



Cancer Research Day 2014 Abstract Book

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POSTER #1

IL-6 AND IL-11 MEDIATE MUSCLE WASTING IN OVARIAN CANCER CACHEXIA

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Cachexia, characterized by skeletal muscle and adipose tissue wasting, is a devastating consequence of cancer that occurs in the majority of cancer patients. Cachexia increases fatigue, reduces performance status, as well as quality and length of life. It is accepted that this condition is a result of tumor-host interactions and often activation of an inflammatory response. However, the molecular causes responsible for cachexia are still unknown. Among other malignancies, advanced stage ovarian cancer (OC) is frequently accompanied by cachexia. OC cachexia is an understudied condition, partly due to the clinical presentation and to the paucity of experimental models. In OC, cachexia associates with high levels of pro-inflammatory cytokines, in particular IL-6. We recently reported that IL-6 signaling to STAT3 in muscle contributes directly to muscle loss in experimental cancer cachexia. However, anti-IL-6 therapies are only partially protective against cachexia, indicating that other mediators are involved. IL-11, an IL-6 family cytokine that binds a distinct alpha receptor (IL-11ra versus IL-6ra) and signals through the common gp130 receptor, is markedly elevated in cachectic mice bearing the C26 tumor. Moreover, IL-11 is known to cause lipolysis and reduce adipogenesis in several human cancers. Despite this, whether IL-11 plays a causal role in OC cachexia remains to be elucidated. Here we show that IL-11 and its receptor (IL-11R) were highly expressed in most human ovarian cancers, such as serous, mucinous, endometrioid ovarian adenocarcinomas and clear cell carcinomas. This was also consistent with previous observations reporting high IL-11R expression in several experimental OC cell lines. Similarly, IL-6 and IL-11 levels were generally elevated in serum and ascites collected from OC patients with advanced disease. In our experimental in vitro model, IL-11, like IL-6, induced fiber atrophy and increased STAT3 phosphorylation in cultured C2C12 myotubes in a time- and dose-dependent manner. Mice implanted with ES-2 xenografts, a high-grade serous (HGS) OC, showed marked cachexia, consistent with muscle and fat wasting and ascites formation. This was also associated with elevated IL-11 and IL-11R localization in ES-2 tumors, high IL-6 and IL-11 levels in serum and ascites fluids and increased STAT3 activation in skeletal muscle. Interestingly, myofibers exposed to HGS-OC-conditioned medium for up to 48h underwent fiber shrinkage that was counteracted by administration of IL-6 and IL-11 neutralizing antibodies. Taken together, these experimental data implicate for the first time IL-11 as a causal agent in muscle wasting and strongly suggest that IL-6 and IL-11 both play a major role in OC cachexia. Moreover, our findings contribute to establish a novel and powerful experimental model of HGS-OC cachexia that might facilitate the advancement of the field. Future experiments are needed in order to validate whether modulation of IL-6 and/or IL-11 signaling can prevent cachexia in OC.

Basic Science Faculty

POSTER #2

WITHDRAWN: AN ARTIFICIAL DESMOPLASIA-MIMETIC MICROENVIRONMENT FOR STUDYING EMT IN PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction: The development of pancreatic ductal adenocarcinoma (PDAC) is heavily influenced by soluble factors, cell-matrix, and cell-cell interactions presenting from the local tumor microenvironment, or desmoplasia. Using conventional two-dimensional tissue culture techniques and animal models, significant progresses have been made toward understanding PDAC. However, PDAC is still difficult to diagnosis and its survival rate is extremely low, in large part due to the limited understanding of the complex interplay between PDAC cells and pancreatic desmoplasia. The inherent limitations associated with current *in vitro* and *ex vivo* tumor cell culture techniques prompted us to design highly defined and tunable biomimetic matrices for studying PDAC cell fate. We have reported bio-orthogonal hydrogels for independent control over hydrogel biophysical and biochemical properties. Built upon our prior work, current project focuses on fabricating synthetic desmoplasia-mimetic microenvironment for examining the influence of extracellular matrix (ECM) and soluble factors on epithelial-mesenchymal transition (EMT) in PDAC cells. **Methods:** COLO-357 (provided by Dr. Murray Korc), a TGFbeta-responsive PDAC cell line, were encapsulated in bio-orthogonal hydrogels cross-linked by multi-arm poly(ethylene glycol) -tetra-norbornene (PEG4NB) and peptide cross-linker KCGPLGLYAGCK (MT1-MMP-sensitive sequence). Collagen 1, the major ECM component in the pancreatic desmoplasia, was incorporated in the gels to evaluate the effect of integrin binding on PDAC cell growth and morphogenesis under the influence of soluble TGFbeta (0.5nM) and EGF (1nM). Cell survival and proliferation were quantified by total DNA content, Edu staining, and AlamarBlue reagent. Cell morphology was observed using Live/Dead staining, immuno-fluorescent staining, and confocal imaging. The expression levels of epithelial and mesenchymal markers were detected using western blot and real time PCR. Gemcitabine was added to evaluate the drug resistance of these cells in 3D culture. **Results:** More than 90% of the encapsulated COLO-357 cells remained alive following the gelation process and the cells proliferated to form clusters with different morphology, depending on the growth factor supplements. Specifically, cell proliferation was suppressed in the presence of TGFbeta and EGF. On the other hand, cells encapsulated in collagen-laden hydrogels exhibited extensive protrusions and adopted irregular shapes when TGFbeta and EGF were added to the culture media. Intensive MT1-MMP expression was detected with the treatment of growth factor. On the mRNA level, expression of mesenchymal markers (e.g., vimentin) was significantly up-regulated in 3D culture while the addition of growth factors led to even higher vimentin expression. We also found that MT1-MMP mRNA expression was independent of collagen but was significantly up-regulated by soluble TGFbeta and EGF. COLO-357 cells encapsulated in hydrogels exhibited drug resistance as compared to the 2D monolayer culture.

Basic Science Faculty

POSTER #3

REDUCED CD1D EXPRESSION (BUT INCREASED ANTITUMOR ACTIVITY) IN A HAPLOINSUFFICIENCY MODEL OF NF1

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Neurofibromin 1 (NF1) is a tumor suppressor gene encoding a Ras GTPase that negatively regulates Ras signaling pathways. Mutations in NF1 are linked to neurofibromatosis type 1, juvenile myelomonocytic leukemia and Watson syndrome. CD1d-dependent natural killer T (NKT) cells play an important role in antitumor immunity. We have previously shown that CD1d-mediated antigen presentation is regulated by cell signaling pathways. To study whether a haploinsufficiency in NF1 would affect CD1d-mediated activation of NKT cells, we analyzed the NKT cell population as well as the functional expression of CD1d in *Nf1*^{+/-} mice. *Nf1*^{+/-} mice were found to have similar levels of NKT cells as WT littermates. Interestingly, CD1d expression on BMDCs from *Nf1*^{+/-} mice was lower than those from WT littermates. There was also a slight, but statistically significant decrease in the splenic B220⁺CD1d^{hi} population from *Nf1*^{+/-} mice. When inoculated with a T-cell lymphoma *in vivo*, *Nf1*^{+/-} mice survived longer than WT littermates. Blocking CD1d *in vivo* significantly enhanced antitumor activity in WT but not in *Nf1*^{+/-} mice. NKT cells from *Nf1*^{+/-} mice were found to be more responsive to CD1d-mediated antigen presentation *in vitro*, which may partly explain the increased antitumor activity observed in *Nf1*^{+/-} mice. In conclusion, our data suggest that NF1 is important to CD1d expression and NKT cell activation. This is manifested *in vivo* in terms of enhanced antitumor activity in mice with a haploinsufficiency in NF1.

Basic Science Faculty

POSTER #4

EFFECTS OF CABOZANTINIB ON BREAST CANCER BONE METASTASES, OVERALL SURVIVAL, AND BONE MASS IN A MOUSE MODEL

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Rapid tumor growth results in tumor hypoxia and the induction of key mediators of angiogenesis including VEGF and MET. Cells in the bone microenvironment, including osteoblasts and osteoclasts, express MET and VEGFRs and respond to HGF and VEGF. Cabozantinib (cabo) is an inhibitor of tyrosine kinases including MET, VEGFR2, and RET. Cabo treatment in preclinical models results in tumor regression and blockade of tumor invasiveness and metastasis, and has shown clinical activity in patients with castration-resistant prostate cancer and breast cancer tumors with bone metastases. To elucidate the mechanisms underlying some of these clinical observations, the effects of cabo were studied in a human breast cancer bone xenograft and in a non-tumor bearing model. Female nude mice were inoculated with MDA-MB-231 cells into the left cardiac ventricle and treated with cabo (10 or 60 mg/kg/day). Treatment was initiated 13 days after tumor inoculation when osteolytic lesions were detectable on x-ray, and continued for 11 days. In a non-tumor model, female nude mice (~5 weeks old) were treated with similar doses for 28 days. Tumor-bearing mice treated with cabo 60 mg/kg showed a reduction in osteolytic lesion area as measured by x-ray ($p<0.05$). Cabo treatment at both doses reduced the intensity of photon emission from tumors as measured by optical imaging using a Cathepsin K-linked fluorescent probe ($p<0.01$). Histomorphometric analysis showed a reduction in tumor burden in mice treated with cabo 60 mg/kg ($p<0.05$). This was accompanied by reduction in osteoclast numbers at the tumor bone interface with cabo 60 mg/kg ($p<0.01$) and 10 mg/kg ($P<0.05$). Mice treated with cabo 60 mg/kg did not exhibit as much weight loss as vehicle-treated mice ($P<0.05$). Mice treated with cabo at both doses showed significantly improved survival compared with vehicle treated mice ($p<0.01$). In non-tumor bearing mice, bone mineral density (BMD) increased at the tibia with cabo 60 mg/kg ($p<0.001$). MicroCT data analysis showed that treatment of mice with cabo 60 mg/kg resulted in an increase in trabecular bone volume (TBV) at the femur ($p<0.001$), tibia ($p<0.001$) and lumbar spine ($p<0.05$). This was accompanied by an increase in trabecular thickness ($p<0.001$) and connectivity density (femur $p<0.001$, Tibia $p<0.01$), a reduction in trabecular spacing ($p<0.001$ tibia & femur), and an increase in structure model index ($p<0.001$). No difference was detected in cortical bone parameters with either dose. In conclusion, cabo reduced osteolytic lesions, reduced tumor burden, reduced osteoclast number and improved survival in mice with established breast cancer bone metastases, and increased trabecular bone volume in non-tumor bearing mice. Studies to further characterize the molecular mechanisms underlying these effects are ongoing.

Basic Science Faculty

POSTER #5

THE HUMANRBPOME:FROM GENES AND PROTEINS TO HUMAN DISEASE

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RNA Binding Proteins (RBPs) play a central role in mediating post transcriptional regulation of genes. However less is understood about them and their regulatory mechanisms. In this study, we construct a repertoire of 1344 RBPs identified from several experimental studies and present a comprehensive analysis to understand their characteristics at a global scale. The domain architecture of RBPs enabled us to classify them into three groups - Classical (29%), Non-classical (19%) and Unclassified (52%). A higher percentage of proteins with unclassified domains reveals the presence of various uncharacterised motifs that can potentially bind RNA. In addition, enrichment of various unconventional superfamilies suggest that RBPs could form an integral part of the cellular architecture. Further, RBPs were found to be highly disordered compared to non-RBPs ($p < 2.2e-16$, Fisher's exact test), indicating a dynamic regulatory role of RBPs in cellular functioning. Evolutionary analysis in 62 different species showed that RBPs are highly conserved compared to non-RBPs ($p < 2.2e-16$, Wilcox-test), reflecting the conservation of various biological processes like mRNA splicing, ribosome biogenesis. The expression patterns of RBPs from Human Body Map 2.0 revealed that ~60% of them are ubiquitously expressed while ~40% are tissue-specific. Additionally, non-classical proteins were found to be higher expressed than the classical proteins across all the tissues ($p < 0.001$, Wilcox test). RBPs were also seen to be highly associated with several neurological disorders, cancer and inflammatory diseases. These analyses are made accessible to researchers in the form of a database called RNA Binding protein expression and disease dynamics database (READ DB)

Basic Science Faculty

POSTER #6

OXIDATIVE DAMAGE-INDUCED EPIGENETIC CHANGES AS A POTENTIAL MECHANISM FOR INITIATION OF CANCER-SPECIFIC EPIGENETIC CHANGES

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Inflammation and the associated increase in reactive oxygen species (ROS) play a key role in the initiation and progression of a majority of human epithelial cancers. Recent work by many groups has demonstrated that inflammation causes alterations in DNA methylation, microRNA expression, and, on a global level, histone marks. Since by definition these epigenetic changes are mitotically heritable and affect gene expression, they likely play a role in establishing disease phenotypes. During carcinogenesis, aberrant gains in promoter DNA methylation transcriptionally silence tumor suppressor genes, linking DNA methylation directly to tumorigenesis. However, it is unknown what the mechanisms of targeting and initiation are for these stable disease-specific epigenetic marks. Many toxins, including those produced by bacteria, cause an increase in inflammation and/or reactive oxygen species (ROS) that oxidize lipids and proteins and damage DNA by base oxidation as well as single and double strand breaks. We find that oxidative damage induces the formation of a large epigenetic silencing complex containing DNA methyltransferases (DNMTs) and members of the Polycomb Repressive Complex 4, including SIRT1 and EZH2. By examining chromatin on a genome-wide scale, we demonstrate that oxidative damage results in the relocalization of these epigenetic silencing proteins from non-GC-rich to GC-rich areas of the genome, including CpG-islands of which some are DNA hypermethylated and silenced in cancer cells. This relocalization results in histone mark and nascent transcription changes and, specifically at the CpG island-containing promoters of low expression genes, gains in DNA methylation. Furthermore, using a mouse model of inflammation-induced tumorigenesis, we demonstrate that, in inflamed tissue, oxidative DNA damage and enrichment of epigenetic silencing proteins occurs in CpG island-containing promoters of key genes. We are currently using this model to study the molecular progression from acute oxidative-induced chromatin changes to permanent epigenetic silencing events during tumorigenesis. This work uniquely links oxidative damage to genome-wide changes in the binding of epigenetic silencing proteins, describing a potential mechanism for the initiation of epigenetic changes in cancer. Understanding the mechanism of initiation and the timing of cancer-specific epigenetic changes will allow for the rational development of treatments that reverse epigenetic changes after exposure to oxidative damage and therefore reduce disease.

