or aged (20-23 months) mice.

Basic Science ***Graduate Student***

POSTER #40

IMAGING GENOMICS: CORRELATING MEDICAL IMAGES TO GENOME INFORMATION

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Purpose: Cancer patients have genomics information and imaging information. There are some research works have been done on genomics as well as medical imaging. However, there is no systematical and integrated investigation on the correlation between genomics and medical imaging. The goal of this project is modeling and analyzing the imaging phenotype and genotype features and discovering the association between them.

Materials and Methods: 72 GBM patients from the Cancer Imaging Archive (TCGA) are selected for the research as all these 72 patients have the corresponding medical images and genomic information. 34 out of 72 patients with T1 weighted FLAIR and post gadolinium (GD) image sequences in axial viewpoint are selected. The acquired brain MRI images are used with the following steps. First, set a seed point in the region of interest (ROI) and include its neighboring pixel as the initially region. The region's intensity value mean and plus a defined factor alpha construct as a threshold. Second, neighboring pixels whose intensity values fall inside the threshold will be included in the connected region. When no more neighbor pixels are found that satisfy the criterion, the mean of included all pixels is recomputed and plus defined factor alpha to generate the new threshold. This iterative process is repeated until no more pixels are found. In the end, the connected region will be treated as the tumor region. The tumor volume will be determined after the iterative process.

Results: A prototype has been developed and preliminary experiments have been performed. The mean value of 34 patients' tumor region's pixel intensities is 103.1 in the range of 0 to 255. As per preliminary analysis, Pearson correlation testing with genome expression profile and the mean of tumor intensity was performed, significantly associated 680 genes (77 genes -vely, 603 genes +vely correlated at $p<0.01$) were obtained.

Conclusion: The proposed approach allows getting the image features from the patient image of tumor region, which is potentially useful for connecting tumor genotype to imaging phenotype and/or other metadata features.

Basic Science ***Graduate Student***

POSTER #41

RAS-MEDIATED EVASION OF DETACHMENT-INDUCED CELL DEATH INVOLVES DIFFERENTIAL SIGNALING PATHWAYS FOR METABOLISM AND ANOIKIS

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In order for successful metastasis to occur, cells must overcome anoikis, a caspase-dependent cell death process triggered by detachment from the extracellular matrix (ECM). In addition, recent studies have revealed that ECM-detached cells must also rectify detachment-induced metabolic defects that compromise cell survival. However, the precise signals involved in the inhibition of anoikis and the restoration of proper cell metabolism during ECM-detachment are poorly understood. Of particular interest to our studies is the oncogene *Ras*, which is constitutively active in approximately 30% of all cancers and is well known to be regulate cell death pathways and metabolism. We have discovered that Ras facilitates the survival of ECM-detached cancer cells by utilizing distinct signaling pathways to block anoikis and regulate metabolism.

Using MCF-10A cells engineered to overexpress oncogenic Ras and HCT116 cells (which contain an activating Ras mutation), we investigated the signaling pathways downstream of Ras that facilitate the survival of ECM-detached cells. Interestingly, we discovered that while Ras-mediated PI(3)K signaling is critical for rectifying metabolic defects during ECM-detachment, the downstream effector is not Akt, but rather SGK-1. SGK-1 stimulates glucose uptake, enhances ATP generation, promotes luminal filling in 3-dimensional cell culture, and drives anchorage-independent growth in soft agar. Interestingly, our data also indicate that oncogenic Ras utilizes an entirely distinct signaling pathway to block anoikis. We discovered that Ras diminishes the expression of the phosphatase PHLPP1. This inhibits the dephosphorylation-induced activation of a signaling cascade that culminates in the activation of pro-apoptotic p38 MAPK. In aggregate, these data unveil a novel survival strategy utilized by ECM-detached cancer cells and implicate both SGK-1 and PHLPP1 function downstream of Ras during ECM-detachment. The molecular mechanisms unveiled here could be utilized for the design of novel therapies that eliminate ECM-detached, metastatic cancer cells with Ras mutations through simultaneous modulation of SGK-1 and PHLPP1.

Basic Science ***Graduate Student***

POSTER #42

APE1/REF-1 REGULATES SURVIVIN-MEDIATED DRUG RESISTANCE IN PROSTATE CANCER CELLS

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Background: A key feature of prostate cancer progression is the induction and activation of the survival proteins, including survivin. Targeting survivin directly has proven problematic clinically, therefore understanding the mechanisms of its induction within prostate cancer cells may prove critical for novel blockade therapy. Apurinic/apyrimidinic endonuclease 1 (APE1), also known as redox factor 1 (Ref-1), is upregulated and activated in human prostate cancer and is essential for prostate cancer cell survival. Its redox function regulates the activity of a number of oncogenic transcription factors. We sought to assess its redox function role in parental and docetaxel-resistant prostate cancer cell survival.

Methods: We assessed the expression of APE1/Ref-1, survivin, Mcl-1 and Bcl-2 in docetaxel-resistant PC3 cells by immunoblotting. The effect of APE1/Ref-1 inhibition on docetaxel-resistant and parental cells was determined by siRNA and treatment with the redox function-specific inhibitor E3330 (10, 30, 50 μM). Direct interaction between APE1/Ref-1 and its redox targets, STAT3/p65, was determined by co-immunoprecipatation. Localization of APE1/Ref-1 in human prostate cancer tissue was performed by immunofluorescence and co-localization was performed by co-staining with survivin or APE1-specific antibodies.

Results: We found that docetaxel-resistant cells showed an induction of APE1/Ref-1, survivin, Bcl-2 and Mcl-1 protein expression. Inhibition of APE1/Ref-1 redox function by siRNA or E3330 inhibited prostate cancer cell growth and induced cell death. APE1/Ref-1 redox inhibition decreased survival proteins expression in prostate cancer cells. We also found that APE1/Ref-1 immunoprecipatates with the transcription factors p65 and STAT3. Finally, we found that APE1/Ref-1 is highly expressed in human prostate cancer and co-localizes strongly with survivin.

Conclusions: These data indicate that docetaxel-resistant cells exhibit induced survivin and APE1/Ref-1 expression and that APE1/Ref-1 inhibition attenuates survivin induction. Future studies in vivo and ultimately in clinics will determine if targeting specifically the redox activity of APE1/Ref-1 may allow for the specific targeting of drug resistance while leaving other functions of this protein intact.

Basic Science ***Graduate Student***

POSTER #43

STATISTICAL ANALYSIS FOR THE IMPACT OF VIGOROUS EXERCISE OVER PARKINSON'S DISEASE PROGRESS

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Introduction:

Parkinson's disease (PD) is typically considered as a chronic, slowly progressive neurological movement disorder or disorder of the brain. It is the second most common neurodegenerative disease in United States. PD is not fatal but progressive and incurable disease and, with time, severely affects the quality of life. Currently there is no one specific test to diagnose PD and the care and management are very difficult and costly for the patients and their healthcare providers. The aim of our study is to determine the impact of vigorous exercise for the patients with PD.

Materials and Methods:

Data Resources includes Healthcare Cost and Utilization Project (HCUP) and Parkinson's Progression Marker Initiative (PPMI) data. The HCUP Data is used to identify the current treatment pattern and medication/treatment cost. Data is drawn from all States participating in HCUP, representing more than 95% of the inpatient samples of US population containing more than 7 million records on hospital stays but still protects the privacy of individual patients, physicians, and hospitals because state and hospital level identifiers are no longer provided. The PPMI data includes clinical data collected at PPMI Clinical sites, including subjects throughout the US and EU. The entire dataset contains 400 Parkinson diseases - PD patient and 200 healthy control- HC records.

Statistical approaches (e.g. Pearson Correlation, Pairwise T-Test, Hypergeometric test) will be used to determine the correlation of clinical features including diagnosis, current medical conditions, exercise habits, current health care costs and outcomes.

Results:

Some basic/preliminary analysis has been done using PPMI data on current medical conditions and type of exercise in which the patients are involved.

The most frequently occurring medical conditions among the PPMI participants were found to be Musculoskeletal and Cardiovascular followed by Ophthalmological types.

Among the PPMI participants, it was found that almost half of them did not exercise at all as part of their leisure activities, about one quarter of them exercised leisurely. The rest exercised rarely.

The following household activities were tracked: Light housework, Heavy housework, Lawn work, Outdoor Gardening, Home repairs, Volunteer work, Caregiving.

Most participants were engaged in Light Housework, Heavy housework volunteer work and lawn work. Comparatively lesser numbers of patients were engaged in Home repairs and Caregiver roles or Outdoor gardening.

Conclusion:

Its large sample sizes of HCUP and PPI data enables us to analyse rare conditions, uncommon treatments and special patient populations. The preliminary study has support the hypothesis that intense physical exercise can lead to the improvement of health and quality of life by lowering the progression of disease and treatment and medication cost accordingly.

Basic Science ***Graduate Student***

POSTER #44

IDENTIFYING PROTEINS AND RNAS THAT INTERACT WITH ONCOGENIC ETS

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Of the twenty-eight ETS transcription factors, four are oncogenic and found as chromosomal rearrangements in about 50-75% of prostate cancers. However, the molecular mechanism underlying the oncogenic phenotype remains unknown. Identifying molecular interaction networks could unveil clues to oncogenic ETS functions. Previous mass spectrometry analysis has revealed interactions between ERG, an oncogenic ETS protein, and RNA binding proteins. Our lab is using RNA Immunoprecipitation followed by next-generation sequencing or qRT-PCR (RIP-seq, or RIP-qPCR) to probe the interaction between RNAs and ERG. Additionally, published reports indicate that Androgen Receptor (AR), a critical regulator of prostate cancer, interacts with ERG, but the specificity of this interaction across the ETS family is unknown. We are currently performing in vitro biochemical and functional assays to measure the specificity of AR-ETS interactions and to assess the role of the interactions in prostate cancer cell migration. Together these approaches can identify potential therapeutic targets for ETS-positive prostate cancer.

Basic Science ***Graduate Student***

POSTER #45

KIF14 OVEREXPRESSION ACCELERATES TUMOR DEVELOPMENT IN THE TAG-RB TRANSGENIC MODEL OF RETINOBLASTOMA

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The mitotic kinesin *KIF14* is a molecular motor that plays a pivotal role in the final stages of cytokinesis. In most retinoblastoma tumors the *KIF14* locus at 1q32.1 is gained as an important event after loss of the *RB1* gene. Moreover, *KIF14* is overexpressed in retinoblastoma and a number other cancers, such as breast, ovarian and lung strongly suggesting its role as an oncogene. In these latter cancers, it is a prognostic factor and is required for tumor cell growth in vitro. Despite this, *KIF14's* role in retinoblastoma progression has not previously been studied in vivo. Here we aimed to determine *Kif14*'s role in promoting retinal tumor formation. Understanding the effects of *Kif14* overexpression in vivo will allow for a greater understanding of the biology of post *RB1* loss events and how they contribute to retinoblastoma progression. By crossing transgenic mice constitutively overexpressing *Kif14* into the SV40 large T-antigen retinoblastoma (TAg-RB) model, we generated *Kif14*; TAg-RB double transgenic mice. The Micron III rodent imaging system was used to obtain fundus photographs as well as optical coherence tomography images. Double transgenics and TAg-RB littermates were imaged in both eyes over a time-course to document tumor development. Mice were sacrificed for histological analysis of tumor burden at various ages. Compared to the TAg-RB single transgenic mice, the *Kif14*; TAg-RB double transgenic mice showed accelerated formation of tumor-like clusters of hyper-reflective cells in the inner nuclear layer of the retina from as early as two weeks of age. In the double transgenic mice, these clusters filled the inner nuclear layer, while the clusters remained isolated in the single transgenics. This difference was maintained through development. Tumor burden was significantly greater in *Kif14*; TAg-RB than in TAg-RB eyes (15% vs. 5.0% of retinal area, Mann-Whitney P=0.016) by immunohistochemical analysis at 8 weeks of age.In conclusion the over-expression of the *Kif14* oncogene in the TAg-RB model of retinoblastoma leads to accelerated onset of tumor formation and total tumor burden, providing strong evidence that *Kif14* can promote retinoblastoma formation in susceptible cells in vivo. This increased understanding the effects of Kif14 overexpression in vivo will allow for a greater understanding of the biology of post RB1 loss events and how they contribute to retinoblastoma progression.

Basic Science ***Graduate Student***

POSTER #46

DETERMINING THE MOLECULAR MECHANISM OF A KEY DRIVER OF ALTERED CELL MIGRATION AND RNA EDITING IN GLIOBLASTOMA

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Gene expression is a tightly regulated process in the development of all organisms, allowing for adaptability and versatility of an organism over its lifetime. In cancer, improper control of gene expression results in increased cell invasion and reduced cell death. RNA editing is a process that alters gene expression by specifically changing nucleotides in the RNA from that encoded by the genome. In a normal cell, RNA editing can change codons, alter splice sites, and affect miRNA binding, among other functions. One specific type of RNA editing is the deamination of adenosine (A) to inosine (I), a non-canonical nucleoside that is recognized by the cell's translational machinery as guanosine. Recently, over one million inosines have been discovered in the human transcriptome and misregulation of RNA editing has been shown to promote cancer.

In humans, three proteins have homology with ADARs, the enzymes that catalyze A-to-I RNA editing. While ADAR1 and ADAR2 are ubiquitous, ADAR3 is specifically expressed in the nervous system. Furthermore, ADAR1 and ADAR2 have been shown to catalyze RNA editing reactions, while initial *in vitro* studies of recombinant ADAR3 have suggested that the protein lacks the ability to edit RNA. Currently, there is no known function for the brain-specific ADAR3; however, previous studies have suggested an unelucidated regulator of RNA editing in glioblastoma and my data supports the hypothesis that ADAR3 does not edit mRNA, but is important in regulating editing in neural cells.

In the context of cancer, reduced RNA editing of the glutamate receptor was reported in glioblastoma (GBM) patients. Editing at the Q/R site of the glutamate receptor subunit B (GluRB) renders the channel impermeable to calcium and the editing event is required for survival in humans. In addition, decreased RNA editing of GluRB increases calcium permeability, which in turn activates Akt signaling to increase the invasive growth of GBM cell lines. Although this editing event is known to be carried out by ADAR2, the levels of ADAR2 do not correlate with RNA-editing at this site, implying that a cellular mechanism that regulates ADAR2 editing activity exists and is misregulated in GBM. I have created overexpression and knockdown constructs of ADAR3 in glioblastoma cell lines to test whether ADAR3 regulates RNA editing at GluRB. My data suggests the requirement of balanced ADAR levels to regulate editing in GluRB and that ADAR3 can inhibit GluRB editing when overexpressed. These data are supported in glioblastoma tumor patient samples through the correlation of ADAR2 and ADAR3 levels with GluRB editing. Furthermore, my data suggest that ADAR3 affects glioblastoma cell migration, independent of GluRB editing and Akt signaling.

Basic Science ***Graduate Student***

POSTER #47

TARGETING LNCRNA HOTAIR WITH PEPTIDE NUCLEIC ACIDS IN BREAST AND OVARIAN CANCERS

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The recently discovered long noncoding RNAs (lncRNAs) are emerging as key contributors to cancer biology. The Hox transcript antisense intergenic RNA termed HOTAIR was one of the first identified lncRNA to exert epigenetic repression in trans on distant chromosomes. By shuttling the polycomb repressive complex (PRC2) and lysine specific demethylase 1 (LSD1) to specific gene targets, HOTAIR represses gene expression through trimethylation of histone H3 on lysine K27 (H3K27me3) and uniquely exerts epigenetic repression. We and others have reported that HOTAIR expression is altered in ovarian cancer, the fifth leading cause of cancer death in U.S. women and the deadliest form of all gynecological cancer, and that HOTAIR contributes to tumorigenesis, cisplatinum (CDDP) resistance and is a potential ovarian cancer biomarker. We further identified HOTAIR as a transcriptional target of NF-κB during DNA damage and activate NF-κB, causing a positive feedback activation of this pathway leading to increased IL-6 secretion and senescence. To assess the functional role of PRC2 in NF-κB activation, we utilized a novel strategy that includes sequence-specific peptide nucleic acids (PNAs) targeting the 89-base minimum PRC2-interacting region of HOTAIR. Biological effects of candidate inhibitors were assessed *in vitro* and *in vivo* using ovarian (A2780, A2780_CR5, and Kuramochi) and breast cancer cell lines (MCF-7, SKBR3 and MDA-MB-231). We measured cell proliferation, invasion, gene expression changes and colonogenic survival assays to test the effect of the candidate PNAs. We report for the first time a peptide inhibitor specific for HOTAIR-PRC2 interaction. The use of PNAs to study PRC2-independent roles of HOTAIR in cancer biology may represent a novel therapeutic strategy for overcoming CDDP-resistance in breast and ovarian cancers.

Basic Science ***Graduate Student***

POSTER #48

A BI-SPECIFIC CHIMERIC ANTIGEN RECEPTOR T-CELL FOR TARGETING PANCREATIC ADENOCARCINOMA.

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Chimeric antigen receptor T-cells (CART-cells) are cytotoxic T-cells which have been genetically engineered to recognize specific cell surface antigens. While CART-cells specific to a single surface antigen have proven successful in targeting a number of cancer cells in recent studies, the potential for such CART-cells to engage against normal cells expressing the antigen is a significant safety concern. This so-called "on-target, off tumor" effect has resulted in fatalities in human clinical trials. Recent advances in CART-cell design have led to the inclusion of suicide genes in CART-cells, allowing for total, rapid eradication of the cells at the onset of severe adverse effects. However, activating the suicide gene effectively ends the therapy in addition to addressing side effects. Limiting the ability of the CART-cells to target non-tumor cells in the first place is therefore of significant clinical advantage. Here, we evaluate the potential of bi-specific CART-cells in targeting pancreatic adenocarcinoma, as a CART-cell specific to two surface antigens should activate only when both antigens are recognized, narrowing down the specificity of the CART-cell to the target tumor population. The epidermal growth factor receptor (EGFR) and mesothelin are ubiquitously expressed on the surface of pancreatic adenocarcinoma cells. Therefore, in this study, we evaluate a CART-cell specific for both EGFR and mesothelin. Using GFP+ labeled EGFR+/mesothelin-, EGFR-/mesothelin+, and EGFR+/mesothelin+ target cells co-cultured with mCherry labeled CART-cells specific for (a) EGFR alone (b) mesothelin alone, or (c) EGFR and mesothelin together, we present a flow cytometric method for evaluating the specificity and efficacy of bi-specific CART-cells in comparison with CART-cells specific to a single antigen. We propose the use of the bi-specific CAR in conjunction with the inducible caspase 9 suicide gene, to maximize treatment potential while also retaining the ability to "stop" the cells should adverse effects arise.

Basic Science ***Graduate Student***

POSTER #49

MODELING BREAST CANCER PROGRESSION IN LRECM 3D CELL CULTURE SYSTEM TO STUDY DISEASE MECHANISMS AND DRUG RESPONSE.

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Background: Preclinical cell line models developed from high-risk, non-malignant breast tissue of women genetically predisposed to breast cancer can be a renewable resource for *in-vitro* exploratory, drug screening and response studies. Toward this goal, we utilized contralateral breast tissue of a Li-Fraumeni syndrome (LFS) patient (patient 50, germline *TP53* [M133T] mutation) undergoing breast cancer surgery to generate human mammary epithelial (HME) LFS progression series. This HME LFS50 series comprises of distinct pre-immortal, spontaneously immortalized, hTERT-immortalized, HrasV12-transformed/pre-invasive and tumorigenic cell lines and can be used to model different stages of breast cancer. Incorporating tissues derived from individuals predisposed to cancer can clarify genotype-phenotype associations and accelerate development of physiologically relevant drug response assays that are crucial to design effective drug combination therapies.

Methods and Results: We have characterized the HME LFS50 cell lines in vitro as monolayers, 3D cultures by immunostaining actin and apico-basal markers, and by bioinformatics approach. Gene expression signatures of LFS50 series cells were profiled using HG-U133_Plus_2 Affymetrix chips, principal component analyses (PCA), and hierarchical clustering. Furthermore, using Ingenuity Pathway Analysis (IPA^{TM}) we identified the most differentially expressed genes and canonical pathways that also support the TNBC subtyping results and provide rational targeting for chemoprevention studies. The gene expression profiles show deregulation of genes involved in migratory and invasive programs typically observed in epithelial-mesenchymal transition. We have further determined the effects of p53 rescue agents and chemopreventive agents such as EGCG and resveratrol, and found that tumorigenic HMET cells were most sensitive to these agents as compared to non-malignant HME50-hTERT and MCF10A controls. In addition, anchorage-independent growth could be prevented after treatment with these agents for the HME50hTR pre-invasive cells. As a proof-of-principle for drug targeting, treatment of the LFS50 series with PRIMA-1 using 3D cultures resulted in a reduction in acini size of the pre-malignant and tumorigenic LFS50 cells ($p<0.05$). Finally, we have performed qRT-PCR validation and confocal imaging to understand effect of drugs on HME50 cell lines. We also have grown and morphologically characterized primary breast cells derived from tissues donated by healthy volunteer in 3D lrECM cultures that can serve as normal controls in drug response assays.

Conclusion: We have developed and characterized a breast cancer progression series, which is consistent and reproducible in 3D lrECM system for functional assays, drug screening and drug response. Each of the HME50 cell lines exhibited distinct growth characteristics in monolayer as well as in 3D cultures, with differential gene expression profiles that can model different stages of breast cancer and provide rational targets for drug screening and targeting studies. The use of well-characterized, high-risk patient-derived and physiologically relevant 3D preclinical models in conjunction with genomic profiling can potentially guide the development of safer and more effective therapeutic approaches.

Basic Science ***Graduate Student***

POSTER #50

CRITICAL ROLE OF PHOSPHORYLATION OF SERINE 165 OF YBX1 ON THE ACTIVATION OF NF-&KAPPA;B 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 highly correlated with cancer progression, making it an excellent marker in cancer. The connection between YBX1 and the important nuclear factor kB (NF-kB), 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 dramatically reduced NF-kB activating ability 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 NF-kB-inducible genes, reduced cell growth, and compromised tumorigenic ability 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 to treat colon cancer.

Basic Science ***Graduate Student***

POSTER #51

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 host of distinct downstream signaling pathways that play an important role in disease progression. In particular, oncogenic Ras has been shown to inhibit anoikis, a form of apoptotic cell death caused by detachment from the extracellular matrix (ECM). This inhibition of anoikis by Ras has previously been shown to be due to impaired mitochondrial cytochrome *c* release caused by modulation of Bcl-2 family proteins. In this study, we have found that Ras-mediated anoikis inhibition also occurs downstream of mitochondrial cytochrome *c* release. While the precise mechanisms by which Ras antagonizes anoikis following cytochrome *c* release remain poorly defined, our data suggest that this cell death inhibition is not dependent on changes in the quantity of the downstream effectors Apaf-1, pro-caspase-9, or pro-caspase-3. 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 an enhanced sensitivity to exogenous cytochrome *c*. This sensitization was found to be due to upregulation of apoptosomal proteins in an ERK/MAPK dependent manner. In aggregate, our data support a model whereby the sensitivity of cells containing hyperactivating Ras mutations to cytochrome *c*-induced apoptosisis dependent on the engagement of cells with ECM proteins. Additionally, our data suggest that cells with oncogenic Ras-mutations may be susceptible to therapeutic agents that mimic the actions of cytochrome *c* during period of ECM-attachment.

Basic Science ***Graduate Student***

POSTER #52

REGULATION OF SENESCENCE IN FLT3-ITD-EXPRESSING CELLS BY THE PROTEIN TYROSINE PHOSPHATASE, SHP2

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FMS-like tyrosine receptor kinase-internal tandem duplications (FLT3-ITDs) are found in 25% of acute myeloid leukemia (AML) patients and portend a poor prognosis. We have shown that the protein tyrosine phosphatase, Shp2, is associated with FLT3-ITD and positively regulates proliferation and aberrant STAT5 activation. We also demonstrated that genetic disruption of the gene encoding Shp2 increased malignancy specific survival of animals transplanted with FLT3-ITD-transduced cells, suggesting that Shp2 may regulate the function of the malignancy initiating cell following transplant. As both Shp2 and STAT5 have been shown to play a crucial role in normal HSPC function, and as Shp2 has been shown to regulate senescence through Bmi1 expression, ***we hypothesized*** that FLT3-ITD+ HSPCs are sensitized to senescence upon loss of Shp2 due to downregulation of Bmi1 and upregulation of p16/p19.

To examine the importance of Shp2 in FLT3-ITD-expressing HSPCs, we examined engraftment of WT-FLT3- and N51-FLT3-transduced lineage negative (Lin-) bone marrow cells *in vivo* after genetic disruption of Shp2. WT-FLT3- or N51-FLT3-transduced bone marrow Lin- low density mononuclear cells (LDMNCs) from $Shp2^{flox/flox}$;Mx1Cre+ (CD45.2+) mice were transplanted into lethally irradiated recipients (F1, CD45.2+/CD45.1+), and animals were treated with polyI:polyC to induce genetic disruption of Shp2. Upon genetic disruption of Shp2, chimerism dropped in animals transplanted with N51-FLT3-transduced cells, and remained significantly lower compared to animals transplanted with WT-FLT3-transduced cells. To examine the role of Shp2 in HSPC function in cells expressing physiologic levels of FLT3-ITD, we transplanted LDMNCs from polyI:polyC-treated $FLT3\text{-}ITD^{+/-}$;$Shp2^{flox/+}$;Mx1Cre- or $FLT3\text{-}ITD^{+/-}$;$Shp2^{flox/+}$;Mx1Cre+ mice (CD45.2) into lethally irradiated BoyJ recipients (CD45.1). While chimerism increased steadily in mice transplanted with cells expressing both alleles of Shp2, there was little engraftment of cells heterozygous for Shp2, suggesting that function of FLT3-ITD+ HSPCs is Shp2-dependent.

To determine the cellular mechanism accounting for the reduced function of FLT3-ITD+ HSPCs lacking Shp2, we examined senescence levels. Lin- LDMNCs from polyI:polyC-treated $Shp2^{flox/flox}$;Mx1Cre+ mice were transduced with WT-FLT3 or N51-FLT3 and evaluated senescence by -galactosidase staining. Significantly higher levels of senescence were observed in N51-FLT3-expressing cells compared to WT-FLT3-expressing cells. Accordingly, bone marrow LDMNCs from polyI:polyC-treated $FLT3\text{-}ITD^{+/-}$;$Shp2^{flox/+}$;Mx1Cre+ mice demonstrated significantly elevated senescence compared to $FLT3\text{-}ITD^{+/-}$;$Shp2^{flox/+}$;Mx1Cre- mice as well as downregulation of Bmi1 and compensatory upregulation of p16 and p19 expression.