Basic Science Faculty

POSTER #7

SULFORAPHANE DEPRESSES PROLIFERATION AND INDUCES CELL DEATH IN GLIOBLASTOMA MULTIFORME (GBM) CELLS, GBM STEM CELL-LIKE SPHEROIDS, AND TUMOR XENOGRAFTS THROUGH MODULATION OF MULTIPLE CELL SIGNALING PATHWAYS

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Glioblastoma multiforme (GBM) comprises the largest group of brain tumors which are drug resistant and respond very poorly to the current therapies. In this study, we used sulforaphane (SFN), a multi-targeting agent with cancer preventive and anti-cancer activities and showed that it targets GBM established cell lines, early primary cultures, and CD133+ GBM stem cells as well as in GBM stem-like spheroids. SFN at 5-50 μ M triggered significant inhibition of cell survival and induced apoptotic cell death in GBM cells and CD133+ stem cells isolated from four GBM cell lines. SFN induced apoptosis in U87MG cells was associated with caspase-7 activation. Moreover, SFN triggered formation of intracellular reactive oxygen species (ROS) and when the cells were pre-treated with 10 mM N-acetyl cysteine (NAC), ROS production and cell survival in cells treated with 5-10 μ M were similar to the control untreated U87MG cells, revealing that SFN-triggered cell death is ROS-dependent. Moreover, SFN-generated ROS in U87MG cells were formed at the Mitochondrial Respiratory Chain (MRC) level. SFN also increased expression of the TRAIL receptor DR5 in GBM cells, U87MG and SF767 cells by 24 h post-exposure. Moreover, as revealed by comet assay, SFN increased single and double-strand DNA breaks in GBM. Compared to untreated control cells, a significantly higher amount of γ -H2AX foci and as consequence higher number of DNA double-strand breaks (DSBs) breaks were observed in the SFN-treated sample. In vivo studies, using NOD/SCID mice revealed that SFN administration via oral gavage at 100 mg/kg for 3 cycles significantly decreases the growth of ectopic xenografts established from the early passage primary cultures of GBM10. Our results show that SFN robustly inhibits growth of GBM cells in vitro and in vivo and induces cell death in established cell cultures, early passage primary cultures, as well as it is effective in eliminating GBM cancer stem cells, which play a major role in drug resistance and disease recurrence. These results suggest that use of SFN alone or in combination with other agents, may potentially improve survival of brain tumor patients.

Basic Science Faculty

POSTER #8

COMBINATION CISPLATIN-RADIATION TREATMENT CAUSES SEQUENCE DEPENDENT CYTOTOXICITY AND DELAYS DNA DAMAGE RESPONSE IN NON-SMALL CELL LUNG CANCER CELLS IN VITRO

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Treatment for advanced stage non-small cell lung cancer (NSCLC) often includes platinum-based chemotherapy and ionizing radiation (IR). While synergy has been observed with concomitant cisplatin-IR treatment, the mechanism remains unclear. We hypothesize that the synergistic cytotoxicity observed with combination cisplatin-IR therapy is dependent on the formation of complex cisplatin-double strand break (DSB) lesions and that the degree of radiosensitization is determined by the DNA repair capacity of the tumor. **METHODS:** Two NSCLC cell lines, H460 and A549, were used for these studies. Cisplatin was given at varying time-points prior to and following administration of IR in order to determine sequence and schedule-dependent cytotoxicity of combination cisplatin-IR therapy. Survival was determined by clonogenic survival assays. DNA damage response was evaluated by γ -H2AX as observed by fluorescence microscopy, flow cytometry and immunoblotting at varying time points, ranging from 0-24 hours. DNA damage response was evaluated by immunoblot analysis. Propidium iodide staining determined the effects on cell cycle. **RESULTS:** Sensitization of cultured A549 and H460 NSCLC cells to IR is dependent on the timing of cisplatin administration, with sensitization observed only in cells treated with cisplatin prior to IR. An antagonistic interaction was observed when cisplatin was administered after treatment with IR ($p < 0.01$). The morphology of γ -H2AX foci varied based on the type of DNA damaging treatment. Cells treated with combination cisplatin-IR therapy showed different nuclear immunofluorescent staining patterns depending on the time from IR treatment: early time points showed morphologic characteristics of both cisplatin and IR-induced γ -H2AX foci but by 24 hours, foci were similar to that observed in IR-treated cells. Gamma H2Ax foci formation was significantly increased in both IR and combination cisplatin-IR treated cells at early time points, while resolution of foci was delayed at 24 hours in combination cisplatin-IR treated cells ($p < 0.001$). This effect is not due to treatment-specific alterations in γ -H2Ax de-phosphorylation as measured by immunoblot. The DNA damage response does not differ in cells treated with cisplatin-IR and IR alone, as measured by cell cycle and Chk1 and Chk2 activation. **CONCLUSIONS:** These data demonstrate that sensitization of NSCLC cells to IR is dependent on cisplatin treatment prior to IR. Persistent γ -H2Ax foci in combination cisplatin-IR treated NSCLC cells suggests impaired repair of DNA DSB lesions. Cisplatin-IR treatment was associated with activation of IR-induced downstream damage response pathways independent of cisplatin-induced DNA damage response. This supports a mechanism in which cisplatin-IR synergy is dependent of IR-induced DNA damage but augmented by cisplatin treatment. Taken together with our previous findings, this data are consistent with a model by which that the repair of DNA double strand breaks, such as those induced by IR, is significantly impaired by the presence of a closely approximated cisplatin-DNA lesion.

Basic Science Faculty

POSTER #9

IMPLICATION OF GF11 IN HUMAN MYELOMA CANCER CELL SURVIVAL

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Multiple myeloma (MM) is a plasma cell malignancy and it remains incurable with conventional therapies. MM frequently involves skeleton and causes lytic bone disease. We previously reported that GF11 is induced by MM cells in osteoblast precursors and suppresses osteoblast differentiation, which suggest that GF11 may be a therapeutic target for reversing the persistent osteoblast suppression in MM bone disease. However, GF11 in MM cells has never been studied, although GF11 was shown to repress pro-apoptotic p53 target genes in leukemia cells. In this study, we investigated the role of GF11 in human MM cells. First, we demonstrated that GF11 was inhibited when H929 MM cells were treated with the proteasome antagonist, Bortezomib. Bortezomib down-regulated GF11, which was associated with up-regulation of p53 target genes (*PUMA*, *NOXA*, and *CNKN1A*) and activation of apoptosis as indicated by cleavage of Caspase-3. Next, using lentiviral RNA interference system, we targeted *GF11* in H929 cells and found that cells transduced with *GF11* shRNA showed severe growth inhibition compared with cells transduced with scrambled control shRNA. This phenomenon was seen in both H929 and JJN3 MM cells. Consistent with the repressive effects of GF11 on p53 target genes, we found that p53 target genes (*PUMA*, *NOXA*, and *CNKN1A*) were induced by the knockdown of *GF11* in H929 MM cells. Further, cleavage of Caspase-3 was observed in H929 cells transduced with *GF11* shRNA. Lastly, since GF11 represses gene expression through recruiting histone modifiers such as histone deacetylases (HDACs), we treated cells with HDAC inhibitors. As we expected, inhibition of histone deacetylases strongly induced cleavage of Caspase-3 and up-regulation of p53 target genes (*PUMA*, *NOXA*, and *CNKN1A*) in H929 cells. Taken together, our findings suggest that GF11 plays a critical role in human MM cell survival possibly by repressing p53 target genes, and may mediate the therapeutic effects of MM drugs such as proteasome inhibitors and HDAC inhibitors. Our study suggest therapeutic strategies targeting GF11 may be efficacious in the treatment of MM.

Basic Science Faculty

POSTER #10

CANCER-INDUCED BONE DESTRUCTION LEADS TO SKELETAL MUSCLE OXIDATIVE STRESS AND WEAKNESS

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Cancer-associated muscle weakness is an important paraneoplastic syndrome for which there is currently no treatment. Using a murine model of human breast cancer that is metastatic to bone (MDA-MB-231) we show skeletal muscle oxidative stress and muscle dysfunction in mice with bone metastases. Skeletal muscle weakness occurs without direct involvement of tumor cells in muscle and the tumor-bone microenvironment is a critical determinant of muscle weakness because there was no muscle weakness in the absence of bone metastasis. Tetanic calcium (Ca^{2+}), which directly determines the force of muscle contraction, was reduced in mice with bone metastases (4.91 ± 0.21 v. 2.28 ± 0.28 ; $p < 0.0001$). The ryanodine receptor/calcium release channel (RyR1) on the sarcoplasmic reticulum (SR), a key protein involved in skeletal muscle excitation-contraction coupling, was oxidized and depleted of the stabilizing subunit, calstabin1. Ex vivo contractility of the extensor digitorum longus (EDL) muscle showed a significant reduction in specific force in tumor mice ($213.2 \text{ kN/m}^2 \pm 16.6$ v. $361.1 \text{ kN/m}^2 \pm 9.6$; $p < 0.001$). Inhibiting the RyR1 mediated SR Ca^{2+} leak with a Rycal (S107) restored muscle force production ($431.0 \text{ kN/m}^2 \pm 19.4$ v. $362.8 \text{ kN/m}^2 \pm 7.2$; $p < 0.0001$) without affecting tumor burden. $\text{TGF}\beta$ is released in large quantities from bone during cancer-induced bone destruction and muscle from mice with bone metastases had increased SMAD3 phosphorylation. The degree of muscle weakness increased with increased bone destruction and inhibiting $\text{TGF}\beta$ or preventing bone resorption (using bisphosphonate therapy) reduced oxidative stress and restored muscle function ($418.60 \text{ kN/m}^2 \pm 15$ v. $336.3 \text{ kN/m}^2 \pm 28$; $p < 0.001$). C2C12 myotubes treated with $\text{TGF}\beta$ had elevated NADPH oxidase (Nox4) levels, oxidation of RyR1, reduced calstabin1 binding to the RyR1 complex and SR Ca^{2+} leak ($0.93 \pm 0.8 \text{ sparks/100}\mu\text{ms}^{-1}$ v. $1.44 \pm 1.3 \text{ sparks/100}\mu\text{ms}^{-1}$; $p < 0.05$). Our data show that $\text{TGF}\beta$ released during bone destruction due to bone metastases leads to oxidative stress in skeletal muscle causing SR Ca^{2+} leak and contributes to cancer-associated muscle weakness.

Basic Science Faculty

POSTER #11

SONIC HEDGEHOG PATHWAY INHIBITION INDUCES HYPERTROPHY IN C2C12 MYOTUBES

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Cachexia, or wasting of skeletal muscle and fat, afflicts many patients with chronic diseases including cancer, organ failure, and AIDS. Muscle wasting reduces quality of life and decreases response to therapy. Cachexia is caused partly by elevated inflammatory cytokines. Recently we found that such cytokines induce sonic hedgehog (SHH) activity in muscle, which in turn leads to muscle wasting through dual effects on progenitor cell proliferation and myofiber atrophy. Recently GDC-0449 (vismodegib), which targets the Shh pathway by inhibiting Smoothened (Smo), became FDA approved for basal cell carcinoma. Previously we found that GDC-0449 reduced muscle wasting in experimental cancer cachexia. Here we sought to identify new approaches to blocking cachexia by targeting the SHH pathway. Using C2C12 myotubes, we found that treatment with SHH or a Smo agonist (SAG) induced significant atrophy (-22.35%) versus control. In contrast, Smo inhibitors (Cyclopamine, GDC-0449, and LDE-225) were capable of inducing significant myotube hypertrophy. Furthermore, a Gli1 inhibitor (GANT61) produced even greater hypertrophy (+32.97% versus control myotubes) when compared with the Smo inhibitors. We also measured number of nuclei per myotube. SAG treatment resulted in a 14.51% decrease in nuclei per fiber versus control, while GANT61 resulted in a 32.98% increase. Thus SHH effects might be mediated both through effects on nuclear accretion as well as protein dynamics. Collectively, these results indicate that targeting the SHH pathway may be effective in preserving skeletal muscle during various disease states, or while undergoing certain therapies.

Basic Science

Graduate Student

POSTER #12

COX-2 EXPRESSION AND ACTIVITY IN APC-MUTANT BREAST CANCER

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Breast cancer is the leading cause of cancer-related death in non-smoking women, and the most commonly diagnosed cancer in women. The Adenomatous Polyposis Coli (APC) tumor suppressor gene is mutated or silenced by hypermethylation in 18-70% of sporadic breast cancers depending on subtype; however, the molecular mechanisms downstream of APC loss in the breast remain largely unexplored. While inactivation of APC most commonly leads to increased signaling through the Wnt/ β -catenin pathway, there are multiple, less-investigated functions of APC, including regulation of GSK3 β -mediated signaling, proliferation, epithelial polarity, cytoskeletal organization, and DNA repair. Given that restoration of tumor suppressors is a hurdle in treating tumors arising from loss of gene function, it is critical to investigate and understand the downstream signaling pathways involved in tumorigenesis. Our laboratory is particularly interested in investigating the effect of APC loss or mutation in breast cancer, and the complex Wnt-independent signaling downstream of APC loss in the breast. We previously demonstrated that Apc mutation accelerates the mouse mammary tumor virus-Polyoma middle T antigen (MMTV-PyMT) transgenic model of breast tumorigenesis independently of Wnt/ β -catenin signaling. Interestingly, focal adhesion kinase (FAK)/Src/JNK signaling was up-regulated and required for the enhanced proliferation and squamous phenotype mediated by Apc mutation. Despite the lack of Wnt/ β -catenin pathway activation, Apc-mutant mammary tumor cell lines demonstrated enhanced expression of Cyclooxygenase-2 (COX-2) and production of prostaglandin E₂ (PGE₂), known to be important in triple-negative and inflammatory breast cancers, two aggressive breast cancer subtypes. While treatment with the Src inhibitor (PP2) decreases COX-2 protein expression, there is no effect on the level of phosphorylated JNK, indicating a non-linear signaling pathway. Consistent with this, treatment with an inhibitor of JNK (SP600125) has no effect on COX-2 expression. These data suggest that while Src activation downstream of Apc mutation enhances COX-2 in a Wnt-independent manner, the pathway does not travel through activation of JNK. Therefore, the mechanisms by which Apc mutation initiates this stream of events to enhance mammary tumorigenesis, and the contribution of COX-2 to the phenotypes acquired in APC-mutant breast cancer remain unclear.

Basic Science

Graduate Student

POSTER #13

ANOIKIS EVASION IN INFLAMMATORY BREAST CANCER CELLS IS MEDIATED BY BIM-EL SEQUESTRATION

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Inflammatory breast cancer (IBC) is a rare and highly invasive type of breast cancer, and patients diagnosed with IBC often face a very poor prognosis. IBC is characterized by the lack of primary tumor formation and the rapid accumulation of cancerous epithelial cells in the dermal lymphatic vessels. Given that normal epithelial cells require attachment to the extracellular matrix (ECM) for survival, a comprehensive examination of the molecular mechanisms underlying IBC cell survival in the lymphatic vessels is of paramount importance to our understanding of IBC pathogenesis. Here we demonstrate that in contrast to normal mammary epithelial cells, IBC cells evade ECM-detachment-induced apoptosis (anoikis). ErbB2 and EGFR knockdown in KPL-4 and SUM149 cells, respectively, causes decreased colony growth in soft agar and increased caspase activation following ECM detachment. ERK/MAPK signaling was found to operate downstream of ErbB2 and EGFR to protect cells from anoikis by facilitating the formation of a protein complex containing Bim-EL, LC8, and Beclin-1. This complex forms as a result of Bim-EL phosphorylation on serine 59, and thus Bim-EL cannot localize to the mitochondria and cause anoikis. These results reveal a novel mechanism that could be targeted with innovative therapeutics to induce anoikis in IBC cells.

Basic Science Graduate Student

POSTER #14

ETS/AP-1 SITES CONTROL CELL MIGRATION GENES DOWNSTREAM OF RAS SIGNALING

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ETS/AP-1 sequences are recognized as one of the key responsive elements downstream of the RAS/ERK signaling pathway. ETS and AP-1 represent multi-protein transcription factor families that have many overlapping and unique functions. Some members of both of these families are responsive to RAS/ERK signaling while others are unresponsive to signaling by this pathway. Although some ETS/AP-1 sites have been discovered in regulatory regions of genes that are crucial for cellular invasion, migration, and transformation, such as PLAU, matrix metalloproteases, and integrins, these sites have not been monitored across the genome to determine if they represent a distinct class of potentially oncogenic binding sites. In this study bioinformatic and genomic analyses were performed to determine the landscape of genes that contain ETS/AP-1 sites and to search for genetic characteristics that define how these sites function in living organisms. ETS transcription factors bind to a conserved 5'-GGA(A/T)-3' motif while AP-1 proteins bind as homo- or hetero- dimers with other AP-1 subfamily members (Jun, Fos, ATF, CREB, and MAF), at a conserved palindromic 5'-TGA(G/C)TCA-3' motif. Through genome-wide pattern matching and gene ontology analysis it was determined that the subset of genes that contain ETS/AP-1 sites are enriched for cell migration, cell morphogenesis, and developmental processes. With the access to microarray experiments with RAS inhibition/activation on GEO, and the ability to search open chromatin for ETS/AP-1 binding sites from DNase-seq data on ENCODE, it was possible to correlate the response of genes with ETS/AP-1 sites to the status of the RAS pathway. To identify the role of AP-1 subunits at ETS/AP-1 sequences, we used RNA-sequencing analysis of knockdowns for various members of the Jun subfamily. Based on this analysis it was found that ETS/AP-1 sites represent a unique subset of AP-1 targets where cJUN and JUNB have opposing function to JUND and regulate cell migration phenotypes.

Basic Science Graduate Student

POSTER #15

DEFINING THE MECHANISM BY WHICH POLYPLOIDY CONTRIBUTES TO GENOMIC INSTABILITY

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Changes in cell cycle progression are known to lead to genomic instability. One type of variant cell cycle is the endocycle in which cells go through alternating periods of synthesis (S) and gap (G) phases without an intervening mitosis, which leads to polyploidy. Polyploid cells are one hallmark of cancer, but the mechanisms that induce polyploidy and how they contribute to oncogenesis are not well understood. Previous work from the Calvi lab showed that *Drosophila* cells can be induced into the endocycle by treatments that perturb cell cycle progression, and that cells in the endocycle are resistant to apoptosis. Upon cessation of treatment, these polyploid cells resumed mitotic proliferation, but these mitoses were error-prone, with markers of genomic instability and aneuploidy. Because many chemotherapeutic agents target the cell cycle machinery, we hypothesized that some of these drugs may induce a subset of cells into an apoptotic resistant endocycle. Upon cessation of therapy, these polyploid cells may resume an error-prone mitosis leading to aneuploidy and disease progression. To test this idea we treated MDA-MB-231 cells with multiple anti-mitotic drugs and found that some of these drugs induced polyploidy. Of these drugs, SU6656 reproducibly induced a large fraction of the cells to have higher DNA content, indicative of an endocycle. Live cell imaging after drug treatment showed that cells undergo mitotic-like rounding, but then re-flatten without completing division, resulting in large, multi-lobed nuclei. Analysis of fixed cells in the rounded state showed cells in early but not late mitosis, suggesting that SU6656 causes a specific type of endocycle known as endomitosis. To ask whether cells in the endocycle could resume mitotic proliferation, cells were synchronized, treated with drug, the drug was removed, and cell fate was analyzed by FACS, by time-lapse microscopy, and by fixed cell analysis. After drug withdrawal, polyploid MDA-MB-231 cells were able to resume mitotic divisions and returned to a pseudo-diploid state. Polyploid cells re-entered mitosis with supernumerary centrosomes and formed large multi-polar spindles that contained hundreds of extra kinetochores. Upon cell division there were lagging chromosomes and chromosome bridges, suggesting that mitotic errors in these cells contribute to further aneuploidy. Our results demonstrate that inhibition of normal cell cycle progression can lead to polyploidization and genome instability. Cancer cell polyploidization may represent a survival mechanism for cells in response to cancer therapies and may be a major contributor to disease relapse.

Basic Science

Graduate Student

POSTER #16

ROLE OF CATIONIC AMINO ACID TRANSPORTER 2 IN MYELOID DERIVED SUPPRESSOR CELL FUNCTION

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Inflammatory chronic prostatitis/chronic pelvic pain syndrome has been linked to autoimmune inflammation. Likewise, prostatitis has been linked to prostate cancer development and progression. A better understanding of the mechanisms by which inflammation is regulated may provide the foundation for novel approaches to controlling inflammation. Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immature cells that expand during benign and cancer-associated inflammation. MDSC block T-cell immunity and promote tumor progression by helping tumors to escape antitumor immune responses. Increased metabolism of L-Arginine, through the enzymes arginase 1 (ARG1) and nitric oxide synthase 2 (NOS2), is well documented as a major MDSC suppressive mechanism for controlling T-cell responses. However, the mechanism by which extracellular L-Arginine is transported into MDSC has not been defined. Using murine models of prostate specific inflammation and prostate cancer, we have shown that MDSC are recruited to localized inflammatory sites and tumor sites. MDSC at both sites display increased expression of cationic amino acid transporter 2 (*Cat2*) that is coordinately induced with *Arg1* and *Nos2*. CAT2 mediates the transport of L-Arginine in MDSC and acts as an important regulator of MDSC suppressive function. MDSC that lack CAT2 have significantly reduced suppressive ability and the abrogation of suppressive function is due to low intracellular L-Arginine levels, which leads to the impaired ability of ARG1 and NOS2 to catalyze L-Arginine metabolic processes. Together, these findings suggest that MDSC are important regulators of immune responses in prostate inflammation and prostate cancer. CAT2 is a novel molecule that plays a critical role in regulation of MDSC suppressive function and can be utilized as a target to intervene MDSC suppressive activity in order to improve cancer therapy.

Basic Science

Graduate Student

POSTER #17

PROTEIN DEPRIVATION ALTERS TRAFFICKING OF THE B CELL RECEPTOR AND ENDOGENOUS ANTIGENS IN B CELLS

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Cancer can result in severe wasting and malnutrition, compromising the immune response to tumors and pathogens. Although poorly understood, protein malnutrition severely disrupts cellular and humeral immunity. These studies suggest protein malnourishment may alter antigen presentation and the cognate interaction between T and B cells that are important for mounting host immune responses to tumors. At the cellular level, protein deprivation induces macroautophagy to promote survival and scavenging of molecular building blocks. Furthermore, macroautophagy appears to play a critical role in wasting of lean muscle tissue in cancer patients suffering from cachexia. We found protein deprivation in B lymphocytes dramatically skewed antigen trafficking and MHC class II (MHCII) antigen presentation to CD4⁺ T cells. The preferential presentation of epitopes derived from membrane proteins such as the B cell receptor (BCR), was compromised in response to protein deprivation. Instead, epitopes from antigens targeted for capture by autophagosomes were presented at higher levels with protein deprivation. Protein deficiency did not alter the expression MHC class II proteins but did upregulate lysosomal protease activity. BCR surface levels and trafficking were significantly perturbed with reduced receptor endocytosis. Macromolecular activation of macropinocytosis in B lymphocytes restored antigen presentation and BCR trafficking. These results demonstrate a key role for nutritional sensing in modulating intracellular antigen trafficking and immune recognition. Furthermore, these studies suggest alterations in autophagy may alter other endocytic pathways. While several therapies for cancer alter the autophagy pathway, these studies bring to light the affect these drugs may have on antigen presentation, a critical component of the immune response to tumors. This work was supported by the T32DK007519 and NIH RO1AI079065.

Basic Science

Graduate Student

POSTER #18

IDENTIFY ULTRA-RARE SOMATIC MUTATIONS FROM TARGET SEQUENCING

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Next-generation DNA sequencing technology enables researchers to interrogate the specific genome profiles of individuals, cancer samples or cohorts in nucleotide resolution. Rare point mutations are of particular interest and significance in early detection of cancer predisposition and relapse, thus are important in finding key driver mutations. Despite of its clinical significance, our capacity of accurately identify rare mutations are limited due to both the sequencing technologies and the availability of high quality variant-calling algorithms. Here we present a combination of experimental protocol and analysis pipeline that can be used to accurately identify de novo somatic mutations with extremely low allele frequency. The overall strategy includes four major components, benchmark sample design, target region amplification and sequencing, variant identification, and variant recalibration using machine-learning approaches. The benchmark sample was made by pooling DNA samples from 18 individuals with known genotype information from 1000 Genomes Project. The pooling strategy aims to maximize number of rare variants, with targeted lowest frequency 0.005. Allele frequencies of over 30% variants in the result benchmark sample are no more than 0.01. The preliminary data showed much improved accuracy on the rare variants identification. For point mutations with observed frequency ≤ 0.01 , the precision is $\sim 74\%$ with recall $\sim 55\%$. When observed frequency > 0.025 , the cumulative precision is $\sim 94\%$ with recall at $\sim 93\%$.