To determine the link between Shp2 and Bmi1, we examined the *Bmi1* promoter and found highly conserved STAT5 consensus sequences near the transcription start site. We compared levels of STAT5 bound to the Bmi1 promoter in the presence (MV411 cells) and absence (HL60 cells) of FLT3-ITD by chromatin immunoprecipitation (ChIP). Indeed, we found more STAT5 bound to the *Bmi1* promoter in MV411 compared to HL60 cells. Collectively, this suggests an increased dependence of FLT3-ITD-expressing HSPCs on Shp2 and that Shp2 regulates senescence in HSPCs through STAT5-dependent expression *Bmi1* expression.

Basic Science ***Graduate Student***

POSTER #53

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. Both options generally 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. In here we present our advances in the preservation of the bone microenvironment and the survival of our colonizing mammary epithelial cancer cells after 4 weeks in co-culture.

We propose to use this model to understand fundamental questions of bone metastases and to test therapies prior to use in patients. We will use this *ex vivo* bone culture bioreactor to identify the molecular factors that contribute to develop bone metastases and to aid in the screenings of new drugs aimed at targeting bone metastasis in breast cancer 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 #54

CHARACTERIZATION AND METHYL-CAPTURE SEQUENCING OF CELL-FREE DNA IN PLASMA OF PLATINUM-RESISTANT OVARIAN CANCER PATIENTS

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OBJECTIVES/SPECIFIC AIMS: Diagnostic biomarkers for ovarian cancer are urgently needed and cell-free DNA (cfDNA) in plasma offers an attractive, non-invasive alternative to tumor tissue biopsies.

METHODS/STUDY POPULATION: Plasma samples (124) were obtained from patients (n=10) participating in an ongoing Phase II clinical trial using SGI-110, a DNMT inhibitor, to re-sensitize recurrent platinum-resistant ovarian cancer to carboplatin. cfDNA was extracted from 1mL plasma with the Circulating Nucleic Acids Kit (Qiagen), quantified with Qubit Fluorometer, determined size with Bioanalyzer, and subjected to fragmentation.

RESULTS/ANTICIPATED RESULTS: Total cfDNA yields ranged from 2.6ng - 3.2µg with a mean of 85ng. Consistent with previous reports, sizes of cfDNA was found to be both 180bp and approximately 5kb. To achieve a library input target size of 180bp, optimal sonication specification of cfDNA was 30sec On/90sec Off for 120 cycles. Life Technologies MethylMiner a methylated DNA enrichment kit is currently being modified in order to detect low input of DNA (<25ng).

DISCUSSION/SIGNIFICANCE OF IMPACT: Analysis of cfDNA allows the detection of tumor-related genetic and epigenetic alterations relevant to cancer development and progression. More specifically, a signature from the cfDNA methylome can be correlated with patient data to serve as a diagnostic biomarker for ovarian cancer.

Basic Science ***Graduate Student***

POSTER #55

HSC-INDEPENDENT YOLK SAC PROGENITORS BEAR HALLMARKS OF JMML IN A PTPN11-D61Y MOUSE MODEL

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Juvenile Myelomonocytic Leukemia (JMML) is a fatal pediatric myeloproliferative neoplasm (MPN) that develops *in utero*. Patients typically present with anemia and marked hepatosplenomegaly and they succumb to bleeding or infection. JMML is caused by somatic mutations in Ras-Erk signaling genes, most commonly gain of function mutations in PTPN11, which cause growth hypersensitivity to the hematopoietic cytokine GM-CSF. Chemotherapy is not an effective JMML treatment and 50% of patients relapse following the only curative therapy allogeneic hematopoietic stem cell (HSC) transplantation. The reasons for this markedly high relapse rate are unknown. Tissue macrophages are unique among hematopoietic cells in persisting after HSC transplantation. As such, their involvement in the pathogenesis of JMML could explain the high relapse rate following HSC transplantation. Recent studies have shown tissue macrophages to descend from embryonic HSC-independent progenitors in the yolk sac. In this study, we sought to determine whether these HSC-independent yolk sac progenitors bear hallmarks of MPN in a mouse model of JMML.

Using the Vav1 promoter-directed Cre recombinase, we generated a mouse model of JMML that expresses the $PTPN11^{D61Y}$ gain of function mutation in all waves of embryonic and adult hematopoiesis. $PTPN11^{D61Y/+}$; VavCre+ mice are viable, born at expected Mendelian ratios, and develop peripheral blood monocytosis as early as 4 weeks of age. E14.5 fetal liver progenitors from $PTPN11^{D61Y/+}$; VavCre+ embryos displayed GM-CSF hypersensitivity in colony forming assays at all measured doses of GM-CSF ($p<0.05$, $n=7$; all statistical analyses performed using two-tailed Student's t-test) and possessed hyperactive Ras-Erk pathway signaling by western blotting. Since the E14.5 fetal liver contains all waves of embryonic hematopoiesis, we restricted our subsequent analysis to the E9.5 yolk sac, which contains only HSC-independent hematopoietic lineages. Compared to littermate controls, $PTPN11^{D61Y/+}$; VavCre+ E9.5 yolk sac progenitors demonstrated marked GM-CSF hypersensitivity in colony forming assay at GM-CSF doses between 0.01-1.0 ng/ml ($p<0.05$, $n=8$). Purified $Ter119^{-}$, $cKit^{+}$, $CD41^{DIM}$ erythro-myeloid progenitors from the yolk sac recapitulated this GM-CSF hypersensitivity ($p<0.05$, $n=6$). Additionally, cultured $PTPN11^{D61Y/+}$; VavCre+ yolk sac progenitors possessed hyperactive Ras-Erk signaling by intracellular flow cytometry using antibodies against pErk and pSTAT5 ($p<0.05$, $n=6$).

We have demonstrated that HSC-independent myeloid lineages from the murine yolk sac possess GM-CSF hypersensitivity and Ras-Erk pathway hyperactivation in a mouse model of JMML. These findings suggest that HSC-independent macrophages may be involved in the development of JMML *in utero*. They further highlight the need to assess the role of bone marrow independent myeloid lineages in pediatric MPN in order to begin the development of therapeutics that can specifically target these populations.

Basic Science ***Graduate Student***

POSTER #56

FUNCTIONS OF P53 ISOFORMS IN STRESS RESPONSE AND DEVELOPMENT

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p53 is a tumor suppressor and plays a major role in the cellular responses to genotoxic stress that preserve genome integrity. A majority of cancers harbor mutations in the p53 gene or a defect in the p53 pathway. In vertebrates, p53 and its paralogs p63 and p73, produce numerous protein isoforms as a result of alternative splicing, alternative promoters and different translation start sites. Emerging evidence suggests that these protein isoforms have different functions in stress response and development. Evidence also suggests that p53 family protein isoforms are differentially expressed in and may contribute to different cancers. However, the roles of these isoforms are not fully defined. We have used the model organism *Drosophila melanogaster* to study the role of different p53 isoforms. The *Drosophila* p53 gene encodes four different protein isoforms- p53A, p53B, p53D and p53E. Historically, investigations have focused on p53A, but the physiological functions of the other isoforms have not been defined. We found that the longest isoform, p53B, is the most potent inducer of cell death when overexpressed. In contrast, overexpression of the short p53E or p53D isoforms inhibits the apoptotic response to radiation. Analysis of isoform-specific loss-of-function mutants indicated that p53A is necessary and sufficient for the apoptotic response to radiation. The p53B isoform was not required, even though it is the most potent inducer of apoptosis when over-expressed. What then is the physiological function of p53B? To address this, we examined the expression of p53A and p53B in fly strains transformed with fluorescent GFP-p53A and mCherry-p53B BAC clones. We found that p53A and p53B co-localize with each other in nuclear compartments and form complexes composed of p53A/p53B heterotetramers. Although p53A is expressed in both somatic and germline cells, p53B is predominantly expressed in the germline. It was previously shown that the expression of p53 target genes is transiently increased during meiosis, likely induced by DNA double strand breaks during recombination. We have found that this "pachytene checkpoint" in meiosis is dependent on the p53B isoform. Our findings are in agreement with those from mouse, which showed that p53 family members have important functions during early mammalian oogenesis. In both flies and mice, specific p53 family members may have important developmental functions to coordinate meiotic cell cycles and cull out defective oocytes. In addition to dissecting the role of p53 isoforms during meiotic recombination, we are also investigating their function in germline stem cells. Our investigation of p53 isoforms in the tractable genetic model *Drosophila* will provide insight into the functions of human p53 family proteins during development and cancer.

Basic Science ***Graduate Student***

POSTER #57

ROLE OF EIF3A EXPRESSION IN CELLULAR SENSITIVITY TO RADIATION TREATMENT

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Translation Initiation in protein synthesis is a crucial controlling mechanism of gene expression involving eukaryotic translation initiation factors (eIFs). eIF3a, the largest subunit of eIF3 complexes, has been shown to regulate protein synthesis and cellular response to cisplatin treatment. Its expression has also been shown to negatively associate with prognosis. In this study, we tested a hypothesis that eIF3a regulates synthesis of DNA repair proteins, which in turn regulates cellular response to ionizing radiation (IR) treatments. We found that eIF3a overexpression sensitizes cellular response to IR while its knockdown causes resistance to IR. We also found that eIF3a over-expression increases IR-induced DNA damage and decreases Non-Homologous End Joining (NHEJ) activity by suppressing expression level of NHEJ repair proteins such as DNAPKcs. eIF3a knockdown decreases IR-induced DNA damage and increases Non-Homologous End Joining (NHEJ) activity by enhancing expression level of NHEJ repair proteins such as DNAPKcs. Together, we conclude that eIF3a plays an important role in cellular response to DNA-damaging treatments by regulating synthesis of DNA repair proteins and, thus, eIIF3a likely plays an important role in outcome of cancer patients treated with DNA-damaging strategies.

Basic Science ***Graduate Student***

POSTER #58

INHIBITION OF SURVIVIN IN PROSTATE EPITHELIUM REDUCE INFLAMMATION INDUCED PROSTATE EPITHELIUM HYPERPLASIA

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Background

Survivin is highly expressed in inflammation-associated proliferative conditions including prostate cancer and Benign Prostatic Hyperplasia (BPH), but not in non-inflamed prostate. Survivin expression is highly regulated during cell cycle and is needed for cells to survive noxious conditions. However, the function of survivin in prostatic inflammation-induced reactive hyperplasia is under studied. The purpose of this study was to determine the role of survivin in inflammation-induced prostate reactive hyperplasia.

Methods

We initiated inflammation in mouse prostates by instilling urogenital pathogen *E.coli* strain 1677 via urinary tract catheter. Prostates were harvested daily for 5 days after inflammation initiation; these were compared to uninflamed control prostates. Survivin inhibitor LQZ-7F (25mg/kg) or vehicle was intraperitoneal injected 24h before bacteria instillation and at Day 2 after instillation. Mice were BrdU labelled and sacrificed 3 days after instillation, previously determined to exhibit maximal epithelial proliferation and hyperplasia in this model. Reactive hyperplasia was evaluated by histological staining (H&E) and BrdU labeling. Expression of survivin was determined by immunofluorescence. The effects of pro-inflammatory factors IL-1 and IGF-1 were examined in IL-1R1 KO mice and in the presence or absence of IGF-R1 antagonist picropodophyllin (PPP), respectively. Anti-proliferation effect of LQZ-7F on prostate non-cancer cell lines E6 and E7 was evaluated by MTS assay.

Results

The percentage of survivin positive prostate epithelial cells increased from 0.5% of total epithelial cells in uninflamed controls to 50.2% in inflamed mouse prostates 5 days post instillation. Selective expansion of survivin-positive epithelial cells was dependent on IL-1 signaling as inflammation-induced survivin was attenuated in IL-1R1 KO mice. The IGF-R1 antagonist PPP also attenuated inflammation-induced survivin expression by 50% (21% of prostate epithelial cells compared to 44% in vehicle-treated mice). Treatment of epithelial cell lines E6 and E7 with LQZ-7F (5uM) entirely blocked cell proliferation *in vitro* with or without the presence of IGF-1. Further, inflamed mouse prostates exhibited 89% co-localization of survivin+ cells with BrdU incorporation. Finally, *in vivo* treatment with LQZ-7F entirely blocked the expansion of survivin-positive cells and reduced the percentage of BrdU-labeled cells by 80%.

Conclusions

Our data indicate that inflammation-induced survivin is required for epithelial cell survival and proliferation during the proliferative phases of repair and recovery. Further, our data indicate that this survivin-mediated epithelial expansion is dependent upon IL-1 to IGF-1 signaling interactions. The development of superior survivin inhibitors is ongoing, and may result in improved therapy options in proliferative diseases of the prostate such as prostate cancer and BPH.

Basic Science ***Graduate Student***

POSTER #59

IGFBP FAMILY REGULATION OF CANCER CELL SURVIVAL

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Carcinoma-associated fibroblasts (CAFs) are an integral part of the tumor microenvironment and are now widely appreciated to contribute to tumor progression. However, the ability of CAFs to regulate cancer cell survival, such as evasion of detachment-induced cell death, termed anoikis, is poorly understood. To date, we have discovered a novel role for CAFs in blocking anoikis in multiple cell lines, facilitating luminal filling in 3D cell culture, and promoting anchorage-independent growth. In addition, we have uncovered a novel mechanism underlying this anoikis inhibition. Our findings demonstrate that CAFs secrete elevated quantities of insulin-like growth factor-binding proteins, IGFBPs, that are both necessary for CAF-mediated anoikis inhibition and sufficient to block anoikis in the absence of CAFs. Furthermore, our data reveal a unique anti-apoptotic mechanism for IGFBPs: the stabilization of the anti-apoptotic protein Mcl-1 within tumor cells. Finally, the presence of CAF-derived IGFBP-2 promotes robust tumor formation *in vivo*. In aggregate, these data reveal a novel role for CAFs in promoting cell survival during detachment and unveil an additional mechanism by which the tumor microenvironment contributes to cancer progression.

In addition to these findings, we have also discovered that factors secreted by normal mammary fibroblasts (NMFs, which are not carcinoma-associated) can actually promote the induction of apoptosis in breast cancer cells independently of other death stimuli. Our preliminary data suggest that the factors secreted by NMFs may antagonize the aforementioned IGFBP-mediated protection and that the relative quantities of IGFBP proteins may serve as a rheostat to influence the ultimate survival of breast cancer cells. These data suggest that different types of fibroblasts can utilize different secreted factors to alter the ratios of pro-and anti-apoptotic proteins within tumor cells. Importantly, modulation of these secreted factors could serve as an attractive strategy to specifically eliminate cancer cells.

Basic Science ***Graduate Student***

POSTER #60

HYPOXIA-INDUCED LONG NON-CODING RNA AFFECTS BREAST CANCER METASTASIS THROUGH MODULATION OF CELL METABOLIC PROGRAMS

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Long noncoding RNAs (lncRNAs) have been shown to exhibit a large variety of regulatory roles on virtually all cancer-relevant pathways. Moreover, that aberrant expression of specific lncRNAs modulates tumor development and progression. Hypoxia, as a key feature of tumor microenvironment has been shown to regulate coding transcripts and microRNAs, however there is very little information regarding the possible involvement of lncRNAs in the response to low oxygen. To bridge this knowledge gap, we performed full-transcriptome analysis of normoxic and hypoxic MCF-7 cells and identified a subset of hypoxia-induced lncRNAs. Among these, lnc-MKL2-1, a previously uncharacterized transcript was confirmed as the most robustly and consistently induced by hypoxia across a diverse panel of cancer lines and untransformed cells. Unlike classic hypoxia-inducible genes, lnc-MKL2-1 induction is independent of Hypoxia-Inducible Factors (HIF) and affected by the presence of additional microenvironmental components, in particular glucose and TGF-beta. Meta-analysis of level 1 data from The Cancer Genome Altas revealed strong correlations between lnc-MKL2-1 expression and hypoxia signatures in triple negative breast cancer and pancreatic adenocarcinomas. Additional correlations with metabolic genes were identified, in particular fatty acid synthesis and cholesterol metabolism, further indication of a functional connection between lnc-MKL2-1 and cell metabolism. In *in vitro*, knocking down lnc-MKL2-1using multiple siRNAs and shRNA showed that this transcript has the characteristics of a tumor suppressor, as its inactivation promoted cell invasion, while ectopic over-expression achieved an opposite effect. RNA-seq on MCF-7 cells following knockdown or overexpression of lnc-MKL2-1 revealed that is a candidate negative regulator of cholesterol biosynthesis pathway, in particular the mevalonate kinase branch. In vivo orthotopic studies using MDA-MB-231 cells with stable knockdown or overexpression in NSG mice confirmed and extended the in vitro effects, indicating that lnc-MKL2-1 is a repressor of metastasis. The mevalonate pathway genes showed similar alterations in response to lnc-MKL2 manipulation in vivo as in vitro. Moreover, blockade of cholesterol biosynthesis pathway with low doses of simvastatin fully reversed the biological effects observed in vitro. These data are consistent and extend the recent findings regarding the importance of the mevalonate kinase pathway and cholesterol metabolism in tumor growth and invasion, and may lead to a deeper understanding of the cancer metabolic networks. Finally we propose that lnc-MKL2-1 is part of a molecular feedback mechanism that integrates microenvironmental, metabolic and cellular motility pathways.

Basic Science ***Graduate Student***

POSTER #61

THE IMPACT OF MT1-MMP ON OVARIAN CANCER INTRAPERITONEAL METASTASIS

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Ovarian cancer is the fifth leading cause of cancer death in women. It is estimated that 21,290 women will be diagnosed with and 14,180 women will die from ovarian cancer in the United States in 2015. The majority of patients have metastasis or eventually will die of metastatic disease within the abdominal cavity. However, the early molecular and cellular events that promote ovarian cancer metastasis remain poorly understood. Nearly 90% of ovarian cancer is epithelial in origin. Epithelial ovarian cancer (EOC) metastasis occurs through a unique anchorage-independent mechanism, which involves shedding of both single cells and multi-cellular aggregates (MCAs) into the peritoneal cavity followed by intra-peritoneal implantation, and is often associated with peritoneal ascites. Recent data showed that serially selected MCAs exhibit a 10^4 increase in tumorigenicity relative to the same number of parental single cells. The factors that regulate the transition from free-floating MCA to peritoneal anchored metastatic lesion are currently unknown.Membrane type 1 matrix metalloproteinase (MT1-MMP, MMP-14) is a transmembrane collagenase highly expressed in ovarian tumors and correlates with poor survival. Our recent studies using wild type MT1-MMP and a cytoplasmic tail phosphomimetic (T567E) demonstrated that acquisition of MT1-MMP expression and/or phosphorylation promotes cellular detachment and MCAs formation. In the current study, we investigated the potential role of MT1-MMP in promoting EOC adhesion to intact mouse peritoneal mesothelium. MCAs produced using the hanging drop method were evaluated using light and scanning electron microscopy to assess aggregate area and overall morphology. The kinetics of MCAs dispersal on collagen surfaces was compared and quantified. Furthermore, a three dimensional *ex vivo* system with mouse peritoneal tissue explants was used to evaluate EOC MCAs interactions with intact peritoneal tissue. MCAs:mesothelium adhesion was significantly increased in EOC MCAs expressing phosphomimetic MT1-MMP-T567E mutants compared with EOC MCAs comprised of cells that express wild-type MT1-MMP, phosphodefective MT1-MMP-T567A, or catalytically inactive MT1-MMP-E240A. Our results suggest a role for MT1-MMP in regulating EOC MCAs adhesion to peritoneal mesothelium and sub-mesothelial anchoring. This initial event will eventually facilitate EOC progression to metastasis.

Basic Science ***Faculty***

POSTER #62

ELUCIDATING THE ROLE OF THE MTSS1 TUMOR SUPPRESSOR GENE IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related deaths in the United States with a 5-year survival rate of 6%. This dismal outlook is largely due to the inability to diagnose the disease before metastasis occurs. 53% of patients afflicted with pancreatic cancer are diagnosed at the metastatic stage. These patients are offered treatment regimens that are unsuccessful or palliative care to ease their pain. PDAC deaths will continue to rise unless meaningful research is undertaken to both uncover new gene targets that suppress metastatic progression and to augment current treatment strategies that are being found to be ineffective in the clinic. One potential therapeutic target is the tumor suppressor gene, MTSS1. Recent work has found that MTSS1 is suppressed in a number of different cancers. Interestingly, though MTSS1 is expressed normally in early phases of cancer, it is lost in the metastatic stages of the disease. Despite the evidence showing that MTSS1 could be important for the suppression of tumor metastasis, the role of this gene in PDAC has not be studied. Our preliminary findings show a correlation between the MTSS1 protein and COX-2 driven inflammation, which is an early hallmark of PDAC initiation and progression.Treatment with non-steroidal anti-inflammatory drugs (NSAIDs), inhibitors of COX-2, has been shown to decrease the risk of pancreatic cancer in early stage patients. Surprisingly, COX-2 inhibition does not increase survival in patients who have already progressed to metastatic disease. However, no studies have explored why COX-2 inhibition is ineffective in these late-stage patients. Our hypothesis is that COX-2 expression causes eventual loss of MTSS1. Once MTSS1 is lost, currently approved therapeutic regimens are no longer effective. We propose to use *in vitro* models and genetically engineered mouse models to elucidate the molecular mechanism behind both the role and regulation of MTSS1 in PDAC in order to uncover not only a novel tumor suppressive pathway in pancreatic cancer, but also to unlock a potentially new biomarker of aggressive disease that could yield significant therapeutic advantages in late stage PDAC patients.

Basic Science ***Graduate Student***

POSTER #63

TRIF AND MYD88 UNCOUPLE THE EFFECTS OF TLR4 MEDIATED INJURY ON HEMATOPOIETIC STEM CELLS AND MYELOID PROGENITORS DURING BACTERIAL INFECTIONS

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Sepsis is a clinical syndrome due to a systemic inflammatory response to severe microbial infection. High mortality rates in sepsis (200,000/yr in the USA) are associated with the host's failure to eradicate pathogens due to the lack of neutrophils, excessive pro-inflammatory cytokines, tissue damage and multiple organ failure. While most studies have focused on the pathological changes in the heart, lung and kidney, little is known about the changes occurring in the bone marrow (BM) at early stages of hematopoiesis and how they affect the hematopoietic response to bacterial infection. Using an animal model of severe sepsis induced by *Pseudomonas aeruginosa*, which closely recapitulate lethal sepsis in patients, we have previously reported that HSC undergo a significant expansion in the BM associated with a block of myeloid differentiation in a TLR4-dependent manner. TLR4 is activated by bacterial LPS and signals through two major pathways: TRIF-dependent and MyD88-dependent. In this study, we further dissected the distinct role of TRIF and MyD88 pathways in maintaining hematopoietic cell homeostasis during sepsis. We found that TLR4/TRIF is involved in the expansion of the HSC pool, but does not play a major role in the myelosuppression, whereas MyD88 activation in the hematopoietic compartment played a dominant role in LPS-induced myeloid suppression. Moreover, we observed that the loss of MyD88 prevents neutropenia both in a cell-autonomous and non-cell autonomous manner. "Septic" hematopoietic stem cells (HSC) showed impaired long-term engraftment (10%) and defective multilineage contribution. HSC self-renewal capability was significantly reduced and HSC exhaustion occurred. Interestingly, loss of TRIF but not of MyD88fully rescued HSC functional injury (long-term engraftment: 65% vs 40%, respectively) upon LPS challenge.

In order to identify the downstream molecules involved in myeloid suppression, we further examined two known downstream molecules (mediators) of the TLR4/MyD88 pathway: miR-21 and p38. Both this molecules are upregulate in a MyD88-dependent manner. Intriguingly, deletion of miR-21 in the BM did not rescue LPS-induced bone marrow dysfunction, demonstrating that miR-21 is not a critical regulator in these processes. Similarly, inhibition of p38 activity, also failed to prevent BM neutropenia. Therefore, further studies are warranted to determine the precise molecular mechanisms involved downstream of TLR4-MyD88/TRIF in the complex pathogenesis of BM response to sepsis.

Taken together, our results show for the first time that the TLR4/TRIF signaling is a key mediator of HSC damage during severe sepsis and that activation of the TLR4/MyD88 signaling pathway play a dominant role in myeloid suppression. These results will provide opportunity to develop therapeutic strategies for time-tuned and selective targeting the two distinct pathways in patients during sepsis. The findings can also be applied to other medical conditions where TLR4 signaling is activated, such as patients with graft-versus-host disease and patients receiving irradiation therapy.

Basic Science ***Graduate Student***

POSTER #64

HYPOTHESIS DRIVING PROTEOMIC STUDY ON THE EFFECT OF DIETARY UNSATURATED FATTY ACIDS ON PROSTATE CANCER

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Introduction and Objectives

Prostate cancer (PCa) is one of the most common cancers in men. In most PCa cases, tumors progress very slowly but in a small portion of patients, PCa develops into aggressive stages and becomes lethal. A line of evidence has suggested that early inference and dietary prevention are beneficial in PCa patient care. Fish oil (FO), which contains mostly omega-3 fatty acid (n-3 FA), is one of the most widely studied candidate supplements for PCa prevention; however, the molecular mechanism is not thoroughly understood. In addition, the function of oleic acid (OA), another kind of dietary oil, is also elusive. The goal of this study is to evaluate the different regulatory effects of FO and OA on a PCa cell line through global proteomic and phosphor-proteomic analyses.

Methods

Cell viability and fatty acid synthase (FASN) activity were assessed in PC3 cell line after different days of treatment by FO or OA. Then, an LC/MS-based label-free global protein quantification method was carried out and discovered that a set of proteins were significantly changed by FO in different stages of treatment. Validation was performed by Multiple-Reaction-Monitoring (MRM) and Western Blot analysis. Additionally, a global phosphor-proteome study was done to investigate the post-modification regulation of FO and OA.