Basic Science

Graduate Student

POSTER #19

ACCURATE IDENTIFICATION OF RNA EDITING EVENTS USING MATCHED RNA AND DNA SEQUENCED SAMPLES UNCOVERS THE CONTRIBUTION OF THE EDITING LANDSCAPE TO DISEASE PROGRESSION IN GLIOBLASTOMA PATIENTS

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RNA editing event is increasingly appreciated as an important posttranscriptional regulatory mechanism in mammals. Adenosine deaminases that act on RNA (ADARs) are the enzymes that catalyze adenosine (A) to inosine (I) editing events. Human brain RNA is reported to have highest number of editing events. Many neurotransmitter receptors and ion channels undergo editing within exonic regions, which generates a different protein that that encoded by the genome. In addition, ALU repeats in introns and untranslated regions of brain mRNAs are often targeted by editing events and result in altered splicing and post-transcriptional gene regulation. In this study, we identified the single nucleotide RNA editing events in ~160 glioblastoma patient samples from The Cancer Genome Atlas (TCGA). We developed a robust statistical framework for comparing both RNA and DNA reads in each patient to discriminate DNA level Single-Nucleotide Polymorphisms (SNPs) from A-to-I RNA level editing events. This framework enabled the identification of hundreds of very high confident edit sites with most of them found reproducible across patients. We extracted significantly editing events that were detected in at least 25 patients resulting in the construction of a gene set of ~590 genes affected by the editing in glioblastoma. Analysis of the underlying protein interaction network of this gene set allowed the identification of a small number of hub proteins which might contribute significantly to the rewiring the glioblastoma network. Additional analysis such as survival modelling to identify and prioritize the genes associated with editing levels and their contribution to the cancer phenotype is underway using the TCGA clinical metadata.

Basic Science

Graduate Student

POSTER #20

RPAP2 IS AN ATYPICAL RNA POLYMERASE II CTD PHOSPHATASE IMPORTANT FOR THE REGULATION OF TRANSCRIPTION ELONGATION

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As a cornerstone in the central dogma of molecular biology, transcription is a process which converts genomic information to RNA molecules that can exhibit function autonomously or be translated into proteins. Regulation of genomic information is critical for a variety of cellular process including growth and responses to the external environment in normal and diseased cells. RNA Polymerase II (RNAPII) is a highly conserved enzyme responsible for transcribing the DNA template into a complementary RNA message. A unique feature to RNAPII is its carboxy terminal domain (CTD) that extends from the core enzyme. This disordered "tail" is comprised of the heptapeptide repeat sequence Tyr₁-Ser₂-Pro₃-Thr₄-Ser₅-Pro₆-Ser₇. This highly conserved sequence is repeated up to 26 times in yeast and 52 times in humans. The CTD tail has been extensively studied over recent years due to its intrinsic role in regulating dynamic interactions with initiation factors, mRNA capping, elongation factors, termination factors, and an assortment of chromatin remodelers. These interactions occur through reversible posttranslational modifications of the CTD: serines 2, 5, and 7, as well as tyrosine 1 and threonine 4 can all be phosphorylated, and tyrosine 1 and threonine 4 can be glycosylated. In light of a myriad of publications describing how different CTD proteins regulate these modifications during transcription, there is controversy as to the role of CTD phosphatases, specifically, the phosphatase activity of RPAP2 (human homologue of yeast Rtr1). Shortly after initiation, serine 5 phosphorylation begins to decrease through the action of Rtr1/RPAP2. Several groups, including our own, have reported Rtr1 exhibiting phosphatase activity; however, others have not been able to observe phosphatase activity. Moreover, crystal structures could not reveal an apparent active site in Rtr1/RPAP2 and as a result, the phosphatase activity of this enzyme remains controversial. To overcome this controversy we present data indicating RPAP2 is a novel phosphatase whose interaction with RNAPII requires the CTD interacting domain containing proteins RPRD1A & B. Through CTD phosphorylation, RPRD1A and 1B form a heterodimer and act as a scaffold for RPAP2 dephosphorylation of serine 5. Additionally, RPAP2 phosphatase activity was characterized *in vitro* on the small molecule substrate DiFMUP. We show that RPAP2 is inhibited by divalent metal ions and known phosphatase inhibitors partially explaining data from other groups in which RPAP2 showed no activity. The results from this study stress the importance of understanding basic biological process since RPRD1B is an oncoprotein whose overexpression has been documented in a large number of human cancers including colon, lung, and breast cancer. Therefore, understanding the mechanism of CTD phosphatases may prove fruitful in the development of cancer treatments that target CTD phosphatase activity.

Basic Science

Graduate Student

POSTER #21

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

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Eukaryotic cells utilize a macromolecular machine called the mitotic spindle to ensure faithful progression through mitosis. The spindle is composed of a dynamic array of microtubules and its associated proteins that facilitate the proper attachment, alignment, and segregation of chromosomes. Interfering with microtubule dynamics blocks mitotic progression, eventually leading to apoptosis, and has been a powerful target for chemotherapeutics. However, these drugs often have significant side effects, highlighting the need for new drugs that could have the benefits of anti-microtubule agents with fewer side effects. One idea would be to develop drugs that target microtubule regulators, whose activities are limited to dividing cells. The Kinesin-13, MCAK, is one such regulator. MCAK uses ATP hydrolysis to actively depolymerize microtubules and is required for mitotic spindle assembly and to prevent erroneous kinetochore microtubule attachments to the chromosomes. Interestingly, MCAK is overexpressed in numerous cancers including breast, lung, and gastric, and its expression can be correlated with metastasis and poor long-term survival. These data indicate that development of specific MCAK inhibitors has the potential to selectively hinder tumors overexpressing MCAK without the limiting adverse effects of anti-microtubule agents. To begin to screen for inhibitors of MCAK, we developed two 96-well plate assays to monitor MCAK activity. The first is an image-based screen in which stabilized microtubules are bound to the plate and then incubated with MCAK to induce microtubule depolymerization. A custom image-analysis algorithm was developed to quantify MCAK activity. This assay has a Z' of 0.6, and can readily detect two known inhibitors of MCAK. The second assay takes advantage of our recently developed FRET-based biosensor for MCAK. MCAK undergoes conformational changes during its catalytic cycle, which can be detected by this biosensor. Active MCAK has high FRET whereas inhibition of MCAK reduces its FRET. MCAK's FRET can be detected with a filter-based fluorometer, allowing detection in 96-well plate format. This assay is very robust and has a Z' of 0.89. We are currently finalizing optimization of these assays for two 3000 compound pilot screens to determine which assay will be better suited as a primary screen and which assay would be better for a secondary screen. These assays will position us to proceed with large scale screens for development of new therapeutics targeting MCAK activity.

Basic Science

Graduate Student

POSTER #22

PHARMACOLOGICAL EFFECT ON TARGET SIMULATION: A COMPUTATIONAL APPROACH TO STUDY DRUG'S THERAPY ON BREAST CANCER

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Inspired by Lamb's argument about examining opposite drug-disease profiles in order to treat diseases, we developed a new pathway pharmacological effects on target simulation (pathway-PETS) framework for drug repurposing. The framework includes two supportive tasks. First, we introduced a new method to construct disease-specific pathway models that are more comprehensive and better annotated than previous methods for pathway model construction. Pathway models developed from our procedure are useful in retrieving drug-disease mechanisms and developing repurposing methodologies by examining opposite profiles of drugs and diseases. Second, we developed a model-driven algorithm, PETS, which is able to perform various tasks: predict uncovered drug-protein effects, suggest repurposing candidates by using the disease pathway model and rank the drug candidates. By predicting unknown effects of drugs on proteins, we reveal potential drug's mechanisms on diseases. Our pathway-algorithm methodology has applications in personalized medicine. We suggested 5 candidate drugs for Breast Cancer ER+ repurposing and 8 candidate drugs for Breast Cancer ER-repurposing. We were able to figure out 17 drugs which were therapeutic for one Breast Cancer subtype but not for the other subtype; among them several cases were supported by literature. Our pathway-PETS mechanism could be further applied for drug's evaluation and prediction on other cancer diseases.

Basic Science

Graduate Student

POSTER #23

INHIBITION OF CD1D-MEDIATED ANTIGEN PRESENTATION BY THE TGF- β /SMAD SIGNALING PATHWAY

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CD1d is a Major Histocompatibility Complex (MHC) class-I like molecule, that presents lipid or lipid-based antigens (Ags) to a class of specialized, innate lymphocytes called Natural Killer T (NKT) cells. TGF- β is a potent cytokine with diverse effects on the immune system. In a previous study, we showed that the mitogen-activated protein kinase family member, p38, is a negative regulator of Ag presentation by CD1d. In an attempt to understand what upstream signals could activate p38, we found that several studies had implicated a role for TGF- β in the activation of p38. Therefore, we hypothesized that TGF- β negatively regulates the CD1d-mediated Ag presentation pathway. TGF- β treatment of murine antigen presenting cells (APC) impaired Ag presentation by CD1d to murine NKT cells. However, this inhibition was not through p38 activation, as TGF- β -induced impairment of Ag presentation by CD1d was also observed in APCs expressing a dominant negative form of p38. We then asked whether the signal transducing receptor regulated proteins, Smads 2, 3 and 4, downstream elements of the canonical TGF- β signaling pathway, contributed to the observed effects on CD1d-mediated Ag presentation. Using a lentiviral shRNA-based approach, we found that knockdown of Smads 2, 3 or 4 in APCs resulted in enhanced endogenous lipid Ag presentation to murine NKT cells, as compared to the negative control. Finally, to understand if TGF- β has any effect on lipid Ag processing, we tested the ability of Smad 2-, 3- or 4 shRNA-expressing APCs to present two exogenous lipid Ags: Gal(α 1 \rightarrow 2)galactosylceramide (α -GalGalCer, which requires intracellular processing) and α -Galactosylceramide (α -GalCer, the prototypic NKT cell ligand which does not need processing) to murine NKT cells, in the presence or absence of TGF- β . There was enhanced presentation of α -GalGalCer in the presence or absence of TGF- β in Smad 2, 3 or 4 knocked down cell lines compared to the control, however, α -GalCer was presented to a similar extent by all the cell lines. This suggests that TGF- β impairs lipid Ag processing. In marked contrast to CD1d, TGF- β was found to enhance MHC class II-mediated Ag presentation. Overall, these results suggest that the canonical TGF- β /Smad pathway has distinctly different effects on the regulation of antigen presentation pathways involved in the innate (i.e., CD1d) and adaptive (i.e., MHC class II) immune response.

Basic Science

Graduate Student

POSTER #24

ANALYSIS OF DOMAINS REQUIRED FOR THE ONCOGENIC FUNCTION OF ETS TRANSCRIPTION FACTORS IN PROSTATE CELLS

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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. In cell line models, expression of oncogenic ETS genes increases prostate cell migration and invasion, attributes of aggressive disease. Thus, defining mechanisms by which oncogenic ETS contribute to prostate cancer biology is of high priority, as it may aid in identifying novel targets to treat primary as well as advanced forms of prostate cancer. Our group recently showed that over-expression of these four oncogenic ETS genes, but not other ETS family members, in a cell line derived from normal prostate epithelia (RWPE1), activates a specific transcriptional program that drives prostate cell migration. The objective of the present study is to map and characterize unique domains that allow these four ETS proteins, but not other ETS family members, to drive prostate cell migration. We proposed that oncogenic ETS factors would differ from non-oncogenic ETS factors because they either have unique gene targets, or a unique transactivation potential. To test these possibilities, I attached a heterologous activation domain (HAD) to six full length non-oncogenic ETS proteins representing the full diversity of the ETS family. Interestingly, every ETS tested was able to induce prostate cell migration by 2 to 5 fold when fused to a HAD, compared to their respective wild-type (WT) proteins. This observation favors the model that all ETS proteins, when over-expressed, are able to bind gene targets that promote cell migration, but oncogenic ETS are unique because they have a domain that allows activation of these target genes. To map these critical activation domains, I made a variety of truncations in ERG and ETV5 and tested their ability to drive prostate cell migration. Preliminary results suggest that an activation domain critical for prostate cell migration is located in N-terminal portion of both ERG and ETV5 proteins. In the case of ETV5, this migration activation domain is distinct from ETV5 activation domains previously mapped by reporter assays. Fusions were then made between the N-terminus of ERG and ETV5 and the C-terminus of the non-oncogenic ETS protein FLI1. These chimeric proteins were able to induce cell migration, indicating that the activation domains of ERG and ETV5 are sufficient to confer this function on a non-oncogenic ETS protein. In the future, defined minimal activation domains will help in identifying co-activators which cooperate with oncogenic ETS factors to induce gene expression program critical for prostate cell migration. These studies will provide oncogenic-ETS specific mechanisms to target for future drug development.

Basic Science

Graduate Student

POSTER #25

CADHERIN-DEPENDENT MULTICELLULAR AGGREGATE MATRIX INVASION LIVE IMAGING

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Epithelial Ovarian Carcinoma (EOC) is the fifth leading cause of women's cancer-related death and deadliest of all gynecological cancers with 21,980 new and 14,270 fatal cases predicted in 2014 (American Cancer Society Estimates, 2014), primarily due to detection at late metastatic, prognostically poor stages of the disease. Epithelial ovarian carcinoma metastasizes by shedding of cancer cells from the primary tumor into the peritoneal cavity where they form multicellular aggregates (MCAs). These metastatic units along with single cells travel with the peritoneal fluid flow, adhere to peritoneum, migrate through mesothelial cell layer into submesothelial matrix wherein they subsequently proliferate into secondary tumor masses. EOC progression is accompanied by an increase of E-cadherin (Ecad) expression at early stages of metastasis and abundant expression of N-cadherin (Ncad) later in the disease. The contribution of MCA dynamics to the metastatic success and role of cadherins in MCA formation, survival in ascitic fluid and further fate remain largely unknown. Previously, we reported striking cadherin-dependent differences in cell-cell interactions, MCA generation, aggregate surface morphology and inner ultrastructure between mesenchymal-type DOV13 and SKOV3 (Ncad+) cells which formed stable, highly cohesive smooth solid spheroids, and epithelial-type OvCa433 and OvCa429 (Ecad+) cells that generated less adhesive cell clusters, loosely conglomerated and covered by uniform microvilli. To further address the relevance of MCA formation and phenotype for EOC progression, we performed live imaging of Ecad+ and Ncad+ single cells and MCAs during collagen invasion process. In attempt to mimic cell/aggregate anchorage and invasion into submesothelial matrix in ovarian cancer tumors, fluorescently tagged OvCa433 (Ecad+) and DOV13 (Ncad+) single cells or OvCa433 and DOV13 MCAs (generated in hanging drops) were applied on top of a 3-dimensional Rat Tail Collagen I construct inside a glass-bottom dish and traced using Nikon A1R-MP confocal microscope in reflectance mode for matrix imaging and fluorescence mode for cell imaging. Study revealed that both Ncad+ and Ecad+ single cells retain superficial localization and penetrate into the collagen layer strictly within 1 single cell diameter. On the contrary, MCA application is associated with higher invasion rate, penetration depth and degree of surrounding matrix deformation. Moreover, cohesive Ncad+ DOV13 MCAs demonstrated considerable plasticity and invasive behavior, attracted neighbor aggregates to form multi-aggregate conglomerates and showed clear intra-collagen MCA dispersal. On the other hand, loose Ecad+ OvCa433 MCAs tended to dissociate easier on top of collagen surface and behave less invasively. These preliminary data support the hypothesis that MCA dynamics is important for metastatic success and may largely depend on cadherin composition.

Basic Science

Graduate Student

POSTER #26

THE ROLE AND POTENTIAL USE OF MICRORNA-29 TO TARGET THE PANCREATIC CANCER STOMA

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths, with a 5-year survival rate of less than 6%. It usually remains undiagnosed until after the cancer has metastasized, and the resulting disseminated tumors are resistant to all forms of existing therapeutic modalities. Until recently, therapeutic strategies have mainly focused on targeting the tumor cells themselves. However, pancreatic tumors are characterized by a prominent stromal/fibrous reaction around the tumor called desmoplasia, which is a major obstacle for drug delivery to the tumor bed and plays a critical role in pancreatic cancer progression. Because of its role in cancer progression and invasion, stroma is an important therapeutic target to improve the efficacy of anti-cancer drugs. Although some of the pharmacological based anti-stromal drugs are shown to be therapeutically beneficial in pre-clinical studies, their effects are minimal in the clinic. A growing body of evidence suggests that pancreatic stellate cells (PSCs), stromal cells activated in response to autocrine and paracrine pro-inflammatory cytokines/growth factors such as TGF- β 1, secrete excessive amounts of extracellular matrix (ECM) proteins, a major component in the stroma formation of PDAC. Furthermore, PSCs interact closely with cancer cells to facilitate cancer progression and metastasis.

miRNAs are endogenously encoded small non-coding RNA molecules that regulate global gene expression and are critical in maintaining cellular homeostasis. It is now well established that miRNAs play a critical role in signaling pathways associated with cancer pathogenesis and the fibrotic process of several organs. Numerous functional studies have demonstrated the pro- and anti-tumorigenic/fibrotic activity of specific miRNAs and their therapeutic potential to suppress tumor growth and fibrosis. In an effort to understand the role of miRNAs in PSC-mediated PDAC-stromal accumulation, we challenged PSCs with TGF- β 1 and estimated miR-29 expression, a known anti-fibrotic miRNA family. In our preliminary work, we observed significant loss of miR-29 in TGF- β 1 treated PSCs, in correlation with an increase in pro-fibrotic mediator connective tissue growth factor (CTGF) expression and ECM component proteins such as collagen, fibronectin, and laminin. Both TGF- β 1 and CTGF are known pro-fibrotic inflammatory cytokines/growth factors, and their up-regulation is well documented in PDAC and chronic pancreatitis, a known risk factor for pancreatic cancer. In addition, we observed a loss of miRNA-29 in the pancreata of a PDAC mouse model which carries oncogenic KrasG12D, a frequent mutation found in >90% PDAC cases. Based on these observations, we are currently evaluating the anti-stromal properties of miR-29 in vitro and in vivo to target the pancreatic cancer stroma using gene therapy based approaches.

Basic Science

Graduate Student

POSTER #27

TISSUE TRANSGLUTAMINASE ALTERS THE PANCREATIC CANCER MICROENVIRONMENT

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Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer mortality in the world. Resistance to chemotherapy due to the presence of dense desmoplastic stroma (DS) represents an important cause of PDA related mortality. The DS is comprised of active fibroblasts and stellate cells which interact with extracellular matrix (ECM) proteins, and protects PDA cells from environmental stress. Tissue transglutaminase (TG2), a calcium-dependent protein which catalyzes crosslinking of ECM proteins, is highly expressed in PDA cells and also secreted in the DS. We measured TG2 expression by immunohistochemistry in 52 clinically annotated PDA specimens on the IUSCC tissue microarray noting 2~3⁺ TG2 expression in PDA cells of 36 specimens (69%) and in the DS of 44 specimens (84%). This led us to hypothesize that TG2 secreted from PDA cells promotes PDA progression by modulating the tumor microenvironment. Co-culture of human dermal fibroblasts (hFibroblasts) with PDA cells (AsPC1 and Panc1) engineered to express decreased amount of TG2 (shTG2 transfected cells) caused decreased proliferation of hFibroblasts compared to control (p<0.01). Likewise, culture of hFibroblasts with conditioned media (CM) from shTG2 cells decreased hFibroblasts proliferation in comparison to control (p=0.01). In addition, proliferation of hFibroblasts was promoted when cultured on TG2-mediated crosslinked collagen (p<0.01) compared to non-crosslinked collagen. An orthotopic xenograft mouse model was created by injecting PDA cells +/-TG2 into the tail of pancreas. ShTG2 PDA cell-derived tumors were smaller (p<0.02) than controls, suggesting that TG2 promotes tumor growth. In addition, response to gemcitabine (Gem) was increased in shTG2 cell-derived xenografts (p<0.05), suggesting that TG2 expression modulates response to chemotherapy. The mechanism by which this occurs was studied by comparing stroma composition in tumors. Deposition of fibroblasts was decreased in shTG2 tumors (p<0.03) compared to controls, corresponding to *in vitro* results. Total collagen deposition was similar between control and shTG2 tumors, but crosslinked collagen was decreased (p<0.01) in shTG2 tumors, suggesting that TG2 secreting tumors are associated with a denser stroma. Overall, this study demonstrates that TG2 secreted from PDA cells plays a pivotal role in modulation of PDA microenvironment and tumor progression. Depletion of TG2 in the PDA stroma may improve response to chemotherapy in PDA patients by providing survival cues to cancer cells. Therefore, TG2 is a promising potential therapeutic target within the PDA stroma.

Basic Science

Graduate Student

POSTER #28

ADENOMATOUS POLYPOSIS COLI MEDIATED SIGNALING, SELF RENEWAL AND DIFFERENTIATION OF BREAST EPITHELIAL CELLS

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Breast cancer is the leading cause of cancer-related death in non-smoking women in the United States. Despite tremendous amounts of information about the etiology of breast cancer, many questions remain unanswered. It has been demonstrated that specific subtypes of breast cancer differ in their sensitivity to chemotherapeutic and targeted therapies. The oncogenic events and signaling pathways driving these tumor subtypes are distinct. This indicates that increased knowledge of the molecular events defining the unique subtypes will provide opportunities for novel therapeutic approaches tailored to fit the specific subtypes and will therefore lead to improvement in patient outcomes. The *Adenomatous Polyposis Coli* (APC) tumor suppressor is mutated or hypermethylated in 18-70% of sporadic breast cancers depending on subtype. Oncomine database analysis revealed reduced expression of APC in aggressive estrogen receptor (ER) negative breast cancers compared to ER-positive breast cancer or normal breast. Using the *Apc*^{Min/+} mouse model, we identified pre-neoplastic lesions in the breast and enhanced breast tumorigenesis in the presence of the Polyoma middle T antigen (PyMT) oncogene. *Apc* mutation changed the tumor histopathology from solid to squamous adenocarcinomas, resembling the highly aggressive human metaplastic breast cancer. In this model of mouse mammary tumorigenesis, these changes occurred independent of Wnt/b-catenin signaling and required activation of focal adhesion kinase (FAK)/Src signaling, with a subsequent increase in cyclooxygenase-2 (COX-2). The applicability of these signaling pathways to human breast cancer remains unclear. Using the epithelial cell reprogramming assay for growing normal breast epithelial cells, we have begun to characterize expression of cell surface markers to delineate subtypes of epithelial cells. We have also assessed APC expression and correlated APC expression with risk and well-established clinical markers, such as ErbB2. Future studies will modulate APC expression in specific compartments of breast epithelial hierarchy to accomplish our objectives of dissecting the molecular alterations specifically downstream of APC loss and its role in the self-renewal and differentiation of breast epithelial cells.

Basic Science

Graduate Student

POSTER #29

ADENOMATOUS POLYPOSIS COLI REGULATES EPITHELIAL MEMBRANE PROTEIN 2 IN EPITHELIAL MORPHOGENESIS

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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/ β -catenin signaling pathway, it also binds to microtubules and polarity proteins, such asDlg and Scribble, suggesting functions in regulation of epithelial polarity and cell migration. 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. Previous studies from our laboratory demonstrated that APC knockdown in the Madin-Darby Canine Kidney (MDCK) model altered epithelial morphogenesis and resulted in inverted polarity in 3D culture. While restoration of the β -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 isolated from *Apc*^{Min/+} mice. We hypothesize that the interaction between the c-terminal fragment and epithelial membrane protein 2 (EMP2) plays a key role in regulating 3D morphogenesis and polarity. Interestingly, EMP2 and APC have been shown to regulate FAK signaling, suggesting an interaction in signaling pathways. We have demonstrated that the up regulation of EMP2 in APC knockdown cells is independent of Wnt/ β -catenin signaling. Treatment of APC knockdown MDCK cells with PP2, a Src kinase inhibitor, or AIIB2, an integrin inhibitor, eliminated the drastic cyst size changes produced by APC knockdown. In addition, preliminary studies suggest a role for APC in cell motility as shAPC-MDCK cells exhibited increased cell migration. Future studies will aim to dissect the role of the c-terminal fragment of APC in regulating gene expression, cell migration, and polarity and 3D morphogenesis in MDCK cells, and to analyze the polarity and 3D phenotype of mammary epithelial cells from *Apc*^{Min/+} mice. Investigating the interactions of APC with several targets such as those in the FAK/Src signaling pathway will help identify key players in the role of APC in Wnt-independent tumor development.

Basic Science

Graduate Student

POSTER #30

EFFECTS OF AGING ON THE PERITONEUM AND OVARIAN CANCER METASTASIS

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Epithelial Ovarian Cancer (EOC) is the most fatal gynecological cancer. EOC, which often goes undetected until metastatic stages of the disease, follows a unique form of metastasis. Tumor cells are shed from the primary tumor into the peritoneal cavity. Metastasis progresses when EOC cells adhere to peritoneal tissue and invade through the surface layer of mesothelial cells into the submesothelial extracellular matrix, where they anchor and proliferate. The majority of women diagnosed with ovarian cancer are over 60 years of age and 90% are over 40. However, the role of aging in EOC metastasis has not been studied. Additionally, peritoneal tissues are understudied. Preliminary data on ultrastructure changes in the aged mouse peritoneum and the elevated incidence of EOC in the aged human population have led us to hypothesize that age-related changes in the peritoneum contribute to EOC progression. We aim to identify and characterize differences in peritoneal tissues of young, middle-aged and old mice and to elucidate mechanisms for how these age-of-host differences affect various stages of EOC peritoneal metastasis.

Basic Science

Graduate Student

POSTER #31

DEVELOPING SMALL MOLECULE INHIBITORS TARGETING NUCLEOTIDE EXCISION REPAIR PROTEIN XPA FOR PLATINUM BASED COMBINATION CHEMOTHERAPY

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Platinum (Pt)-based combination chemotherapy has been the front-line treatment for a variety of malignancies including testicular, lung, and ovarian cancer. However, resistance to Pt-based chemotherapeutic agents has been a major limitation for successful treatment for many of these cancers, as is evident in the case of epithelial ovarian cancers (EOC). More than 80% of EOC patients relapse with Pt-resistant disease, where second line therapies are largely ineffective. Cisplatin is the most commonly prescribed Pt-based anticancer drug that cross-links DNA interfering with DNA replication, transcription and cell division; hence it is lethal for actively replicating cells like cancer cells. Repair of cisplatin-DNA adducts occurs primarily via nucleotide excision repair (NER) and homologous recombination repair (HRR). Germline mutations in BRCA1/2 predispose women to hereditary ovarian cancers that are HRR deficient. This genetic defect can be exploited by using a chemical inhibitor that targets a protein function, making the original defect lethal for the cancerous cells. Thus, in order to exploit the concept of synthetic lethality in Pt-based combination therapies; we have targeted the NER pathway in HRR deficient cancers, such as BRCA1 or BRCA2 null ovarian cancer. Towards this end we have recently identified NER inhibitors targeting the DNA binding activity of the Xeroderma Pigmentosum Group A (XPA) protein, a critical component of the NER pathway. XPA binding to damaged DNA duplex is essential for DNA damage recognition and verification in NER and has been described as the rate-limiting step in NER-catalyzed repair. We have employed Electrophoretic Mobility Shift Assays (EMSA) to identify and characterize third-generation XPA small molecule inhibitors (SMIs). The data demonstrate a 100-fold increase in potency with IC₅₀ values of 1 μ M. Analysis of the third generation inhibitors has revealed structure-activity relationships (SARs) that define the chemical and structural features necessary for interaction with XPA and cellular permeability. Analysis of inhibitory activity against a series of DNA binding proteins indicates a range of selectivity. Additional SARs were identified towards enhancing specificity for XPA. Since our inhibitors were modelled against the minimal binding domain (MBD) of XPA, which is the only characterized DNA binding domain in the protein, we will test whether our inhibitors bind to this MBD alone by an isothermal titration calorimetry assay. We have successfully cloned, expressed and purified XPA-MBD and tested its activity by fluorescence anisotropy. Based on these *in vitro* findings, we will pursue cellular cytotoxicity and sensitization to Pt treatment in BRCA1 null and wt ovarian cancer cell lines, which will form the basis for *in vivo* xenograft studies in mouse models for EOC. These fundamental findings shall promise the development of a chemical synthetic lethal approach, targeting the NER pathway in HRR deficient cancers combined with cisplatin therapy for increased efficacy with minimal toxicity.