Results and Discussion

This study shows that FO and OA both suppress FASN activity but only FO inhibits cell viability. Sequestosome-1 (SQSTM1), which is required by autophagy, was found to be over-expressed after one day of FO treatment. Global discovery also found following proteins being significantly changed in FO group after six days treatment: non-specific lipid-transfer protein (SCP2), fascin, integrin beta-1, calnexin and prostate-associated microseminoprotein (MSMP). Further MRM validation showed a decrease MSMP and an increase SCP2 in PC3 cells under FO treatment. The SQSTM1 level increase indicates that autophagy pathways might be involved in the FO regulation, while the decrease of MSMP in FO group may further explain inflammation inhibitory role of n-3 FA. Cell signal pathway analysis reveals that FO modulates pathways associated with cell cycle and glycolysis in different days of treatment. Additionally, the global phosphor-proteome study showed different phosphorylation patterns under different FA treatments.

Conclusions

Overall, this study shows that FO and OA both suppress FASN activity but only FO induces cell death. Global proteomic study and following validation showed that FO modulates protein levels of SQSTM1, MSMP, and SCP2, as well as pathways involving cell cycle and carbohydrate metabolism in different treatment days.

Basic Science ***Graduate Student***

POSTER #65

A20 A NOVEL SELENOPROTEIN-LIKE GENE INDUCED BY TGF-B

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A novel gene, A20 was identified using differential display technique in SMAD7 over-expressing human pancreatic cancer cells (hPCCs) in the presence of transforming growth factor-beta 1 (TGF-B1). A20 contains 4 exons and 3 introns with two putative open reading frames (ORFs). ORF-1 (A20-1) has a consensus Kozak sequence and encodes a 60 amino acid (AA) protein while ORF-2 (A20-2) encodes a 159 AA protein. The aim of our study was to characterize the translational products of A20 and test the hypothesis that A20 functions downstream of TGF-B1 to modulate cell functions. Accordingly, the full length cDNA was cloned in an expression vector and transiently transfected in 293T cells. Antibodies were raised against predicted protein sequences for both ORFs. An 18 kDa protein was detected for A20-2. By contrast, the 6.6 kDa product corresponding to the 60 AA residues for A20-1 was not observed. Instead, a 25 kDa band was detected. *In silico* analysis of the 3′-UTR region of A20 identified a selenocysteine insertion sequence (SECIS) element, a key feature of mRNAs that are translated into selenoproteins. The SECIS could allow read-through translation of the stop codon at the end of the 60th AA residue of ORF-1 to yield a 25 kDa product (233 AA) as confirmed by our findings. Preliminary data for functionality of the SECIS element in 3′-UTR of A20 mRNA are currently being generated. To assess the function of A20-2, ORF-2 was over-expressed in hPCCs, revealing increased ability of these cells to migrate by comparison with sham-transfected cells. Our future research is directed towards confirming the role of A20-2 in migration of hPCCs, determining its mechanisms of action, and analyzing the translation mechanism of A20-1 and its function in PDAC.

Basic Science ***Graduate Student***

POSTER #66

DONOR AGE AND MEDIA EFFECTS ON STROMAL CELL CULTURE

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Normal breast epithelial and stromal cells derived from healthy women with no history of breast cancer were examined for their replicative lifespan and characteristics of mammary gland tissue. These unique cells were generated from tissue collected at the Komen Tissue Bank (KTB) of Indianapolis, Indiana, and the expansion of this repository will provide a valuable resource foundation for any cellular based research. Epithelial denotes a surface cell while stromal refers to the connective tissue, and this pairing aimed to study the environment of breast cancer. We tested the hypothesis of a relationship among age and the cells' in vitro cellular growth as finite or infinite. Cells expressing finite growth, or a Hayflick limit, present clues to a given tissue's life potential. This limited cell growth is due to the shortening of genetic information at the telomere, or capped end of DNA. Results concluded younger donor stromal cells to grow and divide for a longer duration than older donor stromal cells. When grown in varying media, stromal cells divided approximately twice as fast in DMEM/F12 as in DMEM. Within the field of regenerative medicine, telomerase immortalization and cell differentiation provide many promising benefits in both cancer and stem cell therapeutics.

*Rudimentary abstract, would like to edit here sooner within the next few days. Already submitted my primary abstract for this conference though, so if this doesn't qualify without revision, that's fine.

Basic Science ***Medical Student***

POSTER #67

APC IN BREAST CANCER: THE ABCS OF GENE EXPRESSION

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Breast cancer is the most common malignancy and cancer-related cause of death in non-smoking women in the United States. Due to the heterogeneity of this disease, the oncogenic and signaling pathways contributing to these tumors are distinct. The *Adenomatous Polyposis Coli* (*APC*) tumor suppressor is mutated or hypermethylated in up to 70% of sporadic breast cancers; however, the mechanism by which *APC* mutation impacts tumorigenesis remains unexplored. Up-regulation of the zinc finger transcription factor EGR-1 has been associated with cell invasion and enhanced drug resistance, which is an important property of cancer stem cells (CSCs). ALDH1 and STAT3 have been useful in the identification of CSCs, which are associated with poor clinical outcomes including increased tumor survival and invasion. We utilized two human breast cancer cell lines, MDA-MB-157 and DU4475, and cells isolated from the Mouse Mammary Tumor Virus-Polyoma middle T (MMTV-PyMT;$Apc^{Min/+}$ mouse model. APC was knocked down in the MDA-MB-157 cells through shRNA lentiviral transduction, or transfected into DU4475 cells. First we looked at the expression of the transcription factor EGR-1 through Real-Time PCR in the MDA-MB-157 and DU4475 cell lines. Next, ALDH1 and STAT3 were measured in the MMTV-PyMT;$Apc^{Min/+}$ mouse model and in MDA-MB-157 cells through western blots. In addition, an ALDEFLUOR assay was used to measure ALDH1 activity in the mouse model. EGR-1 expression was increased in both MDA-MB-157 APC-knockdown and DU4475 *APC*-mutant cells compared to cells expressing APC. In addition, STAT3 expression was found to be increased in MDA-MB-157 APC-knockdown and MMTV-PyMT;$Apc^{Min/+}$ cells compared to MDA-MB-157 *APC*-wildtypeand MMTV-PyMT;$Apc^{+/+}$ cells. ALDH1 activity and tumor development were enhanced in $Apc^{Min/+}$ cells compared to control cells. Combined, these data suggest that APC knockdown increases tumorigenic properties of cancer cells through EGR-1 overexpression and modulation of known cancer stem cell markers, ALDH1 and STAT3. Future studies will include measuring the expression of EGR-1 in the MMTV-PyMT;$Apc^{Min/+}$ cells as well as ALDH1 in the human cell lines. Furthermore, because of the overexpression of these genes and markers, strategies designed to target positive populations might lead to more effective therapies of *APC*-mutant breast cancers.

Basic Science ***Other***

POSTER #68

CXXC FINGER PROTEIN 1(CFP1) PROMOTES MYELOID DIFFERENTIATION THROUGH EPIGENETIC REGULATION OF NF-KB SIGNALING PATHWAY

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kB signaling pathway is important in both embryonic gastrulation and myeloid differentiation, and ChP-seq analysis conducted in ES showed that CFP1 binds to transcriptionally active regions in NF-kB promoter. *Thus, we hypothesize that CFP1-induced myeloid differentiation through regulation of NF-kB*. To test our hypothesis, a NF-kB luciferase reporter assay was performed to measure the NF-kB activity in CFP1 overexpressed and knockdown cells. The results showed that NF-kB activity is regulated by CFP1 in both PLB-985 cells and embryonic stem cells. Furthermore, by Western and qRT-PCR, we found that NF-kB expression level correlated with CFP1 expression level in both PLB-985 cells and embryonic stem cells. We also found that overexpressing p65 promotes myeloid differentiation of PLB-985 cells. In addition, we observed that the NF-kB inhibitor dimethylamino-parthenolide (DMAPT) blocks the accelerated differentiation in p65 and CFP1 overexpressing cells. Analysis of the Chip-seq database E-GEOD-18578 showed that most of the NF-kB family members including Rela, Relb, c-Rel, Nfkb1, and Nfkb2 exhibit CFP1, H3K4Me3, and RNA polymerase II co-occupancy at their active promoter regions. Finally, analysis of 35 AML patient samples showed that NF-

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

POSTER #69

RECOMBINANT DEK REGULATES HEMATOPOIETIC STEM AND PROGENITOR CELLS IN A CXCR2- GAI PROTEIN-DEPENDENT MANNER

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DEK, a non-histone nuclear phosphoprotein involved in heterochromatin remodeling, is released from cells and can regulate hematopoiesis.Marrow from DEK-/- mice manifest increased hematopoietic progenitor cell (HPC) numbers and cycling status and decreased long-term and secondary hematopoietic stem cells (HSC) engrafting capability. Moreover, recombinant mouse (rmu) DEK inhibited HPC colony formation *in vitro*.We now show that rmuDEK is myelosuppressive *in vitro* in an S-phase specific manner and reversibly decreases numbers and cycling status of CFU-GM, BFU-E, and CFU-GEMM *in vivo*.*In vivo* administration of rmuDEK to DEK-/- mice greatly enhanced the number of phenotypic LT-HSC.Treating marrow cells *in vitro* with truncated rmuDEK by pretreating the DEK with the enzyme DPP4 (which DEK has targeted truncation sites for) also blocked the inhibitory effects of DEK suggesting that DEK must be in its full length form in order to perform its function.Upon our discovery that the DEK protein 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, known to bind and mediate the actions of IL-8 and MIP-2.In order to examine if this is indeed the case we first confirmed expression of CXCR2 on the surface of HSC and HPC.To determine whether rmuDEK's inhibitory function is mediated through the CXCR2 receptor, a neutralizing monoclonal antibody against CXCR2 was utilized.Marrow treated *in vitro* with rmuDEK, rhIL-8, or rmuMIP-2 inhibited colony formation; however pretreating marrow cells with the neutralizing CXCR2 antibody blocked the inhibitory effect of these proteins.Marrow treated with rmuMIP-1alpha (a chemokine that does not bind to CXCR2) also inhibited colony formation; however neutralizing CXCR2 antibody had no effect on the ability of MIP-1a to inhibit colony formation.Neutralizing CXCR4, a chemokine receptor that does not IL-8, MIP-2 or MIP-1alpha, had no effect on the inhibition of colony formation.DEK inhibition of CFU-GM colony formation is dependent on Gai-protein-coupled receptor signaling as determined through the use of pertussis toxin.This is a unique mechanism since IL-8 and MIP-1alpha had been previously reported by us to be insensitive to the inhibitory effects of pertussis toxin. As extracellular DEK can remodel chromatin in non-hematopoietic cells *in vitro*, we next assessed the effects of DEK on the heterchromatin marker H3K9Me3 in the nucleus of purified mouse Lineage negative, Sca-1 positive, c-kit positive (LSK) marrow cells by imaging flow cytometry.DEK enhanced the presence of H3K9Me3 in the nucleus of DEK-/- LSK marrow cells indicating that rmuDEK can be internalized by LSK cells and mediate heterchromatin formation.These results add to emerging evidence that DEK, a unique nuclear protein, plays a role in regulating hematopoiesis through a CXCR2/Gai protein signaling pathway.

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

POSTER #70

LPA INDUCED FOXM1 UP-REGULATION IN OVARIAN CANCER CELLS VIA BOTH THE PI3K AND YAP PATHWAYS

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FOXM1 is one of the most important signaling molecules involved in cancers. However, there are only limited regulatory and functional studies of FOXM1 in EOC cells. To determine the molecular mechanisms of lysophosphatidic acid (LPA)-regulated FOXM1 expression and the functional roles of FOXM1 in epithelial ovarian cancer (EOC), we conducted in vitro mechanistic studies to demonstrate the signaling pathways and components involved in FOXM1 up-regulation by LPA using pharmacological inhibitors, different genetic forms of the signaling molecules, and RNAi-mediated gene knock-down (KD). The role of FOXM1 in vivo was determined using a mouse EOC xenograft model. We show here, for the first time, that LPA time- and dose-dependently up-regulated FOXM1 in human EOC cell lines OVCA433, CAOV3, and OVCAR5. LPA receptors (LPA1-3) and both of the Gi-PI3K-AKT and the G12/13-Rho-YAP signaling pathways were involved in LPA-induced FOXM1 in EOC cells. FOXM1 was regulated at the transcriptional level. FOXM1B was the major isoform in OVCA433 and FOXM1c was the major splicing form in CAOV3 cells. In addition, FOXM1 was functionally involved in cell proliferation, migration, and invasion in vitro and tumorigenesis in vivo in EOC. FOXM1 target genes involved in proliferation, migration, or invasion were significantly down-regulated in FOXM1-KD cell derived tumors, ascites, and/or serum. Collectively, our data have linked the oncolipid LPA, oncogene YAP, and the central regulator of cell proliferation/mutagenesis FOXM1 in EOC cells. The important functional role of FOXM1 network has been demonstrated in vitro and in vivo, supporting its targeting potential in EOC.

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

POSTER #71

DEVELOPMENT OF REPLICATION PROTEIN A (RPA)-DNA INTERACTION INHIBITORS FOR CANCER THERAPY

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Platinum (Pt)-based chemotherapeutics exert their efficacy via the formation of DNA adducts which interfere with DNA replication, transcription and cell division and ultimately induce cell death. DNA Repair of Pt-DNA adducts via nucleotide excision repair (NER) or homologous recombination repair (HRR) can substantially reduce the effectiveness of the Pt therapy, which is a major contributor for cellular resistance. Replication protein A (RPA) is a single-stranded DNA binding protein that plays a crucial role in the NER pathway and makes RPA a novel drug target to develop more efficacious Pt-based cancer therapy. We have previously developed a series of inhibitors targeting the central oligonucleotide/oligosaccharide binding folds in DNA binding domains A and B (DBD-A/B) of the 70 kDa RPA subunit. One of the lead compound TDRL-551 showed 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 NSCLC xenograft model. These data demonstrate the potential in developing novel anticancer therapeutics that target RPA-DNA interactions. Further structure-activity relationships (SAR) of lead compound is underway to develop highly potent RPA inhibitors.

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

POSTER #72

ACTIVATION OF OCT4 ENHANCES EX VIVO EXPANSION OF PHENOTYPICALLY DEFINED AND FUNCTIONALLY ENGRAFTABLE HUMAN CORD BLOOD HEMATOPOIETIC STEM AND PROGENITOR CELLS BY REGULATING HOXB4 EXPRESSION

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Allogeneic hematopoietic cell transplantation (HCT) is well established as a clinical means to treat patients with hematologic disorders and cancer. Human cord blood (CB) is a viable source of hematopoietic stem cells (HSC) for transplantation. However, numbers of nucleated cells retrieved, as well as limited numbers of HSC/progenitor cells (HPC) present, during collection may be problematic for treatment of adult patients with single CB HCT. One means to address the problem of limiting numbers of HSC/HPC is to ex vivo expand these cells for potential clinical use. While progress has been made in this endeavor, there is still a clinically relevant need for additional means to ex vivo expansion of human HSC and HPC.

OCT4, a transcriptional factor, plays an essential role in pluripotency and somatic cell reprogramming, however, the functions of OCT4 in HSC are largely unexplored. We found that CB $CD34^+$ cells treated with OAC1 (Oct4-activating compound 1) for 4 days showed a significant increase (2.8 fold increase, $p<0.01$) above that of cytokine cocktail (SCF, TPO and Flt3L) in the numbers of rigorously defined HSC by phenotype ($Lin^-CD34^+CD38^-CD45RA^-CD90^+CD49f^+$) and in vivo repopulating capacity in both primary (3.1 fold increase, $p<0.01$) and secondary (1.9 fold increase, $p<0.01$) recipient NSG mice.OAC1 also significantly increased numbers of granulocyte/macrophage, erythroid, and granulocyte, erythroid, macrophage, megakaryocyte progenitors above that of cytokine combinations as determined by colony assays. To further confirm the role of OCT4 in human HSC, we performed OCT4 overexpression in CB CD34+ cells using lentiviral vectors and found that overexpression of OCT4 also resulted in significant increase ($p<0.01$) in the number of phenotypic HSC compared to control vectors. Together, our data indicate that activation of OCT4 by OAC1 or lentiviral vectors enhances ex vivo expansion of cytokine stimulated human CB HSC.

HOXB4 is a homeobox transcriptional factor that appears to be an essential regulator of HSC self-renewal. Overexpression of HOXB4 results in high-level ex vivo HSC expansion. We observed that theexpression of HOXB4 was largely increased ($p<0.01$) after culture of CB $CD34^+$ cells with OAC1 compared to vehicle control. siRNA mediated inhibition of OCT4 resulted in the marked reduction of HOXB4 expression ($p<0.01$) in OAC1-treated cellsindicating that OAC1 treatment lead to OCT4-mediated upregulation of HOXB4 expression in HSC. Consistently, siRNA-mediated knockdown of HOXB4 expression led to a significant reduction in the number of HSC in OAC1-treated cells ($p<0.05$), suggesting HOXB4 is essential for the generation of primitive HSC in OAC1-treated cells. Our study has identified the OCT4-HOXB4 axis in ex vivo expansion of human CB HSC and sheds light on the potential clinical application of using OAC1 treatment to enhance ex vivo expansion of cytokine stimulated human HSC.

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

POSTER #73

EXTRA PHYSIOLOGIC OXYGEN SHOCK/STRESS (EPHOSS) BLUNTS HEMATOPOIETIC STEM CELL PHENOTYPE/FUNCTION VIA REACTIVE OXYGEN SPECIES AND THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE

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Hematopoietic stem cells (HSC) reside in hypoxic niches (~1-4% O_2), however, HSC studies are consistently performed using cells isolated in ambient air (~20% O_2), regardless of subsequent processing in low oxygen tension. By collecting/processing stem cells in physiologically native conditions of hypoxia, where all procedures are performed inside a hypoxic chamber, we demonstrate that brief exposure of mouse bone marrow (BM) or human cord blood (CB) cells to ambient oxygen decreases recovery of phenotypic, and functional, self-renewing long-term repopulating HSC and concomitantly increases numbers of progenitor cells, a phenomenon we term Extra Physiologic Oxygen Shock/Stress (EPHOSS). Up to 5 fold greater numbers of long-term (LT)-HSCs (murine BM or human CB) are recovered from cells harvested in 3% O_2 compared to those harvested in air, or even those harvested in 3% O_2 and then exposed to air as little as 15 minutes before analysis. Competitive transplant experiments were completed at 3% O_2 using a custom mouse respirator, and revealed an increase in mouse bone marrow competitive repopulating units (CRUs) when BM is harvested, and retained, in hypoxic conditions compared to air in primary recipients. This suggeststhattrue numbers of HSCs, as well as the transplantation potency of BM and CB, have been consistently underestimated due to rapid initiation of differentiation of LT-HSCs in ambient air (EPHOSS). Mechanistically, we link mitochondrial function/ mitochondrial permeability transition pore (MPTP), ROS and cyclophilin D to EPHOSS. Genetic or pharmacological suppression of cyclophilin D function [CypD -/- mice or collecting cells in Cyclosporin A (CSA), respectively] protects phenotypic, functional, and transplantable HSCs from enhanced ROS levels and EPHOSS during collection in air. Limiting dilution analysis transplants show at least an average of 3-5 fold increase in phenotypic, functional, and transplantable mouse BM and human CB LT-HSCs. Collectively, this suggests that EPHOSS results in irreversible ROS induced differentiation signals during air harvest, due to mitochondrial permeability transition pore (MPTP) opening. Therefore, pharmacological mitigation of EPHOSS (using CSA) during HSC collections may be clinically advantageous for enhancement of patient transplantation.

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

POSTER #74

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 ERb 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 effects 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 will favor unintended tumor recurrence.

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

POSTER #75

MICROENVIRONMENT MEDIATED DOWNREGULATION OF MIR-193B PROMOTES OVARIAN CANCER METASTATIC COLONIZATION

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Ovarian cancer in the most lethal of all gynecologic malignancies and metastasis is the primary reason for poor prognosis of the patients. The microenvironment of the site of metastasis plays a crucial role in successful colonization by the metastasizing ovarian cancer cells. Productive reciprocal interactions with the microenvironment result in changes in gene expression in the cancer cells, which are essential for formation of the metastatic tumor. microRNAs are small noncoding RNAs that regulate multiple target genes by inhibiting translation. While microRNAs have been identified to have key regulatory roles during metastasis, the potential role of the microenvironment in regulating the miRNAs in metastasizing cancer cells has not been studied. In the current study, we use an organotypic three-dimensional culture model which mimics the omentum, one of the main sites of ovarian cancer metastasis, to identify the microRNAs that are deregulated by interactions with the microenvironment. miR-193b was the most downregulated microRNA in the metastasizing ovarian cancer cells. In addition, miR-193b was also decreased in the ovarian cancer cells cultured on the pieces of freshly isolated human omentum. Stable expression of miR-193 in the ovarian cancer cells suppressed their adhesion and invasion in the organotypic culture model, in addition to reducing the proliferation on human omental pieces ex vivo and decreasing the tumor burden in a mouse xenograft model of ovarian cancer metastasis. The opposite effect was observed on inhibition of miR-193b. We determined that direct interaction with the mesothelial cells covering the omentum resulted in miR-193b downregulation in the metastatic cancer cells through DNA methyltransferase 1 (DNMT1). Inhibition of DNMT1 expression and activity caused a decrease in ovarian cancer proliferation and migration, mediated through a concomitant increase in the miR-193b expression. Furthermore, we identified urokinase-type plasminogen activator (uPA) as a functionally relevant target gene for miR-193b. Knockdown of uPA caused a significant decrease in colony formation, and migratory and invasive functions of ovarian cancer cells and rescued the effects of miR-193b inhibition. Our findings here provide evidence for the regulatory role of the ovarian cancer metastatic microenvironment in promoting metastatic colonization by regulating the expression of miR-193b, though an increase in the expression of its target gene uPA. Targeting miR-193b could be a promising approach to treat ovarian cancer patients with metastatic disease.

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

POSTER #76

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. In the current study we aimed to define the role of the vitamin D receptor (VDR) in breast cancer cell growth. We hypothesized that VDR knockdown enhances human breast cancer cell growth.

In vitro: Using stable shRNA expression and single cell clonal selection, VDR expression was knocked down by ~85% in MCF-7 cells (MCF-$7^{VDR-/-}$). Compared to parental (PA) and non-target (NT) controls, induction of CYP24 mRNA expression by $1,25(OH)_2D_3$ was completely abrogated in MCF-$7^{VDR-/-}$ clones, confirming effective disruption of VDR signaling.

Treatment of PA & NT cells with $1,25(OH)_2D_3$ reduced cell growth by 32% compared to untreated cells ($p< 0.001$). In ligand-free culture, surprisingly, the growth of MCF-$7^{VDR-/-}$ clones was reduced by up to 34%, with a 6-fold increase in cell apoptosis compared to controls ($p< 0.001$), suggesting that VDR might have a ligand-independent effect in promoting cell growth. Treatment of MCF-$7^{VDR-/-}$ clones with $1,25(OH)_2D_3$ had no additional effect on cell growth and apoptosis.

In vivo: To investigate the putative effect of VDR on tumor growth *in vivo*, MCF-$7^{VDR-/-}$ and NT cells were xenografted orthotopically into the mammary fat pad of nude mice. VDR knockdown resulted in reduced tumor growth from day 18 to 51 ($p<0.001$). Intra-tibial implantation of NT cells into nude mice resulted in significant osteosclerosis as assessed by microCT (trabecular bone volume:+38%; trabecular number: +500%; compared to sham-injected tibia; $p<0.001$). Tumor-induced osteoblastic bone lesions were significantly less pronounced ($p< 0.001$ compared to NT cells) following implantation of MCF-$7^{VDR-/-}$ clones (trabecular volume: +9%; trabecular number: +30% compared to sham-injected tibia), indicating that VDR knockdown is associated with significantly retarded tumor growth.

Overexpression of a mutant VDR deficient in the nuclear localization signal (mVDR) in NT and MCF-$7^{VDR-/-}$ cells restored cell growth in MCF-$7^{VDR-/-}$ cells to the same level as seen in controls. This was associated with cytoplasmic accumulation of mVDR.

Conclusion: The VDR has ligand-independent functions promoting breast cancer cell growth which contrast with its ligand-dependent, anti-proliferative effects.

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

POSTER #77

APC SELECTIVELY MEDIATES RESPONSE TO CHEMOTHERAPEUTIC AGENTS IN BREAST CANCER

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The *Adenomatous Polyposis Coli* (*APC*) tumor suppressor is mutated or hypermethylated in up to 70% of sporadic breast cancers depending on subtype; however, the effects of *APC* mutation on tumorigenic properties in breast cancer remain unexplored. Using the $Apc^{Min/+}$mouse crossed to the Polyoma middle T antigen (PyMT) transgenic model, we identified enhanced breast tumorigenesis and alterations in genes critical in therapeutic resistance independent of Wnt/b-catenin signaling. Mechanistic studies in tumor-derived cell lines demonstrated that focal adhesion kinase (FAK)/Src/JNK signaling regulated the enhanced proliferation downstream of *Apc* mutation. Despite this mechanistic information, the role of APC in mediating breast cancer chemotherapeutic resistance is currently unknown. We have examined the effect of *Apc* loss in MMTV-PyMT mouse breast cancer cells on gene expression changes of ATP-binding cassette transporters and immunofluorescence to determine proliferative and apoptotic response of cells to cisplatin, doxorubicin and paclitaxel. Furthermore we determined the added effect of Src or JNK inhibition by PP2 and SP600125, respectively on chemotherapeutic response. We also used the Aldefluor assay to measure the population of tumor initiating cells. Lastly, we measured the apoptotic and proliferative response to *APC* knockdown in MDA-MB-157 human breast cancer cells after chemotherapeutic treatment. Cells obtained from MMTV-PyMT;$Apc^{Min/+}$ tumors express increased MDR1 (multidrug resistance protein 1), which is augmented by treatment with paclitaxel or doxorubicin. Furthermore MMTV-PyMT;$Apc^{Min/+}$cells are more resistant to cisplatin and doxorubicin-induced apoptosis, and show a larger population of ALDH positive cells. In the human metaplastic breast cancer cell line MDA-MB-157, *APC* knockdown led to paclitaxel and cisplatin resistance. In conclusion, both models showed that APC loss-of-function significantly increases resistance to cisplatin-mediated apoptosis. Furthermore, cisplatin in combination with PP2 or SP600125 could be clinically beneficial, as inhibition of Src or JNK in an *APC*-mutant breast cancer patient may alleviate the resistance induced by mutant *APC*.