Basic Science Graduate Student

POSTER #32

DEFORMABLE TUMOR VOLUMETRIC MAPPING WITH AN ITERATIVE MORPHING APPROACH

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Purpose: Due to patient free breathing, tumor and other critical organs deform during cancer radiation treatment. The voxel to voxel mapping among the 3DCT images at different time instances will be of great help in dose calculation and treatment verification. To address these challenges, an iterative morphing approach has been developed to define the iso-surface feature points and to map the 3D volumes. **Methods:** To map the tumor (similarly for other critical structures) from the source 3DCT to the target 3DCT, first, the tumor iso-surfaces of both the source and target phases are automatically detected based on neighboring CT intensity patterns. Then, the minimized bounding boxes of the tumor on both the source and target phases are derived based on tumor iso-surface. The feature points (landmarks) on the source iso-surface are selected based on gridding on the minimized bounding box and distances to the iso-surface. Next, for both the source and target phases, the nearest tumor iso-surface intensity and relative positions are projected to the six 2D planes of the bounding boxes. The corresponding landmarks are then mapped from the source to the target phase based on image template matching algorithm. Last, using a modified Shepard morphing approach, the entire tumor volume is mapped from the source phase to the target phase. **Results:** A prototype has been developed and preliminary experiments have been performed with the previously acquired 4DCT. One set of the experiments conducted where phase 0 was selected as the source phase and phases 7, 8 and 9 as the target phases, respectively. On the source phase, 31 landmarks were obtained on the tumor. The landmarks mapping results were evaluated with the similarity of the image intensity histogram and the displacement of the tumor volume. For the experiment demonstrated, the average landmark intensity differences of the predicted and actual 3D volume were 1.09, 1.36, and 1.08 for the phase 7, 8, and 9, respectively, with the average stdev of 0.82. **Conclusion:** We proposed a tumor iterative morphing method for 3D tumor iso-surface and volume mapping from one 3DCT to another with deformation. The dose effects from tumor deformation will be further investigated.

Basic Science

Graduate Student

POSTER #33

THE ROLE OF THE RAS/PI(3)K/SGK-1 SIGNALING AXIS IN THE REGULATION OF CANCER CELL SURVIVAL DURING EXTRACELLULAR MATRIX DETACHMENT

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Cancer metastasis, the spread of cancer cells to distant parts of the body, accounts for approximately 90% of cancer related deaths and represents an inherently difficult challenge for clinicians. In order for metastasis to occur, cells must overcome a cell death process, anoikis, which is triggered by detachment from the extracellular matrix (ECM). The viability of ECM-detached cells also requires the maintenance of proper metabolism through an anoikis independent mechanism. Previous work in our lab has demonstrated that overexpression of ErbB2 results in a PI(3)K/Akt-dependent mechanism that promotes glucose uptake, leads to the neutralization of oxidative stress, and thereby facilitates the survival of ECM-detached cancer cells. These data led us to investigate whether additional oncogenic insults could signal in a similar manner to promote survival in ECM-detached cancer cells. One oncogene of particular interest is Ras due to the fact that it is mutated in approximately 30% of cancers, is known to promote metastasis, and can signal through the PI(3)K pathway. To investigate the role of Ras in the regulation of metabolism during ECM-detachment, we engineered MCF-10A cells, a non-tumorigenic mammary epithelial cell line, to express a constitutively active form of H-Ras(G12V) or K-Ras(G12V). Our results to date indicate that Ras signals through a PI(3)K-dependent, but Akt-independent mechanism to maintain proper metabolism. These data elucidate a novel role for SGK-1, an alternate downstream effector of PI(3)K, in promoting metabolic maintenance in ECM-detached cancer cells. Our results provide evidence that targeting SGK-1 in cancers harboring Ras mutations may be an effective chemotherapeutic strategy to eliminate ECM-detached cancer cells.

Basic Science

Graduate Student

POSTER #34

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

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Background: A key feature of drug-resistant prostate cancer (DRPC) cells is the induction and activation of the survival proteins. Targeting survival proteins directly has proven problematic clinically, therefore understanding the mechanisms of their induction within DRPC 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. Because APE1/Ref-1 has been shown to regulate the expression of survival proteins via STAT3 reduction in other cells, we sought to assess its redox function's role in drug resistant prostate cancer. **Methods:** We assessed the expression of APE1/Ref-1, survivin, Mcl-1, Bcl-2 and STAT3 activity in docetaxel-resistant PC3 cells by immunoblotting. The effect of APE1/Ref-1 on docetaxel-resistant cells was determined by siRNA and treatment with the redox function-specific inhibitor E3330 (10, 30, 50 μ M). The effect of STAT3 inhibition on IL-6-induced APE1:STAT3 complex formation was performed by co-immunoprecipitation and immunoblotting. Localization of APE1/Ref-1 in human prostate cancer tissue was performed by immunofluorescence and co-localization was performed by co-staining with survivin, pGP130, or STAT3-specific antibodies. **Results:** We found that DRPC cells showed a 4-fold induction of APE1/Ref-1 expression, a 12-fold induction of survivin expression, and a 5.2-fold induction of Mcl-1 expression relative to parental prostate cancer cell lines. Co-IP showed that APE1/Ref-1 interacts directly with STAT3 in DRPC cells. We found that inhibition of APE1/Ref-1 redox function by siRNA or E3330 prevented DRPC cell growth and induced cell death, and this treatment sensitized previously resistant cells to docetaxel. We found that IL-6 induced survivin expression and STAT3 activity, an effect that was attenuated by E3330 and siRNA knockdown of APE1/Ref-1. We found that STAT3 inhibition reduces its direct interaction with APE1/Ref-1. Finally, we found that APE1/Ref-1 is highly expressed in human prostate cancer and co-localizes strongly with survivin and pGP130 activation. **Conclusions:** These data indicate that DRPC cells exhibit induced survivin and APE1/Ref-1 expression and that APE1/Ref-1 inhibition attenuates survivin and Mcl-1 induction and sensitizes resistant cells to docetaxel. 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 #35

VBIM TECHNOLOGY IDENTIFIES BETA-CATENIN LIKE PROTEIN (BCLP) AS A NOVEL NEGATIVE REGULATOR OF NF-KB

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Colon cancer is the second leading cause of cancer related deaths in the United States. The nuclear factor kB (NF-kB) is an important family of transcription factors whose aberrant activation has been found in many types of cancer, including colon cancer. Therefore, understanding the regulation of NF-kB is of ultimate importance for cancer therapy. The purpose of this study is to use a novel validation-based insertional mutagenesis (VBIM) strategy to identify novel regulators of NF-kB, and further evaluate their roles in the regulation of NF-kB signaling in colon cancer cells. We infected Z3 cells (293 derived cells with hyper active NF-kB activity) with VBIM virus to cause the overexpression of negative regulators of NF-kB, and then further selected the mutant cells with low NF-kB activity under ganciclovir (GCV) treatment. Targeted gene was then identified by using VBIM specific primers. In a preliminary screen, we identified the novel b-catenin like protein (*BCLP*) gene as a negative regulator of NF-kB. Overexpression of *BCLP* led to decreased NF-kB activity by kB reporter assay, while knocking it down had the opposite effect. Furthermore, we found that overexpression of *BCLP* in HT29 colon cancer cells greatly reduced both the number and the size of colonies that were formed in a soft agar assay, while sh-RNA mediated knockdown of *BCLP* in HT29 cells resulted in an opposite effect, confirming that *BCLP* is a tumor suppressor in HT29 cells. Our future experiments aim to further assess the role of *BCLP* in colon tumor formation in a mouse xenograft model. In summary, by using the novel VBIM technique, we identified *BCLP* as a novel negative regulator of NF-kB. This discovery could lead to the establishment of *BCLP* as a potential biomarker and therapeutic target in colon cancer.

Basic Science

Graduate Student

POSTER #36

ADAR3: ELUCIDATING THE MOLECULAR FUNCTION OF A NEURONAL RNA-EDITING FAMILY PROTEIN

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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. Improper control of gene expression can be observed in various diseases and is considered a hallmark of cancer. Recently, over one million inosines have been discovered in the human transcriptome. These noncanonical nucleosides are a result of RNA editing by ADARs, a process that can regulate gene expression by altering the genomically encoded sequence. Throughout evolution, RNA editing has become more common and complex, with numerous sites of essential editing occurring in the nervous system. Loss of the editing enzymes in model systems, such as worms, flies and mice shows that RNA editing is required for normal development and proper neuronal function. Consistent with this, alterations in editing levels have been observed in a number of human diseases, including epilepsy, Prader-Willi syndrome, amyotrophic lateral sclerosis, and brain cancers. In humans, there are three ADAR family members, ADAR1, ADAR2 and ADAR3. 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. My thesis research is focused on understanding the brain-specific function of ADAR3. Thus far, I have determined that ADAR3 exhibits both a cellular and molecular phenotype when overexpressed in human cell culture, suggesting a previously undetected functional role for the protein. Glioblastoma cell lines exhibit differential protein expression profiles of the human ADARs, making them an ideal system for studying interaction between the ADARs. Using human cell lines that overexpress ADAR3, I have determined that ADAR3 affects editing levels of coding transcripts, such as the DNA repair enzyme NEIL1. Furthermore, ADAR3 overexpression affects proliferation and migration of neuronal cell lines, depending on the ADAR expression profile. The data suggest a dynamic interaction between the human ADARs that is tightly regulated, especially in the nervous system.

Basic Science

Graduate Student

POSTER #37

NON-CODING RNA HOTAIR CONNECTS DNA DAMAGE SIGNALING TO NF- κ B ACTIVATION IN CISPLATIN RESISTANT OVARIAN CANCER

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The Polycomb Repressive Complex 2 (PRC2) has been implicated in cancer and a role for PRC2 during DNA damage response (DDR) has recently been reported. To examine changes in gene expression with DDR in cisplatin (cddp)-resistant ovarian cancer (OC), we performed whole transcriptome RNA-seq analysis of isogenic cddp-sensitive and -resistant OC cell lines (A2780 and A2780-cp). Differential expression of PRC components (SUZ12, EZH1, SIRT1, PHC1 &2) ($P<0.05$), NF- κ B pathway members (NCAM, ABCB9, RAGE, IL4R, IL6R, BCL2L11) ($P<0.05$), and long non-coding RNAs (lncRNAs) known to associate with PRC2 ($P<0.05$), including Hox transcript antisense intergenic RNA or HOTAIR, was observed. In tumors from patients with high grade serous OC at diagnosis, basal expression of HOTAIR was greater ($P<0.01$) compared to normal ovarian surface epithelium and marked overexpression of HOTAIR was observed in tumors obtained from patients who had developed platinum-resistant OC. Ablation of HOTAIR using dsRNA resensitized A2780-cp to cddp, while ectopic over-expression of HOTAIR increased ($P<0.05$) A2780 cell survival after cddp treatment (3-fold vs. control). To further examine HOTAIR regulation, we conducted a promoter analysis using bioinformatics tools and luciferase assays. We identified a putative p65-NF- κ B binding site (906-GGGACACCCC-915) 906 bp upstream of the HOTAIR transcription start site. Treatment of A2780 cells with the NF- κ B activator TNF- α induced HOTAIR (16-fold vs. control) and NF- κ B enrichment (3-fold assessed by ChIP assays) at the HOTAIR promoter. Furthermore, in A2780-cp compared to A2780, total I κ -B α levels were reduced ($P<0.05$) and nuclear p65 levels were increased, indicating that endogenous activation of NF- κ B contributes to cddp resistance and DDR. Consistent with this observation, cddp treatment (20 μ M for 0-24hrs) of A2780 cells increased ($P<0.05$) HOTAIR expression by 5- and 16-fold at 8 and 24 hrs and decreased ($P<0.05$) I κ -B α protein levels at similar time points. Furthermore, inhibiting NF- κ B by either gliotoxin (5 μ M) or Bay-11 (3 μ M) completely abolished cddp-induced HOTAIR expression in A2780 cells, demonstrating that NF- κ B is a HOTAIR transcriptional activator during cddp-induced DDR. Importantly, EZH2 and histone H3 lysine-27 trimethylation (H3K27me3) levels were enriched (6- and 17-fold) in the I κ -B α promoter at 24 and 48 hours post cddp treatment, and HOTAIR depletion using dsRNA reduced the observed EZH2-H3K27me3 enrichment at the I κ -B α promoter, demonstrating that HOTAIR recruits PRC2 complex to the I κ -B α promoter to prolong NF- κ B activation during cddp-induced genotoxic stress. Mouse xenograft studies with A2780 cells overexpressing HOTAIR are ongoing. The results of this study support a role for HOTAIR as a positive regulator of the NF- κ B pathway and PRC2 during cisplatin-induced DNA damage. We further suggest that HOTAIR may serve as a therapeutic target in cisplatin-resistant OC.