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

POSTER #78

USING VBIM TECHNIQUE TO IDENTIFY NOVEL CARBOPLATIN RESISTANCE GENE IN OVARIAN CANCER

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Ovarian cancer (OC) is the most lethal gynecology cancer in the world. Although carboplatin is one of the major drugs used to treat OC, resistance to carboplatin remains a major barrier to successful treatment. To date, the mechanisms of carboplatin resistance are still poorly understood. The purpose of this study is to use the novel validation-based insertional mutagenesis (VBIM) technique to identify carboplatin resistance gene in A2780 OC cells. A2780 cells were infected with VBIM virus to cause the overexpression of drug resistance genes, then were further selected under carboplatin treatment. Targeted gene was then identified by using VBIM specific primers. In a preliminary screen, we identified the novel carboplatin resistance gene 1 (*NCR1*). Overexpression of *NCR1* increased carboplatin resistance in A2780 OC cells, while knocking it down with shRNA had the opposite effect. In an attempt to investigate the molecular mechanism that underlying *NCR1*-mediated carboplatin resistance, we found that *NCR1* is a potential NF-kB activator. In summary, we conclude that using a novel VBIM technique, we discovered a previously unknown carboplatin resistance gene *NCR1*, which may mediate drug resistance via NF-kB signaling pathway. This study is of extreme importance by identifying a potential novel therapeutic target *NCR1* in carboplatin resistance. Development of small chemical inhibitors targeting *NCR1* could ultimately lead to novel therapeutic approach for ovarian cancer treatment.

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

POSTER #79

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.

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

POSTER #80

ETHNICITY-DEPENDENT AND -INDEPENDENT HETEROGENEITY IN HEALTHY NORMAL BREAST HIERARCHY IMPACTS TUMOR CHARACTERIZATION.

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Histologic abnormalities detected in reduction mammoplasty or tumor-adjacent normal raise concerns about their utility as a reference resource for breast cancer studies. Here we report the remarkable inter-individual phenotypic heterogeneity in normal breast cells, which partly correlated with ethnicity and genetic predisposition. Cells from African American women contained a distinct CD44high/CD24- and CD44high/EpCAM- population compared with Caucasian and gene expression pattern in these cells was similar to that in the PROCR+ multipotent stem cells. Indeed, PROCR+ cells were enriched in African American women. ALDEFLUOR+ luminal stem/progenitor cells were lowest in BRCA1-mutation carriers. Heterogeneity in the healthy breast could impact tumor characterization because tumor and adjoining normal from the same patients showed distinct CD49f+/EpCAM+ progenitor and ALDEFLUOR+ profiles. This study highlights the need to define "normal" and "tumor" at the level of the same individual and to use phenotypically similar tumor and normal cells to discern cancer-specific signaling pathway alterations.

Basic Science ***Research Technician***

POSTER #81

THE ROLE AND THERAPEUTIC POTENTIAL OF MIRNAS IN COLORECTAL LIVER METASTASIS

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Colorectal cancer (CRC) is the most common malignancy and leading cause of cancer related deaths worldwide. Liver metastasis occurs in 60% of CRC patients and responds poorly to the available treatments; therefore, it is the major cause of their mortality. MicroRNAs (miRNAs) are highly conserved, endogenously encoded small, non-coding RNA molecules that regulate global gene expression. The role of miRNAs in cancer pathogenesis, including CRC, has been well documented. However, in-depth miRNA expression analysis on a large cohort of CRC tumors is needed in order to identify the clinically relevant miRNAs and explore their potential to target liver metastases.

To this purpose, we have analyzed miRNA expression data of 406 CRC tumors from the publically available colorectal cancer genome sequencing project and identified 58 miRNAs that were significantly downregulated. Using miRNA target prediction analysis, 10 miRNAs were selected for further analysis because they were either known to target genes in cellular pathways associated with CRC liver metastases, or located within a chromosomal loci commonly lost in colorectal liver metastases. qPCR analysis of these 10 selected miRNAs in primary and colorectal liver metastasis tissues and in CRC cell lines demonstrated four miRNAs; miR-132, miR-378f, miR-605, and miR-1976 are significant downregulated with <2 fold change ($p>0.05$). To investigate the anti-tumorigenic and metastatic properties of miR-132, -378f, -605, and -1976, we transfected 3 different CRC cell lines (SW620, HCT-116, and CT-26) with miR-mimics and subjected them to cell proliferation, apoptosis, soft agar, invasion, and migration assays. Ectopic expression of miR-378f, -605, and -1976 suppressed CRC cell proliferation, anchorage independent growth, migration, and invasion and increased apoptosis. Interestingly, CRC patients with high miR-378f and miR-1976 had better survival compared to low expressing patients ($p<0.044$).

In conclusion, our *in vitro* findings suggest the anti-tumorigenic/metastatic properties of miR-378f, -605, and -1976 in CRC. A further understanding of their functions and *in vivo* therapeutic evaluations may help in developing novel therapeutic strategies for this malignancy.

Basic Science ***Research Technician***

POSTER #82

EFFECTS OF APC LOSS ON WNT/B-CATENIN SIGNALING IN PANCREATIC CANCER

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Loss of the *Adenomatous Polyposis Coli* (*APC*) tumor suppressor gene in colorectal cancer elicits rapid signaling through the Wnt/b-catenin signaling pathway. In contrast to this well-established role of APC, recent studies from our laboratory have demonstrated that APC functions through Wnt-independent pathways to mediate *in vitro* and *in vivo* models of breast tumorigenesis. Pancreatic ductal adenocarcinoma (PDAC) has a five-year survival rate of 5-6% and an overall median survival of less than one year. APC is lost in a subset of pancreatic cancers, but the impact on Wnt signaling or tumor development is unclear. Given the lack of treatment strategies for pancreatic cancer, it is important to understand the functional implications of APC loss in pancreatic cancer cell lines. Therefore, the goal of this project is to study how loss of *APC* affects Wnt pathway activation and ultimately tumor development. Using lentiviral shRNA, we successfully knocked down APC expression in six pancreatic cancer cell lines (L3.6, Mia Paca, BXPC3, HPAF2, Aspc1, Hs766T). While no changes were observed in localization of b-catenin or expression of Wnt target genes, reporter assays to assess b-catenin/TCF interaction found an increase in TCF reporter activity in one of the Aspc1 knockdown cell lines. Despite this relatively modest activation of the Wnt/b-catenin pathway, the majority of *APC* knockdown cell lines exhibit an increase in cell proliferation. Future studies will include migration, soft agar, and chemotherapeutic response assays. By performing these experiments, we hope to exploit APC loss in pancreatic cancer as a targetable therapeutic mechanism.

Basic Science ***Research Technician***

POSTER #83

ANTI-MITOTIC KINESIN-13 INHIBITORS: DISCOVERY THROUGH DEVELOPMENT OF AN IMAGE-BASED ASSAY

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Mitotic spindles are macromolecular machines that ensure faithful progression through mitosis. The spindle is composed of a dynamic array of microtubules (MTs) and associated proteins that facilitate the proper attachment, alignment, and segregation of chromosomes. Current drugs targeting MTs are effective anti-proliferative agents but often have significant side effects, highlighting the need for new drugs that are as potent but have fewer adverse effects. Recently, inhibitors targeting MT regulators are emerging as new therapeutic strategies to treat disease including cancer. The ATP dependent Kinesin-13, Mitotic Centromere-Associated Kinesin (MCAK), is one such regulator that has recently gained attention because it is found to be highly overexpressed in multiple cancers and its expression can be correlated with metastasis and poor long-term survival. MCAK activity is confined to mitotically dividing cells suggesting the development of specific inhibitors have the potential to selectively target tumors overexpressing MCAK while avoiding the adverse effects seen with anti-MT agents. Recently, we developed two assays monitoring MCAK activity to screen for potential inhibitors. The first is a novel image-based screen using stabilized MTs which assesses MCAK's ability to induce MT depolymerization. A custom image analysis algorithm was developed to quantify MCAK activity and found this assay can readily detect two inhibitors of MCAK. To determine assay suitability in high throughput screening (HTS), a Z′ factor is calculated between assay controls where values range from 0 to 1 in assays incapable of HTS to assays perfectly suited to identify target compound modulators, respectively. Ideally, assays considered for HTS have Z′ values greater than 0.5 signifying a wide signal window between controls and/or small control variability. We found our image assay has a Z′ of 0.6 showing this assay is sufficient for identification of MCAK modulators. The second assay takes advantage of our recently developed Fluorescence Resonance Energy Transfer (FRET)-based biosensor that detects MCAK's conformational changes throughout its catalytic cycle. In this assay, active MCAK has a closed conformation characterized by high FRET levels whereas inhibition of MCAK has an open conformation displayed as reduced FRET levels and both can be detected in a filter-based fluorometer. This assay is very robust, with a Z′ of 0.9, allowing for HTS detection of compound modulators to MCAK's FRET. To discover potential inhibitors for MCAK we ran a pilot screen where both assays were used in tandem to identify small molecules in a 3000 compound library that modulated MCAK's depolymerization activity, conformation, or both. Complimentary image and FRET based rescreening revealed 14 potential inhibitory compounds at the conclusion of the pilot screen. Further characterization and analyses of these compounds will allow us to develop more specific inhibitors against MCAK activity serving as potential therapeutic agents against cancer.

Basic Science ***Research Technician***

POSTER #84

MOLECULAR DETECTION OF CISPLATIN VIA SLOT BLOT

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Cisplatin (cis-diamminedichloroplatinum) is a chemotherapy drug that executes its cytotoxic activities via interacting with DNA and forming inter- and intrastrand crosslinks. The crosslinks interferes with DNA replication and transcription and ultimately cell division. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible or ineffective. The two major reaction products of cisplatin are the guanine-guanine (Pt-[GG]) and adenine-guanine (Pt-[AG]). However, 90% of the lesions are result of guanine-guanine dimers. In the past, we have used inductively coupled plasma mass spectrometry (ICP-MS) to detect DNA bound cisplatin and its repair. This methodology allows the detection of all platinum-DNA adducts, including both AG and GG dimers, mono adducts and interstrand adducts. This assay is however, limited by its sensitivity and the amount of DNA necessary to generate a robust signal. Towards improving the sensitivity and throughput of the analysis of Pt-DNA repair, a slot blot techniques was used. This assay employs a rat monoclonal anti-cisplatin antibody as a primary antibody that binds to the Pt-GG adduct on DNA. This technique can not only confirm the presence of Pt-GG adducts in DNA, but also enable us to quantify how many Pt-GG adducts have formed on the DNA strand based on the intensity of the bands on the blotted membrane. We have optimized the assay for the analysis of Pt-DNA damage and its repair in H460 NSCLC cells with respect to DNA concentration, antibody dilution and chemiluminescence detection. The data suggests that in this cell line, repair is very efficient with 90% of the Pt-GG adducts repaired in the first 6 hours post-treatment. The analysis of the effect of NER inhibitors on the repair of Pt-DNA damage will also be presented.

Basic Science ***Research Technician***

POSTER #85

TARGETING THE INTERACTION OF XERODERMA PIGMENTOSUM GROUP A PROTEIN WITH CISPLATIN-DAMAGED DNA

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Targeting DNA repair and the DNA damage response for cancer therapy has recently gained increasing attention with inhibitors of the PI3-K-like kinases in early stage clinical trials. The utility of DNA repair inhibitors can be expanded by their use in combination treatment with DNA damaging chemotherapeutics including cisplatin. We have focused on directly targeting the DNA repair pathway responsible for repairing platinum-induced DNA damage, nucleotide excision repair (NER). We have selected the molecular target XPA, whose role is in the identification and verification of the sites of DNA damage. Clinical validation of XPA has been obtained where high XPA expression in lung, ovarian and lung cancer results in decreased efficacy of platinum therapy. We report the continued development of the X80 class of XPA small molecule inhibitors. In a two-step, iterative process we have identified critical structure activity relationships that have resulted in a 100-fold increase in potency with in vitro IC50 values below 1μM. 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. Recent production of a sub-fragment of the XPA protein will allow the identification of the direct binding domain to enable continued structure-based design of more potent XPA inhibitors.

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Basic Science ***Research Technician***

POSTER #86

EXPLORING THE SITES OF INTERACTION BETWEEN SMALL MOLECULE INHIBITORS AND REPLICATION PROTEIN A

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Replication protein A (RPA) is a heterotrimeric protein with subunits of 70, 32, and 14 kDa that is important in the processes of DNA replication, recombination, and repair. All of these pathways are mediated by the binding of RPA to the single-stranded DNA in its initial phases. The binding ensures the recruitment of other proteins and proper stabilization of the ssDNA regions. Previous research has identified small molecule inhibitors (SMI) that interact with and inhibit the binding activity of full-length RPA to ss-DNA .The purpose of the research within is to identify the precise location of where two of these SMIs (MCI 13E and CheSS19) are interacting with the full-length RPA. The SMI CheSS19 has been shown to inhibit the RPA-AB-box while MCI 13E did not show inhibition of the RPA-AB-box. The RPA-CDE-core ($70_{438\text{-}616}$, $32_{43\text{-}171}$ and $14_{1\text{-}120}$) has been prepared and isolated by nickel column chromatography in order to explore inhibitory effects on the trimerization core. The MCI 13E and CheSS19 have shown no significant inhibition of binding of the RPA-CDE-core to ss-DNA. The data indicates that Chess19 may specifically inhibit the AB-box while MCI 13E may require the full-length RPA in order for proper inhibition of RPA's affinity to ssDNA.

Basic Science ***Research Technician***

POSTER #87

THE HEALTH OF THE SERBIAN ROMA: A RESEARCH REPORT

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Introduction: The Roma/gypsy is a relatively reproductively isolated and socially disadvantaged ethnic minority group that that densely populates the Balkan regions. As little is known about the health of this population, the aim of the present study is to investigate the health history of the females and their close kin. Towards that end, we collected general data regarding the health and reproductive patterns of Roma women living in two settlements in the Northern Province of Serbia. As cancer is one of the two most widespread chronic diseases in Europe, we focused on diagnoses of cancer and other chronic diseases and on behaviors that increase risk for these diseases.

Methods: Eighty women living in two villages were interviewed in their homes. Participants were recruited through personal contacts and Roma/Gypsy organizations. Women were eligible if they were Roma and married with at least one child. The final sample consisted of 60 Gypsy women residing in Village A and 22 from Village B. A focus was placed on asking about family history of diseases, especially cancer, and on behaviors and attitudes related to cancer risk factors.

Results: The average age at menarche was 12.2 years, average age at marriage was 25.3, and women began reproducing soon after marriage, with the average age at first birth being 16.9. The average Roman woman had 3.24 children. Only one woman reported being diagnosed and treated for breast cancer. She currently was 22 and pregnant; she was 18 when she was diagnosed. Her mother had died at age 57 from cervical cancer. Four women reported that their mothers been diagnosed with breast, ovarian, lung and benign liver cancer, respectively. The most common complaints were high blood pressure (32.9%), CVD (24.4%), diabetes (20.7%), and an unspecified "nerve condition" (11%). More than half of the women (53.7%) reported that they do not regularly seek medical help when they are sick; 63.4% reported that they smoked during their pregnancies and 5% of the women reported that they consumed alcohol when pregnant and 96% reported that they were not physically active during pregnancy.

Conclusion: Roma reproductive patterns are distinct, with earlier age at first birth Roma birthrates are higher than those of the majority populations. Considering age at first reproduction, Serbian Roma have early age at first reproduction; in this sample, it is 16.9, while the average number of ever born children is 3.24. All mothers in the sample breastfed their babies, the average duration being 12 months. In Europe, for instance, the average duration of breastfeeding was 6.9 months across all age groups.

Chronic disease is highly rampant due to lack of health care utilization, constant stress, lack of access to healthy foods, increased smoking patterns and alcoholism.

Behavioral ***Graduate Student***

POSTER #88

MAMMOGRAPHY ADHERENCE IN AFRICAN AMERICAN WOMEN: RESULTS OF A RANDOMIZED CONTROLLED TRIAL

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Background. Breast cancer is the second leading cause of cancer mortality among women in the developed world. Mammography screening is especially important for African Americans, who experience greater mortality (OR=1.38) than Caucasians.

Purpose. The purpose of this study was to compare the effects of two interventions with usual care on mammography adherence among African American women.

Methods. African American women (n=244) with no mammogram in the last 15 months were randomly assigned to receive: 1) mailed interactive DVD, 2) computer-tailored telephone counseling, or 3) usual care.

Results. The DVD intervention was 5 times more effective than usual care for promoting mammography screening at 6 months follow-up among women who earned less than $30,000 (OR= 5.3). Compared to usual care, neither the DVD nor phone produced significant effects for women with household incomes >$30,000.

Conclusion. Use of a mailed DVD for low-income African American women may be an effective way to increase mammography adherence.

(ClinicalTrials.gov no. NCT00287040)

Behavioral ***Graduate Student***

POSTER #89

TEMPEST IN A TEAPOT: A REVIEW OF HPV VACCINATION AND RISK COMPENSATION RESEARCH

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Background: Some parents and media reports have raised concern about risk compensation following HPV vaccination (e.g., increased risky sexual behavior). Between 16 and 26% of parents have indicated they are concerned that HPV vaccination will increase risky sexual behavior.

Objectives: To synthesize recent literature and examine any evidence of increased self-reported risky sexual behaviors or biological outcomes (e.g., sexually transmitted infections) after HPV vaccination.

Methods: A review of the literature was performed using PubMed, CINAHL, and Embase databases. The following search terms were used: [(sex behavior OR sex behaviour OR sexual) AND (human papillomavirus OR HPV) AND (vaccines OR vaccine OR vaccination)]. Results were limited to studies published from January 1, 2008 to March 31, 2015. Additionally, a cited reference search of the relevant articles was conducted in order to capture studies that might have been missed in the database searches. The search included studies written in English that examined reported behaviors and biological outcomes post-vaccination with both male and female populations. First, studies were reviewed by title and abstract, studies outside of the scope of the review were eliminated, and the remaining studies were examined as full-text articles.

Results: 1,542 articles were identified through the search and 16 met the criteria for this study. Of the studies that compared vaccinated and unvaccinated populations, 3 studies examined biological outcomes (i.e. pregnancy and STI diagnosis), 8 examined reported sexual behaviors, and 3 examined both biological outcomes and behaviors. Two studies examined risk perception and behavior of a vaccinated cohort and found that even when HPV vaccination decreased perceived risk of acquiring another STI, there was no evidence that this misperception led to increased sexual risk behaviors. Of the studies examining reported sexual behaviors and/or biological outcomes, none reported evidence of risk compensation subsequent to HPV vaccination. Furthermore, some of the research showed that, in fact, it was the unvaccinated population that had higher incidence of risky behaviors. Vaccinated individuals were less likely than unvaccinated persons to report vaginal intercourse without a condom over the previous two months (OR=0.5; 95% CI=0.4-0.6), and non-use of contraception was more common among those who were unvaccinated (OR=0.27; 95%CI=0.15-0.48). One study found the unvaccinated participants had higher rates of testing positive for Chlamydia (OR=2.3; 95%CI=1.06-5.00) and being symptomatic at a clinic visit (OR=1.78; 95%CI=1.09-2.92).

Implications and Impact: None of the studies found evidence of risk compensation. These findings should be reassuring to parents, clinicians, and policy makers. However, it is also important to note that that even if risk compensation existed, it would not be a reason to withhold vaccination, but rather a reason to increase education.

Behavioral ***Graduate Student***

POSTER #90

IMPACT OF DEPRESSIVE SYMPTOMS AND FATIGUE ON QUALITY OF LIFE IN BREAST CANCER SURVIVORS

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BACKGROUND: Breast cancer is the most common cancer for women worldwide. Since the 5 year survival rate for all breast cancer stages reaches 77%, there are now more than 2.8 million breast cancer survivors (BCS) in the U.S. While fatigue and depressive symptoms were widely reported as the most distressing symptoms, evidence has not been well established regarding the relationship among depressive symptoms, fatigue, and quality of life (QOL) in BCS after primary adjuvant therapy.

PURPOSE: The purpose of this study was to examine the prevalence and correlates of depressive symptoms and fatigue and their impact on QOL in BCS.

THEORETICAL FRAMEWORK: Ferrell's QOL Model guided this study.

METHODS: A cross-sectional, descriptive design was used. BCS who were post-menopausal, 40 years of age, and older and at least 1-year post-treatment completed self-report questionnaires including depressive symptoms (CES-D), fatigue (FACT-F) and QOL (Ferrell's QOL Scale). Data were evaluated using descriptive statistics. Furthermore, the impact of depressive symptoms and fatigue on QOL was examined by using a general linear regression model after controlling for known covariates of QOL, including age and time post-treatment.

RESULTS: The average age of 88 female BCS participants was 57 years old (range 40-74; SD=8.54). They were on average 5.3 years (SD=4.0) post-treatment which included chemotherapy. The mean score of fatigue was 37.59 (SD=10.53) with 64% of BCS experiencing clinically significant levels of fatigue (cutoff point <43). The mean score of depressive symptoms on the CES-D was 11.77 (SD=9.04) with 25% experiencing clinically significant depressive symptoms (cut-off point ≥16). Both fatigue (r=0 .579) and depressive symptoms (r= 0.67) negatively correlated with QOL ($p < 0.05$). Fatigue and depressive symptoms together explained 46% variances of QOL ($F=20.05$, $p < 0.000$) after controlling age and time post-treatment. Depressive symptoms was the only significant variable to predict QOL (p=0 .000).

CONCLUSIONS: The findings showed a similar incidence of depressive symptoms but a higher incidence of fatigue compared to other studies. It also supported that fatigue and depressive symptoms may impact or even predict BCS QOL. Based on the widely experienced fatigue and depressive symptoms reported as well as their possible impact on QOL, nurses need to evaluate and manage symptoms of fatigue and depression in BCS in order to improve QOL.

Behavioral ***Graduate Student***

POSTER #91

RELATIONS OF MEANING IN LIFE AND SENSE OF COHERENCE TO DISTRESS IN CANCER PATIENTS: A META-ANALYSIS

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Objective: Cancer patients report high rates of distress. The related constructs of meaning in life (MiL) and sense of coherence (SOC) have long been recognized as important factors in the psychological adjustment to cancer; however, both constructs' associations with distress have not been quantitatively reviewed or compared in this population. Informed by Park's integrated meaning-making model and Antonovsky's salutogenic model, the goals of this meta-analysis were: (1) to compare the strength of MiL-distress and SOC-distress associations in cancer patients; and (2) to examine potential moderators of both associations (i.e., age, gender, ethnicity, religious affiliation, disease stage, and time since diagnosis).

Methods: A literature search was conducted using electronic databases. Overall, 62 records met inclusion criteria. The average MiL-distress and SOC-distress associations were quantified as Pearson's *r* correlation coefficients and compared using a one-way ANOVA.

Results: Both MiL and SOC demonstrated significant, negative associations with distress (r = -0.41, 95% CI: -0.47 to -0.35, k = 44; and r = -0.59,95% CI: -0.67 to -0.51, k = 18, respectively). Moreover, the MiL-distress association was significantly smaller than the SOC-distress association (Q_b = 10.42, df = 1, $p < 0.01$). Neither association varied by the tested moderators.

Conclusions: Findings provide support for the clinical relevance of MiL and SOC across demographic and medical subgroups of cancer patients. The strength of the SOC-distress association suggests that incorporating aspects of SOC (e.g., the perceived manageability of life circumstances) into meaning-centered interventions may improve their effectiveness for distressed cancer patients.

Behavioral ***Graduate Student***

POSTER #92

THE IMPACT OF MESSAGE CONTENT AND IMAGES IN PHYSICAL ACTIVITY PROMOTION MATERIALS

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Background: One in eight women will develop breast cancer in their lifetime. The National Cancer Institute recommends that premenopausal women engage in health protective behaviors like physical activity (PA) to reduce future breast cancer risk. Research has demonstrated up to 80% overall risk reduction from PA. This information should be communicated; however, there is a paucity of research examining health promotion messaging to premenopausal women and their reactions to these messages and accompanying images.

Methods: Premenopausal women were solicited for participation via social media using snowball sampling. Participants (n=123, M=21 years, range: 19-39 years) were randomized to view one of five health promotion flyers. All flyers depicted the same image but message type varied by level of risk and amount of information presented. Age, perceived breast cancer risk (PR), perceived informativeness of the flyer (PI), and thought listing data were gathered after message exposure. Qualitative data were analyzed by two independent coders.

Results: ANOVA revealed differences in PI by message type ($F(4,122)=22.188$, $p < .001$) such that the low risk/low information message was less informative than the others. No differences were observed in PR by message type ($p<.05$). Nearly half the sample (40%) appraised the image on the flyer in their thought listing response. As such, differences in outcomes were examined between the two groups. After controlling for message type, image appraisal ($F=4.93$, $p=.028$) predicted PI such that those who did not discuss the image found the flyer to be more informative. There were no differences between the groups on PR after controlling for message type ($p<.05$). Chi-square analyses suggest that participants who received a high risk/low information message were less likely than expected to appraise the image ($z=-1.9$, $p=.057$). Of those who did, 66.7% had a negative opinion of the woman in the picture.

Conclusions: Message content as well as accompanying images may impact PI of health promotion flyers. Those who received high risk/low information messages focused less on the image than others. Future research should continue to explore the impact of both message content and images in health promotion materials to determine the most effective combinations of message features.

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

POSTER #93

LUNG CANCER SCREENING: WHAT DO LONG-TERM SMOKERS KNOW AND BELIEVE?

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Background: Lung cancer is the leading cause of cancer-related deaths in the U.S. with most cases diagnosed at an advanced stage with very low survival rates. Tobacco smoking is the primary risk factor, and guidelines for screening long-term smokers were recently developed. Since lung cancer screening is now recommended for long-term smokers, it is critical that we understand factors that influence screening participation. Because decisions to participate in cancer screening are made by individuals, exploring long-term smokers' knowledge and beliefs about screening is a necessary first step to increasing lung cancer screening participation.

Purpose: The purposes of this study were to: (1) explore long-term smokers' knowledge and beliefs about lung cancer and associated risk factors; and (2) explore long-term smokers' knowledge and beliefs about lung cancer screening.

Conceptual Framework: The expanded Health Belief Model guided this study.

Methods: Four focus groups comprised of 26 individuals were conducted; two groups of long-term smokers who had been screened for lung cancer with low-dose computed tomography (*n=9; n=3*) and two groups of long-term smokers who had never been screened (*n=7; n=7*). Data were collected via audio recordings and transcribed verbatim. Data were analyzed using content analysis.

Results: Regardless of prior screening, long-term smokers identified environmental and occupational exposures as the greatest risk factors for developing lung cancer, in addition to tobacco smoking. Unscreened participants were unsure what constituted lung cancer screening while those who had been screened identified chest radiography, in addition to LDCT, as ways to screen for lung cancer. While most agree that lung cancer is deadly, confusion exists as to the causes and associated risk factors. Awareness of the association of long-term smoking and lung cancer risk remains suboptimal. Further, healthcare system distrust and perceived smoking-related stigma were identified as potential barriers to screening.

Conclusions: Regardless of prior lung cancer screening experience, knowledge related to the existence of lung cancer screening and how it is performed are low. Barriers to lung cancer screening must be addressed as screening becomes more widely implemented. Further, heightened levels of healthcare system distrust and perceived smoking-related stigma may impact successful implementation of screening programs. Future research is needed to explore the impact that individual health beliefs about lung cancer and screening and other factors (such as distrust and stigma) have on screening behavior. These may be important modifiable targets that can inform the development of an intervention to enhance the shared decision-making process between healthcare providers and their high-risk patients about lung cancer screening.

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

POSTER #94

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

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Cancer accounts for more deaths in low and middle-income countries than HIV/AIDS, malaria and tuberculosis combined. Cervical cancer is the most common malignancy in women living in sub-Saharan African countries, including Kenya. Oncogenic types of HPV are the causative agents of cervical cancer. Women who are HIV-infected have a higher incidence of pre-cancerous and cervical cancer than HIV-uninfected women. There are many unanswered questions about cervical cancer in HIV-infected women.The reasons why some, but not all women develop malignant consequences of oncogenic HPV infection are not understood. However, while HIV infection accounts for much of the high incidence and mortality of cervical cancer in Kenyan women, other cofactors are likely to be critical, some of which are modifiable. In addition, the optimal therapies for early and late cervical cancer in the setting of HIV infection among African women are not well understood.

The AMPATH-Oncology Institute (AOI) is a multidisciplinary collaboration of North American universities, led by Indiana University, with Moi University and Moi Teaching and Referral Hospital in Eldoret, Kenya, serving a catchment area of 20 million people in western Kenya. The overall goal of this five-year project, funded by the National Cancer Institute (U.S.A.), is to enhance the research workforce in Kenya to study the natural history of HPV infections in HIV-infected women. Two longitudinal/observational projects to be conducted are 1) define modifiable factors predicting persistence of oncogenic HPV and cervical dysplasia in HIV-infected women, and 2) evaluate current impact of VIA screen and treatment with cryotherapy or LEEP in HIV-infected women with cervical intraepithelial neoplasia (CIN). Both projects will use prospective data collection (clinical and questionnaire), HPV typing of cervicovaginal specimens, and tissue collection for analysis of CIN lesions and cancers. Data will be managed by two cores; the Biostatistics and Data Management Core, and the Translational Biology Core (including the Kenya Medical Research Institute (KEMRI) in Kisumu). Additionally, a Mentoring and Career Development Core will further enhance the research workforce in Kenya, including physicians, scientists and other health care personnel. 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.

Population Science/Epidemiology ***Faculty***

POSTER #95

ONCOGENIC HPV TYPES IN INVASIVE CERVICAL CANCERS FROM WOMEN LIVING IN THE UNITED STATES, KENYA, OR BOTSWANA

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More deaths occur in African women from cancer of the cervix than from any other malignancy. Screening for cervical cancer is rarely performed in sub-Saharan Africa, where more than 50% of global cervical cancer deaths occur. Oncogenic types of human papillomaviruses (types 16, 18, and others) are the causative agents of invasive cervical cancer (ICC). Co-infection with HIV accelerates the natural history of cervical cancer. High quality data on the HPV types causing ICC in African women is limited, and it is not known if the type distribution is similar to that observed in the U.S. A greater prevalence of types other than HPV 16 and 18 in ICCs may support implementing vaccines that protect against other oncogenic HPV types. A study was performed to identify and compare the HPV types in ICCs from women living in western Kenya (n=146), Botswana (n=136), or the U.S. (n=46). DNA was extracted from archived, paraffin-embedded blocks of formalin-fixed ICC specimens, and HPV and HIV were amplified by PCR. HPV genotyping was performed using the Roche Linear Array HPV Genotyping Test. Oncogenic HPV types were detected in a high percentage of specimens with amplifiable DNA from all three countries. HPV types 16 or 18 were identified in 93.5% of HPV-positive ICCs from the U.S., 93.8% from Kenya, and 61.8% from Botswana ($p<0.0001$). Types other than HPV 16 and 18 (non-HPV 16/18 types) were detected in 10.9% of HPV-positive cancers from the U.S., 17.2% from Kenya, and 47.8% from Botswana ($p<0.0001$). HIV sequences were detected in 2.2% of ICCs from the U.S., 31.5% from Kenya, and 32.4% from Botswana ($p=0.0002$). The distribution of all HPV, HR-HPV types, A9 types, HPV 16, non-16 A9 types, A7 types, HPV 18, and non-18 A7 types were not significantly different between ICCs from HIV-infected or HIV-uninfected women. Multiple type infections in ICCs did not differ significantly between the three sites ($p= 0.217$), or between ICCs from HIV-infected or HIV-uninfected women ($p= 0.147$). We conclude that 1) HPV types 16 and 18 are the predominant types in ICCs from women living in Indianapolis, western Kenya, and Botswana, 2) more non-HPV 16/18 oncogenic types were identified in ICCs from women living in Botswana compared to those other countries, and 3) co-infection with HIV does not appear to be the driving force for the higher percentage of non-HPV 16/18 oncogenic types identified in ICCs from women living in Botswana in our data set, 4) currently available HPV vaccines should provide protection against most ICCs in women in the U.S. and Kenya, but a recently developed nanovalent vaccine may be more suitable for Botswana, where types other than HPV 16 and 18 were frequently detected in ICCs.

Population Science/Epidemiology ***Faculty***

POSTER #96

ADEQUACY OF BLOOD CULTURE SAMPLE VOLUMES AT RILEY HOSPITAL

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Background
Blood culture results have a significant impact on clinical decision-making, and it is well established that volume is a major factor in determining blood culture yield. Though standards exist for recommended sample volume based on weight, many institutions report a high proportion of inadequate volume blood cultures.

Objective
The primary aim of this study is determine how many blood culture samples of adequate volume are drawn at Riley Hospital.

Methods
Bottles from inpatient units were randomly selected by investigators, weighed and labeled. After inoculation, labeled bottles were weighed prior to any processing in the microbiology lab. The difference in post-inoculation and pre-inoculation weight was compared with recommended standards. Data regarding patient age, weight, hospital location, source of sample collection, culture result, and hours until positivity were also recorded.

Results
Using a 10% leeway, 46.8% blood cultures were of adequate volume. The lowest proportion of adequate samples was found in the neonatal intensive care unit at 23%. There was a positive correlation between volume and patient age and weight ($p<0.0001$, $p<0.0001$). Inappropriate use of anaerobic pediatric bottles when adult bottles were indicated was also discovered.

Conclusions
Nearly half the blood culture samples obtained at Riley do not meet minimum standards for appropriate blood culture sample volume. Volume collected positively correlated with patient age and weight, a finding that is not universal amongst similar studies. This study also suggests the need for staff education regarding recommended blood culture volumes. Further investigation is required to evaluate for other factors impeding adequate volume sampling.

Population Science/Epidemiology ***Faculty***

POSTER #97

DIETARY INTAKE OF PHYTOESTROGENS AND THE RISK OF PROSTATE CANCER IN THE PROSTATE, LUNG, COLORECTAL, AND OVARIAN CANCER SCREENING TRIAL

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Prostate cancer is the most common non-cutaneous cancer and second leading cause of cancer death among men in the U.S. Although ecologic and migrant studies suggest that diet plays a role in the etiology of prostate cancer, few specific nutrients that alter its occurrence have been identified in case-control and cohort studies. Phytoestrogens are a family of bioactive compounds that are abundant in soy products and some other food groups (e.g. legumes and chick peas). Experimental studies revealed that phytoestrogen intake may modulate the risk of prostate cancer due to their structural similarity to 17B-estradiol and the resulting competitive binding to estrogen receptors. Despite biological plausibility, it still remains elusive whether phytoestrogen intake influences prostate cancer risk in human populations. Therefore, the objective of the present study was to investigate the associations between dietary intake of phytoestrogens and the risk of total and advanced prostate cancer among 30,097 participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). A total of 3628 cases of prostate cancer (including 396 advanced cases) have been documented during a median follow up of 11.5 years. Advanced prostate cancer were defined as stage II cancer with a Gleason score of ≥8, or stage III or stage IV cancer. Dietary intake of phytoestrogens was assessed with a validated food frequency questionnaire. Cox proportional hazards regression was performed to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for dietary intake of phytoestrogens in relation to prostate cancer risk. After adjustment for confounders, an increased risk of advanced prostate cancer was found for the higher dietary intake of isoflavones (HR: 1.58; 95% CI: 1.11, 2.24), genistein (HR: 1.42; 95% CI: 1.02, 1.98), daidzein (HR: 1.62; 95% CI: 1.13, 2.32), and glycitein (HR: 1.53; 95% CI: 1.09, 2.15). Conversely, it appears that the higher dietary intake of genistein was associated with a reduced risk of non-advanced prostate cancer (HR: 0.88; 95% CI: 0.78, 0.99) and total prostate cancer (HR: 0.90; 95% CI: 0.81, 1.00). The risk estimates presented above were obtained for comparisons between the quintile 5 and quintile 1 of respective phytoestrogen intake. In summary, our analysis of this national prospective cohort study suggests that dietary intake of phytoestrogens modulated the risk of prostate cancer and that some of these effects may differ by the aggressiveness of the disease.

Population Science/Epidemiology ***Faculty***

POSTER #98

EPISODIC DETECTION OF HIGH RISK HPV 16

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Background: Most high risk (HR) HPV cervical infections do not cause cancer, however progression to cancer is associated with persistent HR-HPV infection. These persistent infections may be acquired early, after first sexual intercourse, but data are lacking. Many incident infections become undetectable within a few months, and are therefore believed to "clear". Our longitudinal data in adolescents suggest that HPV is not always persistently detected, but for some, is episodically detected. The purpose of this study was to collect preliminary data to examine whether high risk HPV infections detected in adolescent women could be also detected in early adulthood.

Methods: A sample of 30 women (from a larger cohort of 146 women) were recruited from our prior longitudinal research project, The Young Women's Project (YWP). None were vaccinated against HPV during YWP. At the re-enrollment visit, participants were given a structured interview about sexual behaviors and underwent a pelvic exam for Pap smear and HPV testing (Linear Array, Roche) from a cervical swab. At a second visit, approximately 6 weeks later, Pap smear and HPV results were given to the participant, and the participant provided a self-collected vaginal swab for a second HPV test.

Results: The mean age of YWP enrollment was 15.7 years (SD=1.1) and re-enrollment was 27.8 years (SD=1.9); the majority (90%) were Black. There was an average of 6.0 (SD=.66) years between YWP and re-enrollment. All 30 had at least 1 or more HR HPV infections and 27 (90%) had HPV 16 detected during the previous YWP observation. At re-enrollment, 22 of 30 women (73%) had at least one HR-HPV detected. Of the 27 women who had HPV 16 detected during YWP observation, 11 (40.7%) had redetection of HPV 16. While 9 of the women had episodic detection, only 2 may have had persistent detection of HPV from YWP to re-enrollment. Six of the 11 met study criteria of having "cleared" during the previous observation period (2 consecutive negative tests prior to the last observation for YWP). The mean duration of HPV 16 detection during YWP was 2.58 years (SD=2.46), and 13 had episodic detection (as opposed to a single period of detection) of HPV 16 as defined as detection followed by non-detection of > 6 months, then redetection.

Discussion: HPV 16 was redetected in women followed from adolescence through young adulthood. Episodic detection occurred frequently. Although differentiation between reactivation of latent HPV 16 infection or a newly acquired infection could not be made, persistent HPV infections may be episodically detected due to fluctuations in viral copy number. Our data continue to support early HPV vaccination.

Funding Source: The Center for HPV Research http://pediatrics.iu.edu/center-hpv-research/about-us/

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

POSTER #99

ADULT BMI CHANGE AND RISK OF BREAST CANCER: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

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Objective: Breast cancer is the second leading cause of cancer mortality among women in the developed world. This study assessed the association between occurrence of breast cancer and Body Mass Index (BMI) change from age 25 to age closest to breast cancer diagnosis while exploring the modifying effects of demographic variables.

Methods: The National Health and Nutrition Examination Survey data were used. Women included were ≥50 years, not pregnant and without a diagnosis of any cancer but breast. The total sample included 2,895 women (172 cases and 2,723 controls with no cancer diagnosis). Multivariate logistic regression was used to estimate the OR and 95% CI's and interaction evaluated by including an interaction term in the model.

Results: Women whose BMI increased from normal or overweight to obese compared to those who remained at a normal BMI were found to have a 2 times higher odds (OR=2.1; 95% CI: 1.11-3.79) of developing breast cancer. No significant association was observed for women who increased to overweight. However, a more pronounced association was observed in non-Hispanic Black women (OR=6.6; 95% CI: 1.68-25.86) and a significant association observed when they increased from normal to overweight (OR=4.2; 95% CI: 1.02-17.75).

Conclusions: Becoming obese after age 25 is associated with increased risk of breast cancer in women 50 years, with non-Hispanic Black women being at greatest risk.

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

POSTER #100

CHRONIC HEALTH CONDITIONS (CHCS) FOLLOWING CISPLATIN-BASED CHEMOTHERAPY (CHEM): A MULTI-INSTITUTIONAL STUDY OF 680 TESTICULAR CANCER SURVIVORS (TCS)

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Background: Platinating agents are among the most commonly used group of cytotoxic drugs worldwide. Few studies, however, have comprehensively examined the number and range of co-morbidities following CHEM in uniformly treated patients. Given the limited number of CHEM regimens used to cure TC and high long-term survival rates, TCS represent a unique population to provide new knowledge. We examined CHCs in an ongoing North American multi-center study of TCS given CHEM (NCI 1R01 CA157823-02).

Methods: Eligible TCS were aged <50 y at diagnosis and treated with only first line cisplatin CHEM after 1990. Questionnaires regarding co-morbidities and prescription drugs were completed. Evaluated CHCs included tinnitus, hearing loss, peripheral neuropathy (PN), balance/vertigo, renal disease, hypertension (HTN), Raynaud's syndrome, diabetes (DM), thyroid disease, hypogonadism, erectile dysfunction (ED), anxiety/depression, pain, and others. For PN, responses of "a little", "quite a bit", or "very much" regarding tingling, numbness or shooting/burning pain were scored as "yes". Yes/no variables assessed ototoxicity (i.e., tinnitus, problems hearing words, sounds, or language in crowds', hearing aid use, and deafness).

Results: We evaluated 680 consecutively enrolled TCS. Median age at diagnosis was 31 (range, 15-49 y); median time since CHEM completion was 52 mos. (range, 1-30 y). Only 15% of patients reported no CHCs, with 21%, 23%, 17%, and 24% reporting 1, 2, 3, or 4+ CHCs, respectively. 47% reported any ototoxicity including tinnitus in 36% of all TCS. Ten patients reported hearing aid use. 55% reported PN, while 29% had both PN and ototoxicity. Medication use for HTN, hypercholesterolemia, DM, ED, pain, or anxiety/depression was reported by 8%, 11%, 4%, 4%, 7%, and 13% of patients respectively.

Conclusions: Several years after CHEM, nearly a quarter of TCS in this study reported 4 or more CHCs. We may have overestimated the number of CHCs, since PN included "a little" symptomatology, and ototoxicity was based on limited binary variables without finer gradation of symptoms. Future studies will continue to identify important CHCs following CHEM in TCS.

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

POSTER #101

CANCER SURVIVORS AS ENGAGED PATIENTS: THE RELATIONSHIP BETWEEN CANCER HISTORY AND PATIENT ACTIVATION

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Background: In 2014, there were approximately 14.5 million cancer survivors in America; many of whom experience physical, psychosocial, and economic sequelae. A central goal of survivorship research is to improve the quality of follow-up care as many survivors report feeling "lost" in the medical system after active treatment. Managing and coordinating follow-up care with multiple providers, and being knowledgeable of their cancer history often become the patients' responsibilities. Therefore, survivors may need to become more activated patients; however, there is lack of literature examining how a personal or family history of cancer is related to patient activation (PA).

Methods: For this analysis, we used data from the 2013 fielding of The Health Information National Trends Survey (HINTS), a nationally representative survey of how American adults acquire and use health information (N = 3185, response rate = 35.19%). Participants reported how often they engaged in 7 PA behaviors such as researching and taking information/lists into visits on a 4-point scale from always to never. This was averaged and median split into high and low PA.

Results: 14.4% of the sample reported a personal history of cancer; 52.2% of the sample had a family-only history of cancer; and 33.4% had no cancer history. We ran fully adjusted, unweighted, logistic regression models to explore the relationship between cancer history and PA. Consistent with past research, female gender (OR=1.62, 95% CI 1.39 to 1.90), older age (OR=2.16, 95% CI 1.64 to 2.86), and college education (OR=1.52, 95% CI 1.11 to 2.08) predicted high PA. Controlling for demographic and clinical characteristics, survivors were more likely to report high PA compared to those with no cancer history (OR = 1.41, 95% CI 1.10 to 1.81). A family-only history of cancer was not associated with high PA.

Conclusions: Future research should examine the mechanisms and motivations for this increased PA in cancer survivors. Given the difficulties survivors report when navigating complicated survivorship care, we should seek to understand not only the positive consequences of increased PA, but also potential increased burden to survivors.

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

POSTER #102

PSORIASIS AND RISK OF NON-MELANOMA SKIN CANCER AMONG WOMEN IN THE UNITED STATES: A POPULATION-BASED COHORT STUDY

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Background: Previous clinical-based studies have linked psoriasis with risk of non-melanoma skin cancer (NMSC), particularly squamous cell carcinoma (SCC), but the associations have been attributed to systemic treatment among moderate to severe psoriasis. There are scant data available in common psoriasis patients. **Methods:** We prospectively examined the association between psoriasis and risk of NMSC, based on 157,933 participants from two cohorts of women, the Nurses' Health Study (NHS, n=63,053) and NHS II (n=94,880).Diagnoses of NMSC, including basal cell carcinoma (BCC) and SCC were obtained by self-reported questionnaires biannually and all SCC cases were confirmed. Information on clinician-diagnosed psoriasis and diagnosis year was collected in 2008 (NHS) and 2005 (NHS II) and validated with a supplementary questionnaire. **Results:** We included 2,645 women with psoriasis, including 1,137 in the NHS and 1508 in the NHS II. In the NHS, we documented 1,204 SCC cases and 8,908 BCC cases after around 740,000 person-years of follow-up. In the NHS II, we documented 521 SCC cases and 7,176 BCC cases after around 1,740,000 person-years of follow-up. For the combined cohorts, psoriasis was associated with an elevated risk of SCC, with a multivariate-adjusted relative risk (RR) of 1.51 (95% CI 1.11-2.05). The associations appeared stronger with increasing psoriasis severity with RR of 1.42 (95%CI, 0.94-2.15) in the mild psoriasis group and RR of 1.98 (95%CI, 0.74-5.32) in the moderate-severe psoriasis group (P for trend=0.03). There was no association between psoriasis and the risk of BCC (RR, 0.95; 95% CI 0.75-1.18). **Conclusions:** A personal history of psoriasis may be associated with an increased risk of SCC. Further investigations are warranted to understand the underlying mechanisms.

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

POSTER #103

NO ASSOCIATION BETWEEN SMOKING BEHAVIORS-RELATED ALLELES AND THE RISK OF MELANOMA.

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Background: Several studies have reported that cigarette smoking is inversely associated with the risk of melanoma. Consistent evidence has suggested genetic basis for smoking behaviors. We hypothesized those genetic alleles associated with smoking behaviors may play a role in the melanoma development.

Methods: We investigated the association between single-nucleotide polymorphisms (SNP) selected from genome-wide association studies (GWAS) on smoking behaviors and risk of melanoma using 2,298 melanoma cases and 6,654 controls.

Results: Among sixteen SNPs,three**(**rs16969968 [A], rs1051730 [A] and rs2036534 [C] in the 15q25.1 region) reached nominal significance for their associations with melanoma risk (0.01<=*P* values <= 0.05; 0.91<= Odds Ratios (ORs) <= 1.14). These associations were not significant after the Bonferroni correction for multiple comparisons. There was no association between the genetic scores based on the number of smoking behaviors-risk alleles and melanoma risk.

Conclusions: We found no strong evidence that smoking behaviors-related SNPs were associated with risk of melanoma.

Impact: Our study suggests that smoking behaviors-related SNPs are less likely to play a major role in melanoma development.

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

POSTER #104

PSYCHOTROPIC AND STIMULANT MEDICATION (PSM) USE AMONG TESTICULAR CANCER SURVIVORS (TCS): A MULTI-INSTITUTIONAL CLINICAL STUDY OF 680 PATIENTS GIVEN CISPLATIN-BASED CHEMOTHERAPY (CHEM) (NCI 1R01 CA157823-02)

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Introduction

TCS are known to be at increased risk for acute and chronic medical conditions, but few studies have examined barometers of their psychological health.

Objectives

To characterize the prevalence of PSM use and associations with demographics, health behaviors, and treatment-associated toxicities among TCS.

Methods

TCS aged ≤49 years at first-line CHEM completed a questionnaire regarding co-morbidities and prescription drug use, including PSMs. For co-morbidities, peripheral neuropathy (PN) responses of "a little", "quite a bit", or "very much" were scored "yes".Fisher's exact test was used to examine the significance of various associations.

Results

Among the first 680 consecutively enrolled TCS, median age at TC diagnosis was 31y (range, 15-49y) and

median time since CHEM completion was 52mo (range 12-360mo). 85 TCS (12.5%) reported PSM use, including antidepressants (N=65 [76.5%]), anxiolytics (N=23 [27%]), and stimulants (N=21 [25%]) with 20 TCS on ≥2 PSMs (23%). Compared to non-users, more PSM users were unemployed (11.8% vs. 4.4%%; P<.01), self-rated their health as fair/poor (12.2% vs 4%; P<.01), and had gained >20lb since CHEM (39.8% vs 23.4%;P<.01). PSM users were more likely to have tinnitus (49.4% vs. 36.4%; P<0.04), both tinnitus and PN (43.5% vs. 27.2%; P<0.01), cardiovascular disease (26.2% vs. 15.6%; P<.02), and greater use of prescription medications for pain control (20% vs. 4.7%; P<0.01), hypertension (16.5% vs. 7.1%; P<0.01), diabetes (8.3% vs. 2.9%; P<0.02), and testosterone replacement (10.6% vs. 5.0%; P=0.048).

Conclusions

Future studies should aim for identification of high-risk patients in need of intensified preventive and therapeutic interventions.

Population Science/Epidemiology ***Research Technician***

POSTER #105

MANAGEMENT OF CISPLATIN-INDUCED PERIPHERAL NEUROPATHY WITH OXCARBAZEPINE PLUS MORPHINE: A CASE PRESENTATION.

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Introduction: Multiple adverse events are associated with the use of morphine for the treatment of chronic cancer pain, including opioid-induced hyperalgesia (OIH). Mechanisms of OIH are independent of opioid tolerance and may involve the morphine metabolite morphine-3-glucuronide (M3G). Approximately 60% of morphine is glucuronidated to M3G which may aggravate preexisting pain conditions. Accumulating evidence indicates that M3G signaling through neuronal Toll-like receptor 4 (TLR4) may be central to this proalgesic signaling event by increased voltage-gated sodium (NaV) activity. The M3G-dependent excitability and potentiation of NaV current in these sensory neurons could be reversed by the addition of oxcarbazepine (OXC), a known inhibitor of NaV currents. Our observations demonstrate a potential therapeutic use of morphine and OXC as a combination treatment for severe cancer and neuropathic pain which may improve pain control, reduce morphine use and improve the quality of life.

Case presentation: We report a case of 59-year-old gentleman with a history of relapsed testicular cancer with no evidence of recurrent disease. He had completed chemotherapy in 2004. Since then he had suffered from painful neuropathy for the last ten years. He had tried multiple treatments for his neuropathy without improvement. The patient was enrolled in a IU Simon Cancer Center Phase Ib study to study the pharmacokinetics of OXC in combination with morphine. The patient has tolerated the maximum dose, and has reported significant improvement in pain relief and quality of life.

Conclusions: In this case of refractory pain,the combined use of OXC with morphine was safe and resulted in complete pain relief, reduction of morphine use and improved quality of life. Pharmacokinetic study of this patient supported our hypothesis.

Translational/Clincal Research ***Faculty***

POSTER #106

TMPRSS2-ERG GENE FUSION IS RARE BUT PTEN DELETIONS ARE MORE COMMONLY OBSERVED IN STAGE T1A PROSTATE CANCER

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Background: T1a prostate cancers are indolent tumors that are derived from the transition zone. The overexpression of ERG and the inactivation of PTEN have been shown to be important drivers of carcinogenesis in large series of prostate cancer, but the genetics of transitional zone tumors have not been well characterized.

Design: We evaluated the status of ERG and PTEN in formalin-fixed paraffin-embedded tissue using immunohistochemical and FISH analysis in 47 pT1a tumors that arose within the transition zone. The protein expression of ERG was determined using a rabbit monoclonal antibody (clone EPR 3864, Epitomics Inc) and nuclear staining was scored as positive or negative. The genomic status of ERG was determined using 3 colored FISH using an ERG-TMPRSS2 tri-color probe set (5′ ERG, green, 3′ ERG gold, TMPRSS2 Aqua).The protein expression of PTEN was determined using a rabbit monoclonal antibody (D4.3, Cell signaling technology) and cytoplasmic and nuclear staining was scored as positive or negative. The genomic status of PTEN was determined using dual color FISH with a PTEN probe and a CEP10 probe.

Results: We found ERG rearrangement and coordinate protein overexpression in 1 of 47 tumors (2.1%). No adjacent benign tissue showed ERG overexpression or rearrangement. We found PTEN inactivation in 8 of 47 tumors (17.1%). Seven of the 8 PTEN alleles were inactivated by deletion. No homozygous PTEN deletion was observed. PTEN deletion and ERG rearrangement were mutually exclusive.

Conclusions: ERG rearrangement and PTEN loss are both underrepresented in pT1a transition zone tumors, however, PTEN loss is relatively common in pT1a prostate cancers.