Basic Science

Graduate Student

POSTER #38

THE TRANSCRIPTION FACTOR ETS1 MEDIATES RAS/ERK SIGNALING GENOME-WIDE THROUGH ETS/AP-1 SEQUENCE MOTIFS PROMOTING INCREASED CANCER CELL MIGRATION

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Constitutive RAS/RAF/MEK/ERK (RAS/ERK) signaling is common in cancer and leads to transcriptional activation of genes that promote phenotypes such as cell invasion, migration, and proliferation. Adjoining binding sites for ETS and AP-1 transcription factors are prototypical "RAS-responsive elements" and confer RAS/ERK responsiveness on genes that promote cell migration/invasion. A subset of ETS transcription factors can be phosphorylated by ERK and, when over-expressed, activate RAS/ERK dependent transcription of reporters via ETS/AP-1 sites. However, the endogenous ETS protein that binds ETS/AP-1 sequences across the genome and mediates the RAS/ERK response has not been identified. Here, we used an unbiased approach to identify the ETS factor that mediates this response in prostate cancer cell line with a KRAS mutation, DU145. Kinase assay identified all ETS proteins that were expressed in DU145 cells and could be phosphorylated by ERK. Knockdowns of each candidate ETS protein revealed that only ETS1 was required for DU145 cell migration. When over-expressed, wild-type ETS1 increased migration of DU145 cells, but a mutant ETS1 lacking ERK phosphorylation sites decreased migration. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) of candidate ETS factors showed that only ETS1 bound to regions with ETS/AP-1 sequences. Transcriptome-wide analysis (RNA-seq) showed that ETS1 and RAS/ERK regulate overlapping gene expression programs activating genes controlling cell migration during specialized processes such as wound healing. This role of ETS1 extends beyond prostate cancer, as ETS1 bound to a common set of ETS/AP-1 targets in RAS-active lung, pancreatic, and prostate cancer cell lines. In summary, ETS1 mediates RAS/ERK signaling by activating an ETS/AP-1 regulated gene expression program that regulates cancer cell migration.

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Basic Science

Graduate Student

POSTER #39

THE ROLE OF CELL-CELL CONTACTS IN THE SURVIVAL OF EXTRACELLULAR MATRIX DETACHED MAMMARY EPITHELIAL CELLS.

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Normal epithelial cells require attachment to the extracellular matrix (ECM) in order to survive. When detached from the ECM, normal cells will undergo apoptosis through a process known as anoikis. The ErbB2 receptor tyrosine kinase is well known to antagonize anoikis although the precise molecular mechanisms are not entirely known. Interestingly, we have discovered that the overexpression of ErbB2 in non-malignant mammary epithelial cells (MCF-10A) results in substantial aggregation of cells that coincides with protection from anoikis. Therefore, we were interested in understanding if these aggregates could be involved in the evasion of anoikis induction. We found that the disruption of cell-cell contacts in ErbB2-overexpressing MCF-10A cells (using methylcellulose or by antagonizing the formation of adherens junctions) induces caspase activation. Furthermore, in cells that form large aggregates, ErbB2 can physically interact with E-cadherin and EGFR in a fashion that prevents the internalization of EGFR and ultimately its lysosome mediated degradation. The disruption of aggregation causes the ErbB2/EGFR/E-Cadherin complex to fall apart and subsequently results in EGFR degradation in the lysosome. This degradation of EGFR diminishes signaling through the MAPK pathway which prevents the induction of anoikis. In summary, these data suggest that oncogenic signaling through ErbB2 promotes the formation of cellular aggregates that function to prevent EGFR from degradation and subsequently to block the induction of anoikis.

Basic Science

Graduate Student

POSTER #40

SHP2 TYROSINE PHOSPHATASE COOPERATES WITH SRC AND SYK TYROSINE KINASES TO PROMOTE FLT3-ITD-INDUCED ACUTE MYELOID LEUKEMIA

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FMS-like tyrosine receptor kinase-internal tandem duplications (FLT3-ITDs) have been identified in approximately 30% of patients diagnosed with acute myeloid leukemia (AML) and confer a poor prognosis. FLT3-ITDs are characterized by the addition of several amino acids in the juxtamembrane domain of the FLT3 receptor tyrosine kinase, resulting in its constitutive activation, as well as the aberrant hyperactivation of STAT5. The protein tyrosine phosphatase, Shp2, has been shown to interact with Tyr599 on WT FLT3, and Tyr599 is commonly duplicated in FLT3-ITDs. We found previously that treatment of FLT3-ITD-expressing cells with the novel Shp2 phosphatase inhibitor, II-B08, resulted in reduced phosphorylation of Shp2 as well as STAT5, suggesting that Shp2 phosphatase promotes STAT5 activation via the dephosphorylation, and thus activation, of a STAT5-activating kinase. Based on the findings that both Src family kinases and Syk kinase have been shown to interact with STAT5 and FLT3, and that Shp2 dephosphorylation of Src enhances its kinase activity, ***our over-riding hypothesis is that the duplicated Tyr599 on FLT3-ITD permits increased Shp2 recruitment, allowing Shp2 to dephosphorylate, and thus activate, Src and Syk, leading to constitutive phosphorylation of STAT5 and promoting leukemogenesis.*** To address this hypothesis, we transfected 32D cells with mutant and control constructs of FLT3 (WT-FLT3, FLT3-ITD, and FLT3-ITD-Y599F1/2) and measured proliferation via ³H-thymidine incorporation assays. Mutation of both Tyr599 residues (FLT3-ITD-Y599F1/2) corrected the hyperproliferation of FLT3-ITD-expressing cells. In immunoblot analyses, FLT3-ITD-expressing cells demonstrated elevated levels of phospho-STAT5, phospho-Shp2, and active Src and Syk kinases compared to WT-FLT-expressing cells, and, as predicted, mutation of the Tyr599 residues on FLT3-ITD corrected the hyperactivation of STAT5, Src, and Syk. These findings suggest that interaction of Shp2 with FLT3-ITD Tyr599 promotes STAT5 activation via Src and Syk. Accordingly, in immunoprecipitation analysis, we found decreased Shp2, STAT5, and Src bound to FLT3 in FLT3-ITD-Y599F1/2-expressing cells compared to FLT3-ITD-expressing cells. We next examined the effect and putative cooperation between a Shp2 phosphatase inhibitor and a Syk kinase inhibitor. Using primary patient AML samples, we found that FLT3-ITD+ cells are uniquely sensitive to the Shp2 phosphatase inhibitor, II-B08, implying that leukemia cells bearing FLT3-ITD mutations are distinctly dependent on Shp2 signaling, and suggesting that FLT3-ITD+ AML patients may benefit from Shp2 inhibitor therapy. Additionally, we found an additive effect of II-B08 with the potent Syk kinase inhibitor, R406, on the proliferation of primary FLT3-ITD+ AML cells. Together, our findings indicate a novel signaling relationship between the tyrosine phosphatase, Shp2, and the tyrosine kinases, Src and Syk, in FLT3-ITD+ AML, and suggest that targeting this pathway at multiple points may provide a novel therapeutic approach for treating patients with FLT3-ITD+ AML.

Basic Science

Graduate Student

POSTER #41

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. Roughly 73% of women with breast cancer at death 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 these cancer patients. Currently, the methods used to study bone metastasis are limited to *in vitro* tissue culture models, which lack the 3-D environment of heterogeneous cell types of the bone and marrow, and to *in vivo* animal models, which often are limited by the confounding primary tumor burden and also are not applicable to rapid screening aimed at targeting bone metastases. In this multidisciplinary project, we use a novel bone bioreactor to grow mouse and human bone explants, study bone metastases, and develop therapies to help breast cancer patients. The objective of this research is to develop an experimental platform using primary bone and cultured breast cancer cells to recapitulate the complex interactions between the primary actors (tumor cells, bone, and bone marrow) during breast cancer bone metastasis and to begin to use this platform to study therapies that reverse metastatic tumor growth in bone. Our culture system preserves the 3-D environment and heterogeneous culture conditions within the physiological context of an intact bone environment and is applicable to faster screening techniques than are available in current animal models. We will use this *ex vivo* bone culture bioreactor to help us identify the molecular factors that predispose some breast cancer patients to develop bone metastases and to aid in the screenings of new drugs aimed at targeting bone metastasis. We will validate the bioreactor as a means to understand metastasis, specifically the stages of metastatic tumor colonization, progression, and response to therapies. We first will compare metastatic bone growth of cultured bone metastases to *in vivo* mouse bone metastatic models. Later, we will use the bone bioreactor to study the effects of tumor metastasis on human bone that we will obtain from waste products of human orthopaedic surgical procedures. If successful, our bioreactor makes it possible to study metastatic cancer progression temporally and independently from primary tumor growth. Because this system is amenable for investigating bone colonization by multiple cancer types, this study also has general application beyond breast cancer. Because this bioreactor utilizes human bone as well as vibrational technology that is currently available to patients, this study has high translational value.

Basic Science

Graduate Student

POSTER #42

KIDNEY SPECIFIC REGULATORY NETWORK IN MOUSE UNCOVERS FUNCTIONAL, EVOLUTIONARY AND DISEASE DYNAMICS.

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Transcription factors (TFs) operate in a combinatorial fashion to regulate the expression of a gene or a group of genes; however their tissue specific regulatory interactions are not fully characterized. In this study, we construct and investigate kidney specific regulatory (KSR) network for mouse. We obtained upstream regions of genes in the mouse genome from ENSEMBL and extracted DNase I Hypersensitive sites (DHS) for 8 week mouse kidney from ENCODE project. Similarly, the position weight matrices (PWMs) for TF binding motifs (BMo) were extracted from JASPAR, Jolma, TRANSFAC® and mapped in the mouse genome using FIMO. These BMo were integrated with obtained DHS signals (narrow peak) in 5 KBs upstream regions. The resulting TFs and their targeted genes were modeled as directed interaction network comprising of 619 TFs and their corresponding 13500 target genes. We trimmed the resulting network by only keeping the genes that function as TFs. Resulting TF-TF network (of 619 nodes) was analyzed to provide a holistic picture of TF-TF interactions in mouse kidney tissue while the global network was studied for conservation across 61 species and relevance in kidney associated diseases. We observed that genes related to diseases were significantly enriched in second and third layers in network hierarchy. Conservation analysis of Mouse KSR revealed >50% conservation in close relatives such as rat, human, dog, squirrel and less conserved in invertebrates and yeast, thus elucidating network complexity increases with increase in kidney functionality from lower to higher species. In addition, mouse KSR was examined in its closest relative, rat for segments of nephron — TAL (Thick ascending limb), PT (Proximal tubules), IMCD (Inner medullary collecting duct), which revealed a significant enrichment of TFs for their corresponding original group in mouse KSR. Further, this network was investigated in diverse model kidney diseases such as hypertension, diabetes and kidney renal clear cell carcinoma (KIRC). The compendium of the network reported in this study can form a roadmap for increasing our understanding of the variations in regulatory wiring in kidney diseases.

Basic Science

Graduate Student

POSTER #43

MODULATION OF MDM2 IN CONTEXT OF DNA DAMAGE ENHANCES CELL DEATH IN A METASTATIC BREAST-TO-LUNG XENOGRAFT MODEL

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Metastatic breast cancer is highly refractive to current treatment strategies, and new multi-targeted treatments need to be elucidated. In metastatic disease, inhibiting key protein-protein interactions with the murine double minute 2 (MDM2) could be beneficial for developing new treatment modalities since this signaling pathway is a critical regulatory point in cancer progression. Inhibition of protein binding to the hydrophobic pocket of MDM2 by Nutlin-3a can activate pro-apoptotic proteins such p73 and E2F1 as well as decrease pro-angiogenic Hif-1 α . Since the DNA damaging agent carboplatin is currently being studied in clinical trials of triple-negative breast cancers (TNBCs), our objective was to evaluate the effects of carboplatin and Nutlin-3a in combination in TNBC in a mutant p53 background. Using a TNBC cell line TMD231 derived from the MDA-MB-231 human breast cancer cell line, we performed combination studies using different ratios of carboplatin to Nutlin-3a *in vitro* to evaluate the range of carboplatin-mediated DNA damage required to obtain synergism with inhibition of MDM2 function. A fixed ratio of 1:1 carboplatin:Nutlin-3a was strongly synergistic with a combination index of <0.5. In cell proliferation assays there was increased sensitivity to the drugs when given in combination ($p < 0.05$). TMD231 cells implanted into the mammary fat pad of NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice showed enhanced tumor growth, and metastasis was evident in the lungs. Dose-finding studies were performed to determine an optimal carboplatin dosing schema. NSG mice were randomized based on fluorescent imaging of E2-crimson expressing TMD231 cells allowing for a sensitive measurement of early tumor burden. Following Nutlin-3a and carboplatin combination treatment *in vivo*, there was a statistically significant reduction in tumor volume and lung metastases compared to vehicle and single drug treated mice ($p < 0.001$). Following Kaplan-Meier analysis, the combination treated mice had a significant increase in survival, (54.3 \pm 1.5 days) compared to the vehicle (39.3 \pm 0.6 days) and each single drug (Nutlin-3a: 39 \pm 1 and carboplatin: 47.5 \pm 1.8 days) ($p < 0.001$). While there was a decrease in bone-marrow cellularity, this did not lead to bone-marrow aplasia, and body weights recovered to normal levels within 7 days post-treatment. Pharmacodynamic studies are ongoing to further understand at the molecular level how the DNA damage response and repair is modulated by MDM2 resulting in a robust synergistic response. These studies will lead to a better understanding of how to potentiate DNA damage and may lead to new clinical therapies in the future for metastatic breast cancer.