Translational/Clincal Research ***Faculty***

POSTER #107

KNOCKDOWN OF SPLICING FACTOR ESRP1 AFFECTS MULTIPLE SPLICING FACTORS

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Introduction:

Tissue-specific alternative splicing (AS) is an important mechanism for regulating gene expression. Epithelial Splicing Regulatory Proteins 1 and 2 (*ESRP1* and *ESRP2*), two RNA-binding proteins (RBPs) that promote splicing, are potential candidates that contribute to breast cancer recurrence and resistance to therapies(1,2). In our prior studies, we have shown that *ESRPs* are associated with endocrine resistance. In this study, we seek to investigate the impact of the *ESRP1* knockdown in endocrine resistant breast cancer cells.

Methods:

Expression of *ESRP1* was analyzed in endocrine resistant-LCC2 and LCC9 cells. Knockdown of *ESRP1* expression in LCC2 and LCC9 cells was achieved using lenti-viral based shRNA approach (Mission TRC human shRNA constructs, Sigma). To determine the alternative splicing events regulated by *ESRP1*, we performed Human Transcriptome Array (HTA 2.0) of LCC2 and LCC9 cells transfected with control and clones representative of shRNA knockdown according to the manufacturer's instructions (Affymetrix).

Results:

ESRP1 knockdown was confirmed using qRT-PCR analysis in LCC2 (clone 2C3- 95% reduction) and in LCC9 cells (clone 9C2- 90% reduction). HTA analysis demonstrated that *ESRP1* regulates the expression of several genesincluding other splicing factors. Furthermore, HTA revealed a possible role of altering important pathways related to nuclear receptor meta-pathway and miR pathway related to epithelium, lymphocytes and muscle. This data was similar to that obtained in knockdown of LCC9 cells.

Conclusion:

ESRP1 regulates splicing of multiple genes in ER+ breast cancer. Targeting splicing could be a novel modality for treating endocrine therapy resistant breast cancer.

Translational/Clincal Research Faculty

POSTER #108

A RETROSPECTIVE ANALYSIS OF PATIENTS WITH METASTATIC GERM CELL TUMOR (GCT) TREATED AT INDIANA UNIVERSITY (IU) FROM 2000 TO 2012.

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Background: Metastatic GCT's have been classified by the International Germ Cell Cancer Collaborative Group (IGCCCG) in 1997 into good, intermediate, and poor risk with 5-year progression free survival (PFS) of 88, 75 and 41%, respectively and 5-year overal survival rate (OS) of 91, 79 and 48%, respectively.

Methods: We conducted a retrospective analysis on outcomes of all patients with GCT who were diagnosed and received initial chemotherapy at IU between 2000-2012. We limited our analysis to patients with >1 year of f/u. 5 years PFS and OS were analyzed using Kaplan Meier methods.

Results: 403 patients were evaluable. 240 (58%) good, 46(11%) intermediate and 127 (30%) poor risk. Median time of f/u 5 years. Median age at diagnosis 29. The 5-year PFS of good, intermediate, and poor risk groups were 91, 80 and 52% (P value <0.01) and 5-year survival rates were 95, 89 and 71%, (P value <0.01) respectively.

Conclusions: There was improvement in OS for men with poor risk metastatic GCT in this contemporary cohort of patients, possibly due to improved salvage chemotherapy compared to patients treated from 1975-1990 reported by the IGCCC.

Translational/Clincal Research ***Faculty***

POSTER #109

A P38 MAP KINASE INHIBITOR EXACERBATES OSTEOLYTIC BONE METASTASES DUE TO BREAST AND PROSTATE CANCERS AND HAS NO EFFECT ON OSTEOBLASTIC METASTASES

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Breast and prostate cancer often metastasize to bone, where they can either destroy bone or stimulate disorganized bone formation.Substantial data support a central role in osteolytic metastases for bone-derived transforming growth factor-beta (TGF-B), which activates the Smad signaling pathway in tumor cells. TGF can also act through Smad-independent pathways including p38, a member of the mitogen-activated protein (MAP) kinase family, which is activated in response to inflammatory and environmental stresses. Overexpression of a dominant negative form of p38MAPKa decreased MMP9 secretion and bone metastases in a breast cancer model . We tested an ATP-competitive inhibitor selective for p38 MAP kinase, the indole-5-carboxamide SD-282 in several animal models of bone metastases. SD-282 prevents bone loss and inhibits osteoclastogenesis in several experimental settings and decreases tumor growth in an animal model of multiple myeloma, where it decreases the phosphorylation of p38.

We tested SD-282 on bone metastases caused by MDA-MB-231 breast carcinoma cells and PC-3 prostate cancer cells (both giving osteolytic lesions) and the osteoblastic prostate cancer xenograft LuCaP 23.1. Nude mice were treated with 30 or 90mg/kg/twice/day of SD-282 by gavage. Treatment was started after detection of lesions by x-ray. In MDA-231 tumor-bearing mice, SD-282 increased osteolytic lesion area, as assessed by computerized image analysis of radiographs, at either 30mg/kg ($p=0.0056$) or 90mg ($p=0.0012$) doses. Histomorphometry showed that in SD-282 treated mice there was a tendency towards increase in tumor burden accompanied by a reduction in total bone area. No change in osteoclast number at the tumor:bone interface was noted. In PC-3 tumor-bearing mice, SD-282 similarly increased osteolytic bone destruction, as assessed by computerized image analysis of radiographs, with either 30mg/kg ($p=0.0152$) or 90mg ($p=0.0419$) doses of the drug. However, in mice with LuCaP23.1 prostate xenografts SD-282 had no effect on bone lesions, as assessed by x-ray. When areas unaffected by MDA-MB-231 tumor were examined, BMD was increased relative to vehicle-treated mice, confirming previously reported systemic effects of SD-282 on the skeleton.

The results with the two osteolytic metastasis models are opposite to those predicted from the known effects of SD-282 on bone and on myeloma cells. They suggest that p38 MAP kinase may not be a useful drug target for treatment of bone metastases, and small molecule inhibitors of p38 MAPK may worsen osteolytic metastases by unknown and tumor-specific mechanisms.

Translational/Clincal Research ***Faculty***

POSTER #110

ESTRADIOL-INDUCIBLE DEPENDENCE RECEPTOR UNC5A RESTRICTS ESTROGEN RECEPTOR ACTIVITY AND IMPARTS ESTRADIOL DEPENDENCE TO BREAST CANCER CELLS.

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Signaling by Dependence Receptors (DRs) is a new paradigm in cell signaling and recently has been recognized to play a major role in cancers DRs are cell surface receptors that function as tumor suppressors or induce apoptosis when disengaged from their ligands. In the presence of ligands, these receptors transmit proliferation, migratory, and anti-apoptotic signals thus creating a cellular state that is dependent on ligands for survival. This study was focused on regulation of their expression in breast cancer. We observed that UNC5a, a DR, and its ligand Netrin-1 (NTN1) are estradiol (E2)-inducible depending on cell types. Since E2 and its receptor estrogen receptor alpha (ERa) are major signaling hubs in ERa-positive breast cancers, we examined the function of UNC5a in ERa-positive breast cancer cells. Knockdown of UNC5a resulted in deregulated expression of E2-regulated genes, E2-independent and anti-estrogen-resistant growth in vitro, and E2-independent tumor formation in xenograft models. RNA-seq analyses of parental and UNC5a knockdown cells with or without E2 treatment revealed ~ 10-fold increase in number of E2-regulated genes in UNC5a knockdown cells compared with parental cells. The basal expression of E2-inducible anti-apoptotic BCL-2 was elevated >30-fold at mRNA and/or protein levels in UNC5a knockdown cells compared with vector control cells. In addition, UNC5a knockdown resulted in aberrant NF-kB signaling, which is typically under negative regulation by ERa:E2. Furthermore, UNC5a knockdown cells showed elevated expression of p63, a p53 family transcription factor that promotes breast epithelial stem cell maintenance and basal-like breast cancer. Consistent with the known role of NF-kB and p63 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 parental cells. 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 observed frequently 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.

Translational/Clincal Research ***Faculty***

POSTER #111

RAP1GTPASES REGULATE THE RETENTION AND ENGRAFTMENT OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Rap1, a small-molecular-weight GTP-binding protein that belongs to the Ras-like superfamily of GTPases, is involved in signal transduction cascades. Rap1 cycles between a GDP-bound inactive and a GTP-bound active form, and this switching is regulated by specific GEFs (Guanine Nucleotide Exchange Factors) and GAPs. Rap1 exists in two isoforms - Rap1a and Rap1b which are necessary for migration, adhesion as well as functions of mature hematopoietic cells. While Rap1 has been implicated in regulating several hematologic disorders including chronic lymphocytic leukemia, myeloproliferative stem cell disorders, polycythemia vera and sickle cell anemia, its role in the development and function of hematopoietic stem and progenitors (HSC/Ps) has not been investigated. To study the role of Rap1 in HSC/Ps, we generated a mouse model in which both Rap1a and Rap1b isoforms were conditionally deleted in HSC/Ps (Rap1a/b -/- double knock out (DKO) using polyI: polyC inducible cre-lox system. Our data demonstrate that deletion of both isoforms of Rap1 resulted in enhanced peripheral blood leukocyte counts, mobilization of primitive hematopoietic stem cells Lin-ckit+Sca1+ (LSK) and decrease in erythroid parameters including red blood cells, hemoglobin and hematocrits. In the marrow, Rap1ab deficiency shows reduced cellularity but increased frequency of LSK CD150-CD48- cells (long term hematopoietic stem cells) along with increase in granulocyte-macrophage progenitors population. In contrast, spleen cellularity, size, weight and LSK cells in the spleen were significantly enhanced in DKO mice relative to controls.Given these findings, we hypothesized that perhaps Rap1 plays an essential role in the retention of HSC/Ps in the bone marrow and that loss of Rap1 might inhibit the interaction of HSC/Ps with the niche resulting in the egress of HSC/Ps and thus creating empty space(s) in the marrow for enhanced engraftment of donor cells when transplanted under non-myeloablative conditions.

To test this possibility, we performed bone marrow transplantation using Rap1ab DKO mice as recipients and GFP expressing bone marrow cells as donors in the absence of any conditioning.Our results demonstrate significantly greater donor derived reconstitution of GFP positive cells in DKO recipients compared to WT controls, suggesting that loss of Rap1ab creates a functional vacant niche(s) in the bone marrow.

We next assessed the potential of Rap1ab deficient cells to engraft in a lethally irradiated host. We performed competitive repopulation assay. Rap1ab-/- HSC/Ps showed a defect in engraftment as well as multi-lineage reconstitution when transplanted into lethally irradiated hosts compared to WT controls. To asses if the defect in engraftment could be attributed to impaired homing of DKO HSC/Ps, we performed a homing assay using DiD fluorescence dye and the data showed a significant homing defect Rap1ab deficient cells compared to WT controls. To assess which specific isoform of Rap1 is essential for mobilization and engraftment/homing of HSC/Ps, we induced deletion in Rap1a and Rap1b separately (single knock out mice) and assessed these mice for peripheral blood counts. We found no significant changes in the leukocyte counts in single KO mice relative to controls, suggesting that mobilization of HSC/Ps was relatively unperturbed in these mice. In contrast, engraftment of HSC/Ps derived from the single KOs of Rap1a and Rap1b was impaired to the same extent as DKO HSC/Ps. These data suggest that loss of single Rap1 isoform contribute similarly to the engraftment of HSC/Ps, whereas the combined loss of both isoforms are required for mobilization of HSC/Ps.

Translational/Clincal Research Faculty

POSTER #112

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

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Androgen deprivation therapy (ADT) as a treatment for prostate cancer (PCa) contributes to weaker bone, and predisposes patients to an increased risk of fractures, including hip fractures. The overall quality of life for the patient and his family are negatively affected on a permanent basis following a hip fracture. Bone remodeling is characterized by osteoblast-mediated bone synthesis and osteoclast-mediated bone resorption. Imbalances in this process, whereby resorption outpaces synthesis, result in pathological conditions such as osteoporosis. In contrast to anti-resorptive therapies, anabolic therapies are largely underdeveloped and form the highest clinical need in the treatment of ADT-associated osteoporosis. Ca^{2+}/calmodulin (CaM)-dependent protein kinase kinase 2 (CaMKK2) plays a role in both the anabolic and catabolic pathways of bone remodeling, such that its inhibition positively impacts osteoblasts and negatively impacts osteoclasts. Pharmacological inhibition of CaMKK2 using STO-609 protects against in ovariectomy (OVX)-mediated and age-associated bone loss in mice. Whereas CaMKK2 is not expressed in normal prostate, 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. Moreover, treatment with STO-609 significantly impaired the growth of PCa cells xenografted into the caudal flank region of nude mice. However, the exact downstream mechanism by which CaMKK2 regulates PCa growth and/or migration remains unknown.

We hypothesize that the inhibition of a single target, CaMKK2, will result in the therapeutic alleviation of two major complications in advanced-stage PCa, i.e., ADT-induced bone loss and bone metastatic tumor burden. To test this idea, we performed sham or bilateral orchiectomy (ORX) on 5 week old male athymic nude mice that were pre-treated with three intraperitoneal (i.p.) injections or saline or STO-609. Two weeks after the surgery, castrate-resistant C4-2B PCa cells were injected into the right tibiae of all mice. Tri-weekly i.p. injections of saline or STO-609 were continued for six additional weeks and tumor progression was monitored by bi-weekly X-ray analyses. Trabecular and cortical bone osteolysis were assessed by micro-CT imaging and tumor occupancy within the tibiae as well as bone parameters were measured by histomorphometry. The effect of STO-609 on bone volume as well as osteoclast and osteoblast numbers will be assessed and the correlation of these data with those of tumor occupancy in tibiae will be discussed. Our studies represent a highly novel and unique approach in the treatment of PCa patients with advanced stage disease.

Translational/Clincal Research ***Faculty***

POSTER #113

APURINIC/APYRIMIDINIC ENDONUCLEASE-1 AND HEPATOCELLULAR CARCINOMA

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The enzyme apurinic/apyrimidinic endonuclease-1 (APE1) is associated with protection against DNA and oxidative damage congruent with cancer emergence and resistance to chemotherapy. The purpose of this study is to investigate APE1 levels in association with hepatocellular carcinoma (HCC). This will be achieved by comparing APE1 levels in patients with underlying liver disease (HepC) that have not developed HCC with patients that have been diagnosed with both HepC and HCC. In addition APE1 levels were determined in $MDR2^{-/-}$ mice that spontaneously develop HCC. Our goals are to (1) improve stratification of patients with underlying liver disease for the risk of HCC development (2) describe novel targets to improved therapy options, and (3) identify a murine model for future studies. **Methods:** Hepatic and serum APE1 levels were determined by immunohistochemistry staining and/or ELISA in patients with HepC HCC that had received standard of care. In addition, APE1 levels were determined in untreated HCC tumors by ELISA. Hepatic APE1 staining was quantified by immunohistochemistry in $MDR2^{-/-}$ mice with HCC and in $MDR2^{-/+}$ mice controls that do not develop HCC. **Results:** Hepatic APE1 levels and staining was significantly lower in patients with HepC and HCC when compared to patients with HepC that had not developed HCC. However, APE1 levels were significantly greater in HCC tumors when compared to livers from patients with HepC irrespective of HCC development. Correspondingly, serum APE1 levels were greater in patients with HepC and HCC. Hepatic APE1 staining in $MDR2^{-/-}$ mice with HCC was lower when compared to $MDR2^{-/+}$ mice, correlating well with the human data. In addition, APE1 staining was increased in HCC tumors of $MDR2^{-/-}$ mice when compared to adjacent liver. **Conclusion:** Increased APE1 is a possible biomarker of HCC risk in patients with underlying liver disease and is a novel target for therapy in patients with established HCC. By blunting APE1 protection against DNA and oxidative damage and redox control of transcription factors, targeted APE1 inhibition has the potential to augment chemotherapy response. However, additional studies are required to better understand the risk of generalized APE1 inhibition exacerbating hepatic sensitivity to chemotherapy due to increased DNA damage. The $MDR2^{-/-}$ mouse model of HCC appears to be a suitable model for future studies.

Translational/Clincal Research ***Faculty***

POSTER #114

ASCORBIC ACID COMBINED WITH THE ANTI-DIABETIC DRUG PHENFORMIN SYNERGISTICALLY INDUCE CELL DEATH IN NON-SMALL CELL LUNG CANCER CELLS

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An exceptional characteristic of many cancer cells is increased glucose uptake and elevated glycolysis coupled with an increased flux through the pentose phosphate shunt, and enhanced activity of the mitochondrial TCA cycle. This unique phenomenon of tumor cells is responsible for generating high levels of biosynthetic precursors for amino acids, nucleotides and lipids as well as generating ATP that is required to enhance high levels of cell proliferation. Given the high rate of ATP consumption in cancer cells and the limited glucose supply in the tumor microenvironment, intracellular ATP depletion is potentially a target for selective inhibition of tumor growth and survival over that of normal cells. Since, tumor cells already contain higher levels of basal reactive oxygen species (ROS) than normal due to the Warburg effect, additional ROS generation is also a potential target for selective inhibition of tumor growth and survival. Phenformin, an anti-diabetic drug has been shown to have anti-tumor activity and target cancer cells by inhibiting complex I of the mitochondrial respiratory chain, resulting in reduced ATP levels and increased ROS. We have previously shown that pharmacologic doses of ascorbic acid (AA) are selectively toxic to non-small cell lung cancer (NSCLC) cells through a ROS-dependent mechanism and that AA combined with a glycolysis inhibitor synergistically induce apoptosis. We have now extended these studies by investigating the effects on NSCLC cells of AA alone or in combination with phenformin at physiologically low glucose concentrations with differing LKB1 status (wild type, mutant and dominant negative).

Results: Cell viability assays were used to study the effect of AA, phenformin or combinations of both on the NSCLC cell viability. IC50 values showed that some LKB1-mutant cell lines were more sensitive to AA or phenformin when compared to cell lines with wild type LKB1. The combined treatment of AA and phenformin caused a synergistic induction of cell death in both LKB1 mutant and wild type cells at concentrations well below the IC50 of each compound alone. This synergistic induction of cell death was more pronounced in media containing 0.5 mM glucose when compared to 1 mM glucose, indicating that these drugs effectively kill the cancer cells at physiological glucose concentrations. Current studies are focused on determining the mechanism underlying the effects of AA, phenformin or combinations of both in LKB1 negative cell lines.

Conclusions: The major significance of these studies is that, pharmacologic concentrations of AA selectively synergize with the anti-diabetic drug phenformin in killing NSCLC cells. This synergistic induction of cell death is enhanced at the lower glucose levels commonly found in tumor microenvironments and suggests that this combination treatment may represent a promising therapy for NSCLC patients.

Translational/Clincal Research ***Faculty***

POSTER #115

AURICULAR PROSTHETIC: 3DMD FACIAL SCANNING, DIGITAL PROSTHETIC DESIGN, AND 3-DIMENSIONAL PRINTING

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Maxillofacial defects can cause facial disfigurements resulting from congenital abnormalities, surgical resection of tumors, trauma, or a combination of these. Auricular prosthesis is an alternative option, when esthetic and functional demands cannot be surgically fulfilled. The purpose of this report was to discuss facial prosthetic workflow and fabrication by using available techniques. In this clinical case report, we discuss three different possible ways to fabricate auricular prostheses for a 70 years old patient, whose ear was resected due to a basal cell carcinoma, via three methods: traditional, digital, and combination of both. Methods and materials: PVS impression (GC America; Alsip, IL, Factor II; Lakeside, AZ), prosthetic wax (FactorII; Lakeside, AZ) for wax pattern ear prosthetic digital facial scanning (3dMD; Atlanta, GA), computed tomography for soft tissue digital modeling, digital design software (GeoMagic Design X; Cary, NC) to merge scanning and CT data, and 3-dimensional printing (Whip Mix Corp., Louisville, KY). Digital technologies can improve prosthetic design, fabrication position, and esthetic. In addition, time and comforts factors for the patient and the specialists are more appreciated by using these technologies.In this case, a fully digital method utilizing 3dMD facial scanning data, contralateral ear digital mirroring and positioning design, 3-dimensional printed surgical guides and printed mold are highly promising and recommended to obtain high quality of work and less time consuming.

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

POSTER #116

MANDIBULECTOMY PROSTHETIC CASE REPORT: FACIAL SCANNING, DIGITAL DESIGN, 3-DIMENSIONAL PRINTING.

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>Maxillofacial silicone elastomer prostheses are used to replace facial features surgically removed due to disease or lost due to trauma. Traditional prosthetic fabrication (impression, intuitive wax-up, and silicone processing) can be streamlined using digital scanning, computer-assisted design and manufacturing, reducing cost, saving time, and improving prosthetic predictability. In addition, digital technology can eliminate the arduous impression process and reduce required office visits for patients. Current digital design software and 3-dimensional printing hardware is complex and expensive. This limits the viability and practicality of a fully digital workflow. Combining both traditional and digital techniques (hybrid process) overcomes limitations in digital design, improves patient experience, and provides more predictable outcomes. The purpose of this study was to improve traditional facial prosthetic workflow and fabrication using facial scanning and 3-dimensional prosthetic design and printing technology. In this clinical case report, we present multiple facial prostheses for a mandibulectomy patient via three methods: traditional, digital, and hybrid of both. Methods and materials included PVS impression (GC America; Alsip, IL), prosthetic wax (Factor2; Lakeside, AZ) and oil-based clay (NSP by Chavant; Farmingdale, NJ) for prosthetic mock-ups, digital facial scanning (3dMD; Atlanta, GA), computed tomography for soft tissue digital modeling (IU Health affiliate hospital; manufacturer unknown), digital design software (GeoMagic Design X; Cary, NC) to merge scanning and CT data, and 3-dimensional printing (Whip Mix Corp., Louisville, KY). We identified areas of improvement for traditional maxillofacial prosthetics using digital scanning and 3-dimensional printing technology. A hybrid method combining traditional impression, prosthetic mock-up, digital scanning / design, and 3-dimensional printing is recommended to overcome limitations in CT soft tissue data, facial scanning, and digital design software, as well as improve patient experience and provide more predictable prosthetic outcomes.

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

POSTER #117

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

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Breast cancer is the most common cancer among women worldwide. In 2014, over 200,000 cases were diagnosed in the U.S. Of the cases diagnosed, 15-20% of the cases are categorized into one type of breast cancer called triple-negative breast cancer (TNBC), which is determined by the absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2). It is generally associated with poor prognosis, relatively short disease-free survival (DFS), and high risk of local and distal recurrence due to the lack of targeted treatments. In order to provide timely medical intervention, methods of early detection of tumor recurrence are vital to potentially developing interventions that could prevent relapse. The goal of this study is to identify predictive biomarkers for tumor recurrence from patients with TNBC. In this study, we mainly focus on plasma tumor DNA (ptDNA). Clinical samples were collected from triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy from the Hoosier Cancer Research Network Phase II trial BRE09-146. We collected the primary tumors, blood samples and four time points of plasma samples post-surgery from 44 patients. ptDNA is the genetic material released into circulation from tumor cells. The amount of ptDNA in circulation, however, is limited compared to total cell-free DNA (cfDNA), which is mainly contributed by normal cells after necrosis or apoptosis. Thus, amplifying the signal of ptDNA is required. We amplified extracted DNA using the Life Technologies Oncomine Cancer Panel, which is comprised of 150 cancer-related genes, including TP53 and PIK3CA, which are the most commonly mutated genes in TNBC patients. Amplified libraries were sequenced on an Ion Proton next-generation sequencer to at least 2500X coverage. Out of 44 patients, 39 patients with matched tumor, blood and plasma were analyzed. We first detected somatic mutations by analyzing primary tumors and comparing with matched blood sample. We then analyzed sequencing data of plasma to find the same mutation in the previous step. In this study, we were able to detect somatic ptDNA in 4 patients, and all of these patients had a rapid recurrence (0.3, 4.0, 5.3 and 8.9 months), indicating that this method is able to detect rapid-recurrence but limited to detect distant recurrence.

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

POSTER #118

THE ROLE OF PI3K P110DELTA IN GAIN-OF-FUNCTION SHP2 NEUTROPHIL ROS PRODUCTION AND MYELOMONOCYTIC PROGENITOR INFILTRATION OF PERIPHERAL TISSUE

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Juvenile myelomonocytic leukemia (JMML) is a fatal myelodysplastic/ myeloproliferative neoplasm that affects young children. Conventional chemotherapy is ineffective and the only curative treatment is allogeneic bone marrow transplant, yet 50% of children will relapse and succumb to leukemia. JMML patients commonly present with a hyperinflammatory syndrome and mortality is due to infiltration of myelomonocytic progenitors, monocytes, and macrophages into extramedullary tissues, leading to bleeding, infection, and organ failure. Given the hyperinflammatory nature of JMML, we surmise that an altered, hostile bone marrow microenvironment promotes the egress of myelomonocytic progenitors from the bone marrow to peripheral tissues and leads to poor engraftment of normal donor cells following allogeneic stem cell transplant. Previous studies in our lab have shown that the PI3K subunit, p110delta, uniquely promotes JMML progression and hypersensitivity to growth factors. In preliminary studies, we found that mice expressing gain-of-function mutant Shp2 (Shp2D61Y, one of the most common mutations found in JMML) have greater numbers of neutrophils in the spleen, which is normalized upon genetic inhibition of p110delta. Further, Shp2D61Y-expressing neutrophils overproduce reactive oxygen species (ROS) in response to various stimuli. Given the crucial nature of PI3K p110delta in innate immunity, ***we hypothesize*** that specific inhibition of p110delta will reduce hyperinflammatory neutrophils, inhibit neutrophil migration and progenitor cell egress from the bone marrow to peripheral organs, and yield a favorable bone marrow environment for improved donor WT cell engraftment. To address this hypothesis, we stimulated Shp2D61Y bone marrow-derived myeloid cells with serum opsonized zymosan and, using a luminol-based assay, observed that ROS production was decreased by the p110delta-specific inhibitor, GS-9820, in a dose-dependent manner. To examine if increased ROS is causative for increased progenitor egress in Shp2D61Y-expressing mice, we injected mice with a granulocyte-depleting antibody 1A8, and successfully depleted peripheral blood progenitors based on flow analysis. We found that animals with depleted granulocytes retained more hematopoietic progenitors (KIT+) in the bone marrow compared to animals treated with the isotype control, and had an increased number of colony-forming units measured by methylcellulose-based assays. These studies suggest a novel mechanism for p110delta's role in JMML progression and are a step towards generating the necessary pre-clinical studies to introduce p110delta inhibitors into clinical trials for JMML patients.