Basic Science

Graduate Student

POSTER #44

IL-1 AND IGF-1 MEDIATE INFLAMMATION-DRIVEN EXPANSION OF SELECTIVELY SURVIVIN-POSITIVE STEM CELLS IN THE PROSTATE

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Prostate inflammation is extremely common and is one of the most important risk factors for prostate cancer. The IL-1/IGF-1 signaling pathway is known to induce epithelial cell expansion in prostate development, reactive hyperplasia, and prostate cancer, but the mechanisms and cell types expanded within the epithelium are unclear. The purpose of this study is to determine if survivin, a known survival and proliferative factor in prostate cancer, is involved in a specialized protected stem cell population that expands in response to prostatic inflammation. We initiated inflammatory reactive hyperplasia in mice using our bacterially-induced acute inflammation model, harvesting daily for 5 days after inflammation initiation; these were compared to non-inflamed control prostates. We counted previously characterized prostate epithelial progenitor cells (PEPCs) by their characteristic CD133⁺/Sca-1⁺/CD44⁺/CD117⁺/Lin⁻ pattern by flow cytometry. We quantified survivin expression in inflamed prostates with both immunofluorescence and immunoblotting. We then determined the expression of survivin in PEPCs by immunofluorescence post-sorting. Finally, the dependence of inflammation-driven survivin-expressing PEPC expansion was determined by inflaming IL-1R1 (-/-) and IGF-1 antagonist-treated mice (picropodophyllin, [PPP]) and determining PEPC expansion and survivin expression as described above. Our data showed that inflammation induces PEPC expansion 6-fold in the prostate, increasing the percent of PEPCs within the epithelium from 0.2% to 1.2%. Simultaneously, survivin was up-regulated 5-fold as measured by western, and IF showed that survivin expression was increased gradually throughout the inflammatory period, as it was expressed in less than 1% of cells (exclusively basal) in control prostates but increased to 15% of the epithelium by day 2, and 50% of the epithelium by day 5, suggesting that it is preferentially the survivin-positive fraction of cells that expand during inflammatory reactive hyperplasia. Survivin was expressed in 44% of PEPCs sorted by flow cytometry, but only 15% of non PEPC epithelial cells. Finally, pretreatment of inflamed mice with the IGF antagonist PPP attenuated both PEPC expansion and the survivin-percent fraction by 50%, and inflammation in IL-1R1(-/-) completely attenuated the inflammation induced effects in this study. Our data indicate that inflammation induces the expansion of a specialized survivin-expressing progenitor cell population in the prostate. Our data further indicate that this expansion is dependent upon the IL-1/IGF-1 signaling loop described in prostate development and reactive hyperplasia. Given the importance of survivin to cancer cell survival, our data may lead to a better understanding of how inflammation promotes cancer growth, and how prostate cancer cells are able to survive the toxic events of inflammation.

Basic Science

Graduate Student

POSTER #45

CAF-SECRETED IGFBPS REGULATE BREAST CANCER CELL ANOIKIS

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Carcinoma-associated fibroblasts (CAFs) are now widely appreciated to contribute to tumor progression. However, the ability of CAFs to regulate anoikis, detachment-induced cell death, has yet to be investigated. Here, we describe 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 discovered a novel mechanism underlying anoikis inhibition. We demonstrate that CAFs secrete elevated quantities of 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. 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. These results also identify IGFBPs as potential targets for the development of novel chemotherapeutics designed to eliminate detached cancer cells.

Basic Science

Graduate Student

POSTER #46

STRUCTURAL PROTEOME OF THE CANCER GENOME ATLAS (TCGA)

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Identifying druggable targets implicated in cancer is a key challenge for drug development and cancer treatment. Here, we map gene expression data from RNAseq and microarray technologies from 11 cancer types at The Cancer Genome Atlas (TCGA) onto the structural proteome to identify differentially expressed genes with druggable pockets on their protein products. We classify these pockets by annotating their role in enzymatic processes or at protein-protein interaction (PPI) interfaces. In addition, we explore the impact of these proteins in signaling pathways by integrating them onto 21 cancer-related pathways in KEGG. We also overlaid these proteins onto a human PPI network by integrating data from seven major interaction databases to identify highly connected proteins implicated in these individual cancers. Finally, druggable pockets were identified among proteins in the urokinase receptor subnetwork. Thus, these structural and network analyses reveal putative druggable binding sites for drug development in various cancers.

Basic Science

Graduate Student

POSTER #47

CD166 PROMOTES MULTIPLE MYELOMA CELLS HOMING TO THE BONE MARROW AND MYELOMA DISEASE PROGRESSION

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Multiple myeloma (MM) is a plasma cell malignancy characterized by multiple lytic lesions throughout the skeleton, suggesting that trafficking of MM cells from the bone marrow (BM) and lodgment of these cells at secondary sites is important in disease progression. CD166 is a member of the immunoglobulin superfamily capable of mediating both homophilic and heterophilic interactions. Accumulative evidence shows that CD166 is pathologically correlated with disease progression in a variety of cancers including melanoma and breast cancer. However, whether CD166 is involved in MM and how it mediates myeloma disease progression have not been addressed. Studies from our laboratory demonstrate that both MM primary cells and cell lines express CD166. The aims of this study were to elucidate the role of CD166 in MM cell trafficking to the BM and in MM disease progression. H929-GFP MM cells were injected intravenously into NSG mice and BM-homed GFP cells were analyzed 14hr later. The frequency of CD166+ cells contained in BM-homed H929 cells was significantly higher compared to that in total H929 cells prior to injection, suggesting that CD166 plays a critical role in directing homing of MM cells to the BM. When we knocked down (KD) CD166 expression on H929-GFP cells with shRNA, the number of BM-homed GFP cells was significantly decreased for CD166KD cells compared to mock control. We then compared MM progression in NSG mice injected with mock control or CD166 KD H929 cells. Mice receiving control cells showed more rapid disease progression than those receiving CD166KD cells as evidenced by higher serum human IgA (kappa) levels and shortened survival. We next examined the potential role of CD166 in osteolytic lesions using a novel Ex Vivo Organ Culture Assay (EVOCA) in which MM cells were co-cultured over calvarias from 10d-old mice for 7 days. EVOCA data showed that osteolytic lesions were substantially reduced when CD166 was absent on either MM (CD166⁻ fraction) or osteoblast lineage cells (calvarias from CD166^{-/-} mice). Furthermore, co-culturing CD166+ or CD166⁻ H929 cells with bone marrow stromal cells (BMSC) from WT or CD166^{-/-} mice revealed that mRNA levels of RANKL were decreased when CD166 is absent on either MM or stromal cells while those of OPG, an inhibitor of osteoclastogenesis, were not altered. This resulted in decreased RANKL/OPG ratios in cultures containing a CD166⁻ component suggesting reduced MM-induced osteoclastogenesis in the absence of CD166. Together, these results suggest that CD166 plays an important role in homing and retention of MM cells in the BM and promotes MM disease progression as well as bone-lytic disease and that CD166 may serve as a therapeutic target in the treatment of MM.

Basic Science

Graduate Student

POSTER #48

ACTIVATION OF TLR4-MYD88 PATHWAY IMPAIRS HSC FUNCTION DURING ACUTE INFLAMMATION

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Sepsis is 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 late consequences of sepsis, 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 burn patients, we have previously reported that HSC undergo a significant expansion in the BM associated with a block of myeloid differentiation. Furthermore, we found that expanded HSC were unable to generate the downstream progenitors (common myeloid progenitors and granulocytes/monocytes progenitors) necessary to produce neutrophils, and had reduced self-renewal. All these effects were TLR4-dependent. TLR4 is activated by bacterial LPS and signal through two major pathways: TRIF-dependent and MyD88-dependent. In this study, we show the different contribution of the TLR4-TRIF and the TLR4-MyD88-dependent pathways to the BM response to *Pseudomonas aeruginosa* LPS. LPS challenge conducted on TRIF-null and MyD88-null mice demonstrated that TRIF is involved in the expansion of the HSC pool, but does not play a major role in the myelosuppression, whereas MyD88 activation was required for LPS-induced myeloid suppression. Moreover, we observed that the impaired engraftment at long-term observed in wild-type LPS-challenged HSC during transplantation was rescued by MyD88 loss of function. Taken together, our results indicate a distinct role of the TLR4-TRIF and -MyD88 pathways in the regulation of the primitive stem/progenitor pool during sepsis and provide insights for a better understanding of the molecular mechanisms leading to neutropenia.

Basic Science

Graduate Student

POSTER #49

METABOLIC AND MOLECULAR REGULATION OF DIETARY UNSATURATED FATTY ACIDS ON PROSTATE CANCER

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Prostate cancer (PCa) is one of the most common cancers among adult men. Early interference and dietary prevention are crucial in PCa patient care. Primary dietary unsaturated fatty acids (UFAs) have diverse regulatory functions. N-3 FAs, which are major components of fish oil (FO), are believed to be anti-inflammatory and anti-proliferative. The intake of n-3 FA in modern life styles is low and epidemiologic studies show that high n-6/n-3 ratio is associated with higher PCa risk. N-3 FAs have been reported to induce cell death in combination with chemo/radiation therapies by sensitizing cancer cells. Moreover, Oleic acid (OA), an n-9 FA, also known as one of the main components of cooking oil, may also have anti-tumorigenic effects though the conclusions are controversial. Our current study evaluated the regulatory role of FO and OA on PCa cells and sought to discover the global protein level changes with FA treatment. To address the function of FO and OA, cytotoxicity and colony formation assays were carried out and results showed FO and not OA suppress cell viability and growth. Fatty acid synthase (FASN), a known oncoprotein was knocked down in PCa cell line, then expression levels of several enzymes involved in UFA metabolism were measured by quantitative PCR and/or western blot. Cyclooxygenase-2 (COX2), an inflammatory molecule, which plays important role in PCa was found to be downregulated when FASN is depleted. FASN activity assay results showed that both FO and OA inhibit FASN activity, and OA was amore potent FASN inhibitor than FO. This result suggests the cell death inducing effects might be FASN independent. To further study the underlying molecular mechanism of FO action, an LC/MS-based label-free global protein quantification experiment was carried out to measure the differential protein expression in PC3 with different FA treatment through different periods of time. Significantly changed proteins were selected. In day 1 group, sequestosome-1 (SQSTM1), which is required by autophagy, was found to be expressed at a higher level in FO treated group compared to control and OA treated group, suggesting that autophagy may play an important role in FO induced cell death. In day 6 group, proteins with significant expression change were searched in the Protein Atlas and UniProt data base for those related to cancer, prostate, or lipid processing. Candidates will be further validated by biochemical methods. Overall, Our study connects FASN and COX2 directly, and provides useful information to help understand regulatory effects of different dietary FAs on PCa.

Basic Science

Graduate Student

POSTER #50

IDENTIFICATION AND CHARACTERIZATION OF ESTROGEN INDUCED ALTERNATIVE SPLICING IN MCF7 CELLS

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Evidences have shown that pre-mRNA alternative splicing takes place in the development, progression and metastasis of breast cancer, and estrogen is an inducer of alternative splicing for breast cancer cells. However, the extent and biological effects of estrogen-induced alternative splicing remain unclear. Moreover, whether and how estrogen-induced alternative splicing change with time is still unknown. In this study, we implemented an RNA sequencing assay on estrogen treated MCF7 cell line, which captures the MCF7 transcriptomes 0, 1 and 24 hours after estrogen treatment. We identified 650 genes differentially spliced after 1 hour and 610 after 24 hours. Only 150 genes are uniformly spliced on both time points. We interpreted the biological effects of these alternative splicing events from two aspects, 1) the functions of differentially spliced genes and 2) the biochemical features of the peptide encoded by the alternative spliced region, including post translational modification, protein disorder, Pfam domains and protein-protein interaction. We confirmed that estrogen can induce alternative splicing change on a broad range of genes, and the changes are progressive relative to time. These splicing changes may influence protein functions via inclusion/exclusion of protein binding sites and structural units on the alternative regions.

Basic Science

Graduate Student

POSTER #51

COOPERATION OF THE PROTEIN TYROSINE PHOSPHATASE, SHP2, AND PI3K IN FLT-3 ITD+ LEUKEMIA

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Internal tandem duplications in the fms-like tyrosine kinase receptor (FLT3-ITDs) cause constitutive activation of the receptor, hyperactivation of STAT5, and confer a poor prognosis in individuals with acute myeloid leukemia (AML). While constitutive activation of STAT5 is a hallmark of FLT3-ITD-induced leukemia, cytoplasmic signaling molecules such as Shp2 and phospho-inositol-3-kinase (PI3K) have also been found to promote STAT5 activation. Studies have clarified that tyrosine