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

POSTER #119

NOVEL COMBINATION THERAPY OF DNMT INHIBITOR SGI-110 AND PARP INHIBITOR BMN-673 (TALAZOPARIB) FOR BRCA-DEFICIENT AND PROFICIENT BREAST AND OVARIAN CANCERS

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Breast and ovarian cancers 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 were recently indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer, but most ovarian and breast 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 hypothesized that combining DNMTi and PARPi will impair BRCA-mediated DDR, resulting in cytotoxicity in both breast and ovarian cancer cells. A panel of breast cancer cell lines (MCF7 (BRCA-wt), MDA-MB-231 (BRCA-wt), and SKBR3 (decreased BRCA1 expression)) and ovarian cancer cell lines (A2780 (platinum sensitive, BRCA-wild type (wt)), A2780-cp and HeyC2 (platinum resistant, BRCA-wt) and Kuramochi (platinum resistant, BRCA2 mutant) were examined for cell growth using colony formation assays after treatment with DNMTi SGI-110 (5, 20, or 100nM) and PARPi talazoparib (1 or 10nM), alone or in combination. Combination treatment schemas consisted of: 1) "priming": SGI-110 for three days, 24 hour recovery, then talazoparib treatment on Day 5 or 2) "co-administration": talazoparib administration on day 1 and SGI-110 treatment on days 1-3. In the breast cancer cell lines, colony formation was reduced ($P<0.05$) by SGI-110 or talazoparib alone (dose-dependent manner); however, combination drug treatments resulted in greater ($P<0.05$) reduction of colony formation, regardless of BRCA expression. Similarly, in the ovarian cancer cell lines, talazoparib alone reduced ($P<0.05$) colony formation (all cell lines, dose-dependent manner), while combination drug treatments again resulted in a greater ($P<0.05$) decrease in percent survival. To focus specifically on the effect of BRCA status on these treatments, we utilized two ovarian cancer cells lines derived from the same patient, differing notably in BRCA2 status: PEO1 (BRCA2-deficient) and PEO4 (BRCA2-proficent). Western blot analysis of the SGI-110-treated PEO1 and PEO4 demonstrated increased ($P<0.05$) PARP levels and enzymatic activity ($P<0.05$), while talazoparib treatment increased ($P<0.05$) DNMT1 recruitment and decreased PARP activity ($P<0.05$). No change in proliferation was observed after SGI-110 or talazoparib treatment alone; however, the combined drug treatment reduced ($P<0.05$) proliferation. In addition, we observed, by caspase 3 cleavage, increased ($P<0.05$) apoptosis following combination treatment in either PEO cell line, demonstrating that the combination treatment was not only cytostatic but also cytotoxic. In summary, talazoparib combined with SGI-110 displayed increased cytotoxicity in both ovarian and breast cancer cell lines harboring either wt- or mutant-BRCA, indicating that this DNMTi-PARPi drug combination impairs BRCA-mediated DDR and may represent an effective treatment regimen for BRCA-related cancers.

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

POSTER #120

LUNASIN HAS POTENTIAL THERAPEUTIC UTILITY AGAINST MALIGNANT MELANOMA

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Lunasin is a 43-44 amino-acid soy-derived peptide which contains a predicted helix domain homologous to a conserved region found in chromatin-binding proteins and a C-terminal end that includes a RGD cell adhesion motif followed by a poly-aspartic acid tail. Lunasin exhibits both chemoprevention activity as well as therapeutic activity against several cancers including breast cancer, leukemia, non-small cell lung cancer and colon cancers. In these studies, Lunasin's effects have been attributed to modulating histone acetylation and inhibiting integrin signaling, resulting in an antiproliferative or apoptotic response. We have now found that Lunasin also has significant effects on human melanoma cell lines both in vitro and in vivo. A key novel observation is that Lunasin has a selective antiproliferative effect on putative melanoma initiating cells (MIC) identified by elevated expression levels of the aldehyde dehydrogenase (ALDH) enzyme. Previous studies have recognized ALDH as a biomarker for cancer initiating cells (CIC) in several cancer models including breast, colon, and skin. Cells expressing ALDH are enriched in subpopulations displaying stem-like properties including sphere formation and tumorigenicity in immunodeficient mice.

Results: In our study, $ALDH^{high}$ melanoma cells displayed enhanced sensitivity to Lunasin treatment compared to non-sorted samples both in vitro and in vivo. Lunasin inhibited the self-renewal capacity of ALDH expressing cells, and reduced both colony size and formation when plated in soft agar. Lunasin also significantly decreased tumor burden in a mouse xenograft model using A375 cells, with the largest effect seen in tumors initiated using $ALDH^{high}$ A375 cells. Using proximity ligation assays (PLA), we showed that lunasin disrupted interactions between integrin subunits and downstream kinases. Additionally, lunasin treatment of putative MICs induced melanocyte-associated differentiation markers.

Conclusion: Our results suggest that Lunasin may have potential as a component of malignant melanoma treatment that has the ability to specifically target MICs and lessen the risk of patient relapse and subsequent metastases caused by circulating MICs.

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

POSTER #121

DOSIMETRIC COMPARISON BETWEEN 3D CONFORMAL PROTON THERAPY AND IMRT FOR COMPREHENSIVE HEAD AND NECK IRRADIATION

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Introduction: IU Health Proton Center designed a technique for treating cancers of the head and neck using 3D conformal proton therapy without matching to photon treatment fields. IMRT plans were later created to compare the two modalities.

Materials and Methods: Proton plans utilized a monoisocentric technique with a 30 cm snout. Cervical lymphatics were treated with three fields: a right and left posterior oblique field and a posterior-anterior field with a midline block. These were matched to the primary tumor fields and the matchline was staggered by 0.5 cm. Sequential intensity modulated photon (IMRT) plans were designed for 12 patients previously treated using the above-mentioned proton therapy technique. The comparative IMRT plans sought to provide adequate coverage of target volumes while matching or improving upon the sparing of organs at risk (OARs) achieved by the proton plans. Resulting dose to OARs was evaluated and compared between the two modalities.

Results: The head and neck irradiation utilizing proton therapy provided significant dose avoidance to the oral cavity and midline neck structures. When compared to the IMRT plans, the proton treatment plans yielded statistically significant reductions in the mean and integral radiation dose to the oral cavity, larynx, esophagus, and maximally spared parotid gland. There was no significant difference in mean dose to the lesser spared parotid gland, or in mean or integral dose to the spared submandibular glands between modalities.

Conclusions: A technique for cervical nodal irradiation in head and neck cancers using 3D conformal proton therapy was developed and implemented in clinical practice. Use of proton therapy for cervical nodal irradiation resulted in large dose avoidance of several structures in close proximity including the oral cavity, larynx, and esophagus, as well as lower mean and integral dose to assessed OARs when compared to the competing IMRT plans.

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

POSTER #122

EVALUATING THE IMPACT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY SYMPTOMS (CIPN-SX) ON PERCEIVED ABILITY TO WORK IN WOMEN WITH BREAST CANCER DURING THE FIRST YEAR OF SURVIVORSHIP

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Background: Advances in the treatment of breast cancer are increasing the number of *breast cancer survivors* (BCS) that are returning to work. Symptoms like pain and fatigue are known to interfere with survivor's ability to work, but the impact of one of the most common side-effects of cancer treatment on work *chemotherapy-induced peripheral neuropathy* (CIPN) has yet to be described.

Purpose: To describe the impact of CIPN-sx on BCS's ability to work during the first year after treatment.

Methods: Data were collected from breast cancer patients treated with (Ctx+, N=22) and without (Ctx-, N=22) chemotherapy at three time points, baseline (BL; post-surgery, pre-treatment), one month (1M) post-treatment completion, and approximately one year (1Y) following (13 months post-treatment completion). CIPN-sx were collected using the self-reported FACT/GOG-Ntx 11 item subscale. Ability to work was measured by self-report using the FACT-G questionnaire.

Results: At both 1M and 1Y, Ctx+ BCS reported CIPN-sx which were associated with poor work performance. Ctx- BCS reported an average of five distinct CIPN-sx at both 1M and 1Y. Symptoms were largely sensory and motor in nature, including numbness/tingling in hands and feet, discomfort in hands, feeling weak all over, ringing/buzzing in the ears, and trouble buttoning buttons. Both the severity and total number of CIPN-sx survivors reported were significantly correlated with being less able to work. Analysis of covariance (ANCOVA) testing showed that Ctx+ BCS were less able to work than Ctx- BCS at both 1M ($p<0.010$) and 1Y ($p<0.020$). Results of a regression analysis found that the number CIPN-sx and severity of sensory domain could predict 35.0% of the variance in work scores at 1M for Ctx+ BCS ($r^2= 0.35$, adj. $r^2=0.28$, $p<0.017$). At 1Y, sensory and motor CIPN-sx continued to be the best predictors for work scores, accounting for 48.6% of work scores.

Conclusions: During the first year of survivorship, BCS treated with neurotoxic chemotherapy continue to experience CIPN-sx that are associated with inability to work. Results from the analysis indicate that while the severity of CIPN-sx was a significant predictor of difficulty working, the presence of several mild CIPN-sx can also interfere with work. These findings underscore the need for research directly investigating the impact that CIPN-sx have on work performance in BCS at different stages of survivorship. Doing so will be essential first step towards developing interventions to improve work function in BCS receiving neurotoxic chemotherapy.

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

POSTER #123

EVALUATION OF A PERIOPERATIVE PROTOCOL FOR EARLY DISCHARGE AFTER TRANSPHENOIDAL PITUITARY MACROADENOMA RESECTION

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Introduction: An estimated 10-15% of intracranial tumors are pituitary adenomas. These tumors can be categorized as macroadenomas (>10 mm) and microadenomas (<10 mm). Microadenomas are the most common pituitary adenoma and are more likely to be endocrinologically active, while macroadenomas are more often nonfunctioning. Treatment options typically involve either pharmacological management or surgical resection via an endonasal transphenoidal approach. Patients are generally hospitalized for several days post-operation in order to monitor for pain control, neurologic status, cerebrospinal fluid leak, and endocrine complications. Due to the minimally invasive nature of the surgical approach and success of the procedure, there exists potential for a management protocol that would shorten post-operative hospital stay and speed discharge.

Methods: A retrospective review of medical records identified 24 consecutive patients (13 males, 11 females) who underwent endonasal transphenoidal pituitary adenoma resection between 2013 and 2014 at our institution. Each patient had a single pituitary adenoma, and of the total of 24 tumors, 13 (54.2%) were pathologically designated as macroadenomas and 11 (45.8%) were microadenomas. There were no re-operations after initial tumor removal. All patients returned to baseline GCS, mRS, and GOS scores.

Results: Of the 13 patients with macroadenomas, 9 patients were post-operatively discharged at one day, and 3 patients were discharged after two days. 1 patient remained in the hospital for ten days due to an undocumented complication. 1 patient returned two days post-operation due to a sigmoid perforation unrelated to the neurosurgerical procedure. There were 3 readmissions for adrenal insufficiency and 1 readmission for syndrome of inappropriate antidiuretic hormone secretion. The adrenal insufficiency resolved with time and all patients were weaned off steroids. 4 patients had transient diabetes insipidus and were managed in outpatient with desmopressin.

Conclusions: Macroadenomas tend to be nonfunctioning pituitary tumors that can be safely removed via a minimally-invasive endonasal transphenoidal approach. We recommend that morning cortisol levels be checked and prophylactic hydrocortisone (10 mg BID with tapering) be started to prevent post-operative adrenal insufficiency. A protocol can be implemented to assess neurologic status, pain control, and transient endocrine complications and facilitate early discharge 24 hours after surgery. Patients can be managed and followed-up in the outpatient setting.

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

POSTER #124

FLOW CYTOMETRIC EVALUATION OF FANCA AND FANCC FIBROBLASTS FOR OPTIMIZATION OF CLINICAL DIAGNOSTIC TESTING

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Fanconi anemia (FA) is a heterogenic condition that is associated with bone marrow failure, physical abnormalities, organ defects, hypersensitivity to DNA-damaging agents and an increased risk of leukemia certain cancers. Sixteen complementation group genes of FA have been characterized which include 5 familial breast cancer genes and potentially more remain yet undiscovered. The definitive diagnostic test for FA is a chromosome breakage test. Cells derived from FA patients demonstrate hypersensitivity to DNA interstrand cross-linking agents (ICLs) such as diepoxybutane (DEB), and mitomycin C (MMC). Typically, this test is performed using peripheral blood lymphocytes and is routinely performed in our laboratory. In patients with somatic mosaicism however, the MMC/DEB-induced chromosomal breakage test on peripheral blood cells may be negative. In order to rule out mosaicism, a skin fibroblast culture can be used to perform the chromosomal breakage test. This assay is now being standardized in our laboratory. Although protocols are available in the literature, we found that the mitotic index in FA skin fibroblast cells were variable and inadequate for diagnostic testing with a high level of confidence. Therefore, in order to understand the temporal cycling pattern of these cells, we proceeded to evaluate the cell cycle of FANCA AND FANCC fibroblast cells, with and without exposure to DEB and compared their response with that of normal fibroblasts. Both normal and FA fibroblasts were exposed to 0, 0.01 and 0.1 mg/ml of DEB for 48 hours. The cells were then washed and cultured in medium without DEB. They were fixed with 70% ethanol at 0, 6, 12, 18, 24, 32, and 48h after withdrawal of DEB, treated with RNase A, stained with Propidium Iodide (PI) and analyzed by flow cytometry. Untreated normal fibroblasts peaked in G2/M (4n) stage at 18h whereas the FA cells peaked earlier at 12h. After treatment with 0.01mg/ml of DEB, both normal and FA cells showed a increased number of cells in S phase at 0 h post DEB withdrawal followed by progression of a proportion of cells into G2/M at 6h. A significant fraction of cells continued to be arrested in S phase in both normal and FA fibroblasts, although the percentage was significantly higher in the FA (both FANCA and FANCC mutant) cells. Subsequent progression through the cell cycle was monitored and normal cells were observed to proceed to the G2/M phase at 24 hours and FA cells at 32 hours. This important observation allows for measured coordination of the cell cycle with our FA protocol for maximizing the mitotic index of these cells, essential for a successful FA breakage assay on fibroblast specimens.

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

POSTER #125

A SYSTEMATIC QUALITY CONTROL ANALYSIS OF LINCS DATA

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Library of Integrated Cellular Signatures (LINCS) project, for the first time, provides comprehensive transcriptome profiling of human cell lines before and after chemical and genetic perturbations. The unique LINCS L1000 platform utilized 978 landmark genes to computationally infer to other 14,292 genes expression. The potential biological impact of LINCS L1000 data heavily depends its quality. Our quality control data analyses showed a promising 80% correlation between L1000- and Affymetrix HU133-assessed MCF7 breast cancer cell transcriptomes; the L1000 genetic perturbation ratio reached as high as 67% and 53% for shRNAs and overexpression, respectively; L1000 reproducibility analyses showed that a moderate 30% of differentially expressed genes overlapped between any two selected controls viral vectors. The MAPK, VEGF, and T-cell receptor pathways are pointed out for the most significantly connected breast cancer cell in chemical and genetic perturbations. A control pipeline of L1000 data quality was recommended before addressing biological questions.

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

POSTER #126

PROTEIN EXPRESSION LEVELS OF TNFR1, BUT NOT SK3, CORRELATE WITH SURVIVAL IN PATIENTS WITH CANCER BONE METASTASIS.

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Tumor colonization in bone is a main cause of cancer associated pain and mortality. Despite progress in the understanding of the pathophysiology of bone metastasis, it remains incurable.

Previous studies indicate the specific involvement of the Receptor 1 of Tumor Necrosis Factor (TNFR1) in the onset of bone metastasis and the existence of a ligand-dependent and a ligand-independent signaling through TNFR1.

Recently, the SK3-Orai1 complex has been implicated in human cancer cell migration and bone metastasis in mouse models. SK3 is a Ca^{2+}-activated K^+ channel that pairs with Orai1, a Ca^{2+} channel, in the lipid raft. We hypothesize that the activation of the SK3-Orai1 complex enhances TNFR1 signaling in bone metastasis by an increased shedding of the receptor due to the higher concentration of Ca^{2+} inside the cell. In this study we aim to identify 1) the level of involvement of TNFR1 and SK3 in bone metastasis and effect on survival, 2) prognostic biomarkers, and 3) novel therapeutic targets.

143 bone metastases frozen samples from different types of primary tumors (22 sarcomas and 12 head/neck, 35 urogenital, 15 gastrointestinal, 12 breast, 23 lung, 7 melanoma, and 16 unknown carcinomas) were collected at the Istituto Ortopedico Rizzoli, Bologna, Italy. Each specimen was lysed and lysates were used to quantify the level of TNFR1 and SK3 by Reverse Phase Protein Microarray (RPMA).

Metastases from different primary tumors showed similar levels of expression for TNFR1 and SK3 across tissue types (Kruskal-Wallis analysis), except for a higher expression of TNFR1 in metastases from sarcomas. Spearman's comparison analysis showed no correlation between TNFR1 and SK3 expressions, both in the total data set and in each primary tumor group. TNFR1 expression levels were statistically correlated to patients' survival after diagnosis of bone metastasis (Kaplan-Meier analysis, $p<0.0001$). No correlation was found between SK3 expression and patients' survival ($p=0.2011$).

Our data suggest a major role for TNFR1 in the bone metastatic disease. No direct influence on patients' survival has been seen for SK3. Further experiments will determine whether and how SK3 expression contributes to TNFR1 signaling. Animal models of bone metastasis will be used to investigate the effects of a TNFR1-inhibitor therapy on the onset and progression of bone metastasis.

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

POSTER #127

BIDIRECTIONAL NOTCH SIGNALING ACTIVATED BY INTERACTIONS BETWEEN MULTIPLE MYELOMA CELLS AND OSTEOCYTES DRIVES TUMOR CELL PROLIFERATION AND OSTEOCLAST RECRUITMENT

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Multiple Myeloma (MM) is characterized by growth of monoclonal plasma cells in the bone marrow, increased bone resorption, concomitant reduced bone formation, and increased osteocyte apoptosis. The role in MM of matrix-embedded osteocytes, which comprise more than 95% of bone cells and are major regulators of osteoclast and osteoblast activity, is unclear. We investigated the mechanism(s) that trigger osteocyte apoptosis and its significance for MM cell growth and associated bone disease. We found that apoptosis of murine osteocytic MLO-A5 cells is induced by interactions with MM cell lines of murine and human origin as well as with primary CD138+ plasma cells from MM patients, and is blocked by the caspase3 inhibitor DEVD. In addition, apoptosis measured at 8h-24h is abolished by the Notch inhibitor GSIXX, whereas apoptosis measured at 48h is only fully inhibited with a combination of GSIXX and a neutralizing anti-TNFa antibody. These findings demonstrate that osteocyte apoptosis induced by MM is triggered by Notch activation and sustained by MM-derived TNFa. Further, interactions with MM cells increased osteocytic RANKL expression (3-fold) and increase the recruitment of osteoclast precursors by 50% compared to osteocytes cultured alone. These effects were inhibited by blocking osteocyte apoptosis with DEVD or by the combination of GSIXX and anti-TNFa. CD138+ cells from MM patients also increased osteocytic RANKL expression. Further, the percentage of apoptotic and RANKL-positive osteocytes increased by 100% and 53%, respectively, in tumor bearing bones of a mouse model of human MM. Importantly, interactions with osteocytes reciprocally activated Notch signaling in MM cells and in CD138+ cells from patients. Osteocytic cells increased MM cell proliferation by 50% and cyclinD1 expression by 8-fold, and these effects were blocked in a time- and dose-dependent manner by GSIXX. Moreover, osteocytes upregulated Notch receptor 1-4 in MM cells by 4-8-fold. This altered Notch receptor repertoire could impact tumor growth that is dependent on autocrine and paracrine Notch signaling. Thus, bidirectional Notch signaling between MM cells and osteocytes stimulates MM cell proliferation and induces osteocyte apoptosis, which enhances the osteoclastogenic potential of osteocytes. This study emphasizes the ability of osteocytes to impact the MM niche within the bone marrow and supports development of novel approaches to treat MM bone disease by targeting osteocytes.

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

POSTER #128

DNA METHYLOME ALTERATIONS IN PLATINUM RESISTANT OVAIRAN CANCER TUMORS

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Epigenetic changes, particularly DNA methylation aberrations have been implicated in acquired resistance to platinum in ovarian cancer. An ongoing phase I/II multi-institutional clinical trial uses the novel DNA methyl transferase (DNMT) inhibitor SGI-110 to re-sensitize recurrent platinum resistant ovarian cancer to carboplatin. Tumor biopsies or malignant ascites were collected at baseline and after two cycles of SGI-110 administered daily for 5 days in low dose (30mg/m2). The goal of the current study was to analyze global DNA methylation profiles of platinum resistant tumors and compare them to the methylome of untreated, platinum-sensitive ovarian tumors. LINE1 methylation and promoter methylation of selected genes (MAGE-A2, MAGE-A3, MAGE-A11, NY-ESO, RASSF1, MLH1, and HOXA11) were quantified by pyrosequencing before and after SGI-110 treatment (n=12 paired samples). Epigenetic profiling using the Infinium HumanMethylation450 BeadChip (HM450) revealed extensive methylation changes when comparing recurrent platinum resistant ovarian tumors (n=42) to primary, untreated ovarian cancer specimens analyzed as part of the TCGA project (n=10). Six hundred and four promoters were significantly differentially methylated (adjusted $p<0.05$, absolute methylation changes $B>0.2$), among which, 498 and 106 were hypermethylated or hypomethylated respectively in recurrent platinum resistant ovarian tumors. DNMT1, 3A, and 3B mRNA levels in the tumors were highly variable (n=19). Analysis of a limited number of paired samples (n=7) revealed no significant changes in global methylation or in DNMT expression levels induced by treatment with SGI-110 (adjusted $p>0.05$). However, the DNMT inhibitor induced significant methylome alterations in selected patients. Significant hypomethylation of MAGE-A3 and MAGE-A11 promoters ($p<0.05$) was detected. Correlations between methylation changes and clinical outcomes are being explored.

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

POSTER #129

TARGETED THERAPY FOR COLORECTAL CANCER WITH FOLATE-TETHERED LIPID NANOPARTICLES

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The antitumor nucleoside pro-drug tiazofurin (2-beta-D-ribofuranosylthiazole-4-carboxamide) is converted by cells into tiazofurin adenine dinucleotide (TAD), a potent inhibitor of inosine 5\'-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme for the synthesis of guanylate nucleotides. Since guanylate nucleotides are required for DNA and RNA synthesis and signal transduction by oncogenes, tiazofurin kills cancer cells. Although toxicity prevents its use, tiazofurin received approval for the treatment of leukemia. The goal of this research is to find ways to limit its toxicity and also to expand its use to other cancers.

Overexpression of nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2), the rate-limiting enzyme for the synthesis of TAD, with a pTracer-NMNAT2 vector increases the sensitivity of colorectal cancer cells to tiazofurin. We have confirmed and extended this finding by showing that infecting colorectal cancer cells with an adeno-associated virus 6 vector encoding NMNAT2 (AAV6-NMNAT2) reduced the IC_{50} for tiazofurin from 125 ± 7, 80 ± 5, 130 ± 4 in HeLa, HCT116 and HT29 to 25 ± 2, 21 ± 1, 43 ± 5 respectively.

Since colorectal tumors overexpress folate receptors, folate-tethered lipid nanoparticles have been prepared and tested as a way to target both tiazofurin and AAV6-NMNAT2 to colorectal cancer cells. Successful encapsulation of AAV6 in lipid nanoparticles has not been previously reported. Folate-tethered lipid nanoparticles deliver both tiazofurin and NMNAT2 to HeLa, HCT116 and HT29 cell lines more efficiently than non-folate-tethered lipid nanoparticles.

Our next aim will be to further increase the specificity of tiazofurin for colorectal cancer cells by engineering an AAV6-NMNAT2 with a CEA promoter to take advantage of the overexpression of the carcinoembryonic antigen in these cells.

Novel approaches for targeted therapy for colorectal cancer are being developed by these studies.

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

POSTER #130

A PILOT STUDY COMPARING THE ADDITION OF OLANZAPINE OR APREPITANT IN AN ANTIEMETIC REGIMEN FOR HIGHLY EMETOGENIC CHEMOTHERAPY

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Background: Chemotherapy induced nausea and vomiting (CINV) remains a problem in pediatric oncology. There is a need for new agents aimed at preventing CINV. Adult trials have shown olanzapine to be an effective antiemetic. Olanzapine has also been shown to be safe and well tolerated in children for psychiatric indications.

Objective: We undertook a feasibility study comparing olanzapine and aprepitant as antiemetic regimens for pediatric patients receiving highly emetogenic chemotherapy (HEC). A secondary aim was to obtain preliminary data regarding the effectiveness and tolerability of both olanzapine and aprepitant.

Design: Patients age 4-21 receiving two cycles of the same HEC were eligible. The study is a randomized crossover design. Patients received aprepitant in one cycle and olanzapine in the other cycle; both were administered with ondansetron and dexamethasone as multiple antiemetic agents are typically required for HEC. Patients and caregivers logged episodes of emesis, use of breakthrough antiemetics and daily nausea ratings, for days 1-5. To rate nausea caregivers used a visual analog scale (VAS) (1-100) while patients used the Baxter Retching Faces (BARF) pictorial scale (0-10), with a higher value indicating greater level of nausea. A complete response was considered no emesis and no use of breakthrough medications, measured as acute (Day 1) and overall (Days 1-5).

Results: A total of 14 patients (ages 7-18) have enrolled to date, 9 have completed both cycles, 3 are currently active, 1 withdrew after cycle 1 and 1 withdrew prior to cycle 1. The caregiver/patient log has a current return rate of 89.4% (17/19). The overall complete response rates are: olanzapine 40% and aprepitant 33.3%. The acute phase complete response rates are: olanzapine 70% and aprepitant 66.7%. Mean parent VAS nausea ratings are: olanzapine 15.3 and aprepitant 21.5. Mean patient BARF nausea ratings are: olanzapine 1.87 and aprepitant 1.95. Two patients experienced grade I agitation during olanzapine cycles, no other adverse events were reported.

Conclusion: Olanzapine was well tolerated and demonstrated promise as an effective antiemetic in pediatric patients. There were no significant adverse events. A larger trial comparing olanzapine and aprepitant appears feasible.

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

POSTER #131

THE LANDSCAPE OF DNA METHYLATION IN PERIPHERAL BLOOD DNA OF HEPATOCELLULAR CARCINOMA AND ITS PREDICTIVE VALUE

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Hepatocellular carcinoma (HCC), one of the most prevalent types of primary liver cancer, is the sixth most common cancer worldwide and the third leading cause of cancer death with rising mortality and morbidity rates. As late onset of HCC accounts for late diagnosis and poor prognosis and early detection increases cure rate from 5% to 80%, identifying reliable and quantifiable biomarkers of risk prediction is of high interest. Aberrations in the DNA methylation patterns, an important early event in carcinogenesis, have been shown to differentiate HCC tumors from normal tissues. However, these changes as predictive markers would have a high application in clinics only if detectable by minimally invasive tests like blood test.

In the present study, we performed a comprehensive evaluation of DNA methylation profiles in blood DNA collected before diagnosis with HCC. Aberrant methylation was investigated in DNA isolated from blood of 21 HCC patients (cases) who provided samples between 1-4 years prior to diagnosis and 21 controls enrolled by the Indiana Biobank of Indiana CTSI. Cases were matched with controls for gender, age, ethnicity, hepatitis C infection, and diabetes. We used Infinium Human Methylation 450K BeadChip array for genome-wide DNA methylation analysis and pyrosequencing for validation of DNA methylation differences.

We found 966 probes differentially methylated between cases and controls with $p<0.05$ and intraclass correlation coefficient >0.5. Among these significant changes, 732 CpG sites are hypomethylated in HCC cases compared to matched controls and include 130 CpGs corresponding to 75 genes with delta beta value (differential methylation) ≥ 0.1. Forty six CpG sites out of 234 significantly hypermethylated probes show delta beta value ≥ 0.1 and correspond to 26 genes. Functional analyses using GO, KEGG and DAVID knowledgebase indicate that hypomethylated genes are associated with Wnt signaling, cell adhesion, blood coagulation, and regulation of transcription, whereas hypermethylated genes are enriched with cytoskeleton organization and small GTPase mediated signal transduction. One of the genes hypomethylated in blood DNA of HCC cases prior to diagnosis is TET1 that was found to be 8-fold overexpressed in HCC tumors in our previous studies and implicated in gene-specific hypomethylation. Interestingly, 75% of differentially methylated sites in blood DNA of HCC cases are hypomethylated prior to diagnosis. Validation by pyrosequencing of four hypomethylated probes including CpGs in the CpG island shore of BRUNOL5 that was linked to fatty liver disease in earlier reports and found demethylated and overexpressed in HCC tumors, suggests their predictive potential.

Our results establish the possible predictive value of aberrant methylation, in particular DNA hypomethylation, in blood DNA for risk of HCC. This study was supported by Showalter Trust and Purdue Center for Cancer Research Awards granted to BS.

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

POSTER #132

EXTENDED VOLUMETRIC FOLLOW-UP OF JUVENILE PILOCYTIC ASTROCYTOMAS TREATED WITH PROTON BEAM RADIOTHERAPY

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Introduction

Juvenile pilocytic astrocytomas (JPA) are WHO grade 1 glial neoplasms treated by resection with radiotherapy reserved for inoperable cases or following subtotal resection. Proton radiotherapy minimizes integral dose and is thus preferred in children. We analyzed the MRI follow-up of patients with JPA treated with proton radiotherapy to define the volume changes, response rate, need for post-radiotherapy intervention and survival.

Methods

Fifteen pediatric patients histologically diagnosed with JPA make up this retrospective report. From August 2005 through March 2012, patients were treated to a median dose of 5400cGy(RBE) using proton radiotherapy then followed with serial MRI's for three years. MRI's were imported into Eclipse 11 treatment planning software where contours of the T1 contrast enhancing volumes including cystic components was performed by one clinical radiation oncologist, EMM. Volume in cm^3 was plotted against time since completion of therapy to track volumetric changes. Demographics, prior therapies and post-radiotherapy interventions were catalogued.

Results

This is a retrospective review of 15 patients with a mean age of 10.9 years (4-20) and mean number of MRI's of 8.9 (4-12) . 10/15 (67%) patients had prior R2 resections, with three patients having two R2 resections. 12/15 (80%) patients had CSF shunts prior to radiotherapy. 9/15 (60%) patients received prior chemotherapy, all at least a platinum agent and vincristine. With a median follow-up of 55.3months, 14/15 (93%) patients were alive for an estimated 5-yr overall survival of 93.3%. Median event-free survival was 86.6months with estimated 5-yr EFS of 72.2%. 11/15 (73.3%) patients declared as responders by 6months with 3/11 (27%) demonstrating pseudoprogression (increase in volume followed by spontaneous regression) with mean time to maximum volume of 177days. 4/15 (26.7%) patients were nonresponders including one who died of progression nine months after radiotherapy and another who restarted chemotherapy. Three patients underwent shunt revisions while two received hyperbaric oxygen, one for presumed and another for biopsy-proven radionecrosis (only patient with prior radiation). Stereotactic cyst aspiration was required in one case. One heavily pretreated patient developed a hematologic malignancy requiring further chemotherapy. 8/15 (53.3%) patients required no further therapy or intervention after radiotherapy. Gross tumor volume changes ranged from 91% reduction to 207% increase during the evaluation period.

Conclusions

Pediatric patients with pilocytic astrocytomas can have extended survival following proton beam radiotherapy. This volumetric study illustrates that responders declare within six months but vigilant surveillance is

necessary due to potential need for post-radiotherapy interventions.

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

POSTER #133

SKP2 AS A NOVEL THERAPEUTIC TARGET FOR T-CELL LEUKEMIA

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Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer. In pediatric T-ALL, leukemia relapse and refractory disease (~30% of patients) are often life-threatening, and novel, more effective therapies are much needed. Recent advances in understanding the molecular alterations of ALL have led to identification of new molecular targets, such as the Notch signaling pathway, although this knowledge has yet to translate into effective molecular therapies for relapse patients. Constitutive activation of Notch signaling is involved in more than 50% of human T-ALL, and over expression of activated Notch (ICN) induces T-cell leukemia and lymphoma in murine tumor models. However, disruption of Notch signaling by gamma-secretase inhibitors(GSI) failed to fulfill its clinical promise. Furthermore, despite multiple studies, little is known on the role of Notch downstream mediators in T-cell ALL.

Our previous studies demonstrated that Notch1 activation induces transcriptional activation of SKP2, the F-box protein of the SCF E3-ubiquitin ligase complex. SKP2 is the main F-box protein regulating cell cycle, promoting downregulation of the CKIs ($p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$ and p130). SKP2 overexpression accelerates cell cycle progression in hematopoietic cells and is frequently associated with cancers, in particular lymphomas and leukemia's, correlating with poor prognosis.

We found that *Skp2* expression is dynamically regulated during T-cell development coinciding with the Notch expression pattern. Primary thymocytes cultured in vitro responded to Notch stimulation by increasing *Skp2* expression and entering into cell cycle, whereas SKP2 loss impaired their ability to proliferative selectively in response to IL-7 stimulation. Importantly, we observed increased *Skp2* expression in human T-ALL cell lines and T-ALL patient samples, and in mice with Notch-induced T-cell leukemia (5-fold). Our hypotheses are: i) *Notch activation promotes T-cell leukemogenesis by altering the cell cycle control through upregulation of Skp2* and ii) *selective targeting of SKP2 is a novel, effective therapeutic strategy for childhood T-ALL*.

To test whether SKP2 is a key downstream mediator of Notch in T-ALL, we transduced oncogenic Notch (ICN) in hematopoietic cells from $Skp2^{-/-}$ null mice and from controls, and we determined their ability to induce leukemia in irradiated recipients. Loss of SKP2 significantly delayed the development of T-cell leukemia and increased animal survival by 40%.

To evaluate the effects of targeting SKP2 in T-ALL leukemia, we used a novel small inhibitor (T6) developed by our collaborator, Dr. Hyun-Suk Lim. Blockade of SKP2 by T6 in T-ALL cell lines in vitro resulted in significant inhibition of proliferation and viability in a dose dependent manner. This effect was associated with significant decrease of cells in S-phase (cytostatic effect) and significant increase in apoptotic cells (cytotoxic effect).

Taken together, these results demonstrate a previously unrecognized role for SKP2 in the initiation and progression of T-ALL and its potential role as a therapeutic target.

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

POSTER #134

BRIDGING THE GAP: COMBINING MURINE AND 3D BREAST TUMOR MODELS TO UNDERSTAND ZNF217 INDUCED 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 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 Znf217 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 Znf217 overexpression in vivo contributed to chemotherapy resistance. We treated mice overexpressing Znf217 with a combination therapy of microtubule inhibitor epothilone B, Adriamycin, and cyclophosphamide (EAC). Mice overexpressing Znf217 that were treated with EAC developed a significant increase in tumor volume over control mice within 21 days of EAC treatment. This confirmed that mice overexpressing Znf217 developed resistance to the EAC chemotherapy.

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 Znf217 and also had synergy with doxorubicin in human xenografts. In addition, we have used our preclinical animal models of Znf217 overexpression to elucidate 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 the therapy. We are confirming these results using patient-derived tumor xenografts (PDX) to compare therapeutic response of human tumors with high versus low levels of ZNF217 expression.

We have also generated 3D organoids from murine breast tumors in an effort to bridge the gap between animal models and 2D cell culture. We monitor proliferation/invasion characteristics of the organoids using time-lapse microscopy. Because organoids remain in a 3D environment, this provides a more accurate model to examine the mechanistic details of treatment response and therapeutic resistance. Our future work aims to establish a drug-screening platform to identify combinations of chemo/targeted therapeutics aimed at overcoming resistance to therapy. Utilizing both PDX and 3D organoid models provides an ideal platform to gain mechanistic insight into therapeutic resistance. This work will lead to increased therapeutic efficacy by creating personalized treatment regimens for patients.

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

POSTER #135

TELOMERIC CELL-FREE DNA ABNORMALITY IN PLASMA FOR BREAST CANCER DIAGNOSIS

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Telomeres are unique repetitive structures at the end of chromosomes that are essential for protecting the chromosomes from degradation and end-to-end fusion. Telomere erosion and subsequent end-to-end chromosome fusion underlie telomere impairment. Notably, a number of studies reported that accelerated telomere shortening is observed in breast cancer lesions when compared to adjacent non-cancerous lesions, thereby suggesting that telomere length may represent a key biomarker for early cancer detection. Because tumor-driven, cell-free DNA (cfDNA) is often released from cancer cells and circulates in the bloodstream, we hypothesized that breast cancer development is associated with changes in the amount of telomeric cfDNA and can be detected in the plasma. Prior to testing this hypothesis, we developed a novel, highly sensitive and specific quantitative PCR (qPCR) technique to quantify changes in telomere and centromere DNA contents in human plasma. This new assay improved the sensitivity of cfDNA detection by amplifying repetitive sequences that are abundant and spread throughout the genome (telomere, centromere, and LINE). Therefore, only 150 μL of plasma (less than 1 mL of whole blood) is necessary for this new qPCR-based cfDNA assay. Furthermore, we improved the reliability of the qPCR-based cfDNA assay by including an invariant endogenous control (LINE) to correct for sample-to-sample variations in PCR efficiency and errors in sample quantitation. Our data indeed showed that the repetitive sequence LINE accurately reflects input cfDNA amount and thus is well suited as a reference sequence. By using this new qPCR-based cfDNA assay, our preliminary results clearly showed a lower amount of telomeric cfDNA in breast cancer patients even at the Ductal Carcinoma *In Situ* (DCIS) stage compared to control individuals. Taken together, we propose that this approach may identify breast cancer patients or even women at high risk of breast cancer (e.g., atypical hyperplasia, DCIS) based on abnormal plasma telomeric cfDNA content. Development of this minimally invasive blood analysis of cfDNA could be highly significant for early breast cancer detection and potentially for risk assessment.

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

POSTER #136

IDENTIFICATION OF COMMON AND RARE GENETIC VARIANTS ASSOCIATED WITH RISK OF CHEMOTHERAPY-INDUCED CONGESTIVE HEART FAILURE IN THE BREAST CANCER TRIAL ECOG-5103

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Anthracyclines are a widely used chemotherapeutic agent for a variety of malignancies, including breast cancer, lymphoma, sarcoma and many others. Despite substantial anti-tumor activity, anthracyclines are not without limitation. Cardiotoxicity, including congestive heart failure (CHF), is a dose-limiting side effect of these drugs that can have a mortality rate of up to 72%. Furthermore, patients that followed an anthracycline regimen were found to be five-times as likely to develop cardiac symptoms in a meta-analysis of various cancer types. The ability to identify a population at high risk for CHF would have direct application in the clinical setting.

Known risk factors for developing clinical CHF include cumulative anthracycline dose, age, and previous history of cardiac conditions. Risk of cardiac event is also further increased when anthracyclines are combined with other therapies, including trastuzamab. While the overall incidence of clinical CHF in patients receiving anthracyclines is 2.2%, the severity of the side-effects necessitates a means to identify high risk patients. Blood biomarkers for CHF have been studied, but their ability to predict cardiac risk remains unclear. Identification of a genetic biomarker that is predictive for CHF would be of great clinical value.

Here we report an evaluation for both common and rare genetics variants that could serve as biomarkers for anthracycline-induced CHF through a genome-wide association study (GWAS) and next-generation sequencing (NGS) of the phase III clinical trial ECOG-5103. GWAS have gained popularity in recent years as a means to analyze common genetic variants and determine possible associations with risk or disease among a population. Additionally, NGS has become increasingly available for high coverage analysis of rare variants. Both techniques have allowed for greater insight into genetic factors that contribute to cancer. Currently we are performing statistical analysis of our matched case-control exome sequencing data and will validate preliminary hits by Sanger sequencing. Additionally we are developing *in vitro* functional assays to explore the mechanism of action of top SNPs associated with CHF. We aim to translate our findings towards a clinically useful biomarker and to increase understanding of CHF risk.

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

POSTER #137

TP53 MUTATION IS A BIOMARKER FOR PROGNOSIS IN TRIPLE-NEGATIVE BREAST CANCER PATIENTS TREATED WITH POST-NEOADJUVANT CISPLATIN

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Introduction: Triple-Negative Breast Cancer (TNBC) is a clinical subtype of breast cancer characterized by lack of expression of the clinical biomarkers Estrogen, Progesterone, and HER-2 receptors and is associated with poor prognosis. 75% of patients will have residual disease (RD) after neoadjuvant chemotherapy (NAC). These patients have inferior survival, and no adjuvant therapies are currently indicated for this group. Platinating agents such as Cisplatin are indicated for cancers with frequent homologous recombination (HR) deficiencies and have shown activity in TNBC. BRE09-146 was a post-neoadjuvant clinical trial testing the combination of Cisplatin and the PARP-inhibitor Rucaparib versus Cisplatin alone for patients with TNBC or ER+/HER2- patients with a germ line BRCA1/2 mutation. Cisplatin is associated with relatively severe toxicities, so establishing a biomarker for outcome is necessary. TP53 is the most mutated gene in cancer as a whole and is mutated in 70-80% of cases of TNBC. Here we explore TP53 mutation as a prognostic biomarker in BRE09-146.

Methods: We performed full sequence and copy number analysis of 134 genes from 78 tumors from BRE09-146 using the Oncomine Research Panel along with Ion Proton Next Generation Sequencing. All patients included had RD. Somatic mutations were called by identifying mutations that were present in in the tumor that were not present in the germ line DNA from a normal blood sample. Mutations were annotated using the IARC TP53 somatic mutation database. Copy numbers were established using the Ion Reporter system from Thermo-Fisher Scientific and called as lost or normal based upon a reference range established from the normal blood samples.Survival comparisons were generated using the Log-Rank and Kaplan-Meier methods. For mutation class separation, frame shifts and truncations were compared against all other mutants. Tumor infiltrating lymphocyte (TIL) analysis was conducted by comparing pathologist-verified lymphocyte constituency in the mutated and non-mutated groups.

Results: 81% of cases contained a somatic point mutation. Mutations were most frequently missense mutations in the DNA binding and tetramerization domains. Single copy number loss was observed in 27% of cases. The percentage of cases in which point mutation and/or copy loss was observed was 85%. Survival analysis indicated that patients with TP53 mutations have a shorter DFS and OS ($p<0.021$ and $p<0.017$). No association was observed when mutations were separated by mutation class as well as between mutated status and TILs.

Conclusions: We first confirm findings that TNBC has marked TP53 involvement. TP53 overall mutation status is the most superior biomarker for prognosis for Cisplatin use in BRE09-146, while TP53 mutation class does not correlate with prognosis. Finally, TILs were not associated with mutation status, indicating that differences in outcome based on mutation status are probably not primarily immune-related.

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

POSTER #138

CACHEXIA AVATARS: PATIENT-DERIVED ORTHOTOPIC XENOGRAFTS TO MODEL PANCREATIC ADENOCARCINOMA-ASSOCIATED MUSCLE WASTING

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OBJECTIVE:

To establish patient-derived xenograft models that mimic cancer cachexia in pancreatic adenocarcinoma and to use the models to comprehensively describe the effects of the tumor on body composition.

METHODS:

Patient tumor samples were obtained at surgery from patients undergoing surgical resection for pancreatic adenocarcinoma under IRB IUCRO-0454. The fresh tumor samples were place in RPMI-1640 medium with 1% Pen/Strep, and transferred on ice to laboratory. The tumor sample was divided into fragments of around 3mm3, and dipped in matrigel before xenografting into NOD scid gamma2 (NSG) or athymic nude mice. The tumor fragments were transplanted through sewing of the fragment directly to the pancreas (orthotopic) and/or into the back (ectopic) of the immune deficient mice. Successful grafts were serially transplanted orthotopically to assess effects of tumor growth on cachexia endpoints. After tumor implantation, the mice were monitored for tumor growth, body mass, body composition, and body condition scoring. Blood, muscles, organs, tumors and carcasses were collected and measured.

RESULTS:

A total of 18 pancreatic cancer tissues were orthotopically and etopically transplanted to NSG/nu mice. Seven patient-derived orthotopic xenografts (PDOX) lines (S002, S004, S005, S011, S016, S017, S023) were established. These represented the expected range of histologic differentiation. All xenografts showed high intrapancreatic tumorigenicity and extensive local tumor growth. Although the overall metastatic rate was moderate to high, the metastatic pattern varied; S011 and S017 mice showed ascites at the end of life. Four of the PDOX lines (S002, S005, S017 and S023) were used to study cachexia. All induced wasting, although with differency latency and severity. Quadriceps, gastrocnemius, tibialis muscle and carcass wasting demonstrated 32%, 28%, 28%, and 22% decrease compared to sham control in S002 mouse model, 25%, 22%, 19%, and 15% decrease compared to sham control in S017 mouse model, respectively. Measurement of tumor, muscle and blood gene expression and cytokine levels in patients versus their matched murine "avatar" are in progress.

CONCLUSIONS:

Pancreatic tumors from patients carry the potential to induce cachexia in mice. These PDOX cachexia avatars could provide a platform for preclinical exploration and pharmacological interventions for pancreatic cancer cachexia.

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

POSTER #139

CLINICAL PET/CT EVALUATION OF PATIENTS WITH NEUROENDOCRINE CANCER: EXPERIENCE WITH EXPANDED ACCESS IND PRODUCTION AND USE OF GA-68-DOTA-NOC

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Objective: To provide the ^{68}Ga-DOTA-NOC radiopharmaceutical for use in *clinical* PET/CT evaluation of patients with neuroendocrine tumors. The somatostatin-receptor-targeted ^{68}Ga-DOTA-NOC radiopharmaceutical has been widely employed as a clinical agent in Europe, but is not available in the United States as an FDA-approved drug product.

Methods: Protocols were developed and validated for ^{68}Ga-DOTA-NOC preparation as a sterile drug product, and Expanded Access IND #117255 written and submitted to allow use of this radiopharmaceutical in clinical imaging of patients at the I.U. School of Medicine. Both the TiO_2-based Eckert & Ziegler (EZAG) ^{68}Ge/^{68}Ga generator, and the SiO_2-based ITG generator, have been employed to supply ^{68}Ga for manual on-demand synthesis of ^{68}Ga-DOTA-NOC. The synthesis employs $^{68}Ga^{3+}$ in either 1.5 mL 0.1M ultrapure HCl (fractionated elution of the EZAG generator) or 4.0-mL 0.05M HCl (ITG generator, without fractionation). In both cases, the eluate is buffered to pH ~4.8 by addition of ultrapure NaOAc and reacted with commercial cGMP DOTA-NOC conjugate (60-µg for the EZAG eluate; 30-µg for the ITG eluate). After heating for 10-minutes, the ^{68}Ga-DOTA-NOC product is isolated by C_{18} solid-phase extraction, washed, recovered in ethanol:saline, and then diluted with sterile saline to ≤5% ethanol prior to terminal sterilizing filtration. Quality control measures and release criteria follow the specifications of the published EANMMI Procedure Guidelines (*Eur J Nucl Med Mol Imaging 37*:2004-2010; 2010). Patient imaging is performed 60-minutes after radiopharmaceutical administration.

Results: Over the first two years of the Expanded Access IND, ^{68}Ga-DOTA-NOC was prepared for 83 clinical patient exams (administered dose: 4.7 ± 0.6 mCi; 174 ± 22 MBq). ^{68}Ga-DOTA-NOC radiochemical purity averaged 99.0 ± 0.5% (ITLC-SG strips developed with 0.1M HCl to quantify levels of ionic ^{68}Ga, and with 1:1 MeOH:1M NH_4OAc to quantify colloidal ^{68}Ga-hydroxide). Administered peptide doses averaged 43.2 ±5.2 µg (n = 47) and 23.0 ± 5.9 µg (n = 36), respectively, using the EZAG and ITG generators. ^{68}Ga-DOTA-NOC has provided high quality PET/CT images, delivering diagnostic information that substantially exceeds what was known about these patients from conventional imaging. In approximately one-half the patients being screened for multivisceral transplant, ^{68}Ga-DOTA-NOC imaging has revealed previously unknown disease in locations that preclude transplant.

Conclusions: The ^{68}Ga-DOTA-NOC radiopharmaceutical has been reliably produced for local use employing both the EZAG and ITG ^{68}Ga-generators. PET/CT with ^{68}Ga-DOTA-NOC has often provided unique clinical information, advancing medical care for neuroendocrine cancer patients through improved definition of the location and extent of their disease. The Expanded Access IND has been valuable as a regulatory pathway for providing this investigational agent, which is fulfilling patient care needs, but otherwise would be unavailable for clinical use in the United States.

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

POSTER #140

RAD51 PLAYS A ROLE IN NSCLC METASTASES

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Homologous recombination (HR) is one of the major pathways that repair DNA damage to maintain genome integrity.RAD51 is one of components of the HR pathway with growing evidence of its possible role in cancer. Increased expression of RAD51 has been reported in numerous cancers and has correlated with resistance to a number of therapies. Increased RAD51 expression is a poor prognostic variable in non-small cell lung cancer. Our data showed increased expression of both primary and metastatic non-small cell lung cancer tumor samples. We utilized an *in vivo* metastatic NSCLC rat model to dissect the role of RAD51 in NSCLC metastases. We implanted an H460SM cell line with metastatic potential endobronchially into the right lung of nude male rat. Our model displayed metastases to the contralateral lung, brain and lymph nodes. We then established stable knockdown of RAD51 using lentiviral shRNA in the H460SM cell line. We confirmed significant knockdown of RAD51 by mRNA and protein expression. We found that rats implanted with RAD51 knockdown H460SM cancer cells exhibited significantly longer rates of survival compared to rats without RAD51 knockdown. We in addition show RAD51 knockdown reduces invasion in a matrigel invasion assay. Future studies will characterize the mechanisms that facilitate RAD51's role in metastases. These findings point to RAD51 as a target for the development of cancer therapies.

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

POSTER #141

DUAL PI3K AND WNT PATHWAY INHIBITION IS A SYNERGISTIC COMBINATION AGAINST TRIPLE NEGATIVE BREAST CANCER

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Introduction: Triple negative breast cancer (TNBC) accounts for 15% of all breast cancer cases in the United States, and despite its lower incidence, contributes to a disproportionately higher rate of morbidity and mortality compared to other breast cancer subtypes. Because these tumors lack expression of the estrogen, progesterone, or HER-2 receptors ("triple negative"), TNBC patients do not respond to targeted therapies that have been successfully used against tumors that over-express these proteins. Thus, there exists a critical needto improve the outcomes of TNBC patients through the implementation of novel targeted agents.

Methods: RNA-seq data from 94 TNBCs (from Indiana University and TCGA) and 20 microdissected normal breast tissues (Komen Tissue Bank) were merged and imported into Partek Genomics Suite. The merged transcript RPKMs were transformed and batch effect corrected differential expression. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify therapeutic targets. For cell based studies, we tested a panel of seven TNBC cell lines using Buparlisib and WNT974, a WNT pathway inhibitor, individually. To further enhance antitumor efficacy, we tested these TNBC cell lines using variable dilutions of both drugs in combination with one another. Synergy between the two drugs was calculated using the Chou-Talalay method. Pharmacokinetic and Pharmacodynamic studies were performed using NSG mice and TMD-231 cell line injected into mammary fat pads. Blood samples after initial treatments were taken at 0.5, 1, 2, 6, and 24 hours. Tumors were allowed to grow to an average of 300mm^3.

Results: IPA identified over-expression and hyper-activation of the PI3K/AKT/mTOR and Wnt pathways in the TNBC data set. When anti-tumor efficacy against these pathways was assessed, a significant reduction in cell viability in combination was observed across the panel of cell lines. Using the Chou-Talalay method, we found for MDA-MB-231 and Hs578T, a ~50% reduction in cell viability at 100nM concentration of each drug that was highly synergistic (Combination Index = 0.33, and 0.36 respectively). Furthermore, cell line zenografts saw a 40% reduction in tumor size after 7 days of treatment with the combination and an 80% survival rate of PDX mice compared to 40% with the vehicle and drugs alone.

Conclusion: PI3K/mTOR/AKT and Wnt pathways are a vital target for treatment of TNBC. Using small molecule inhibitors that are in phase trials (Buparlisib and WNT974) we have found that there is a strong synergy when given at low nanomolar doses. *In vitro* studies of inhibitors of these two pathways in a panel of TNBC cell lines demonstrated significant efficacy in reducing cell viability with substantial synergy when used in combination. Furthermore, mouse studies display a similar synergy and reduction in tumor size when given in combination when compared to these two drugs alone.

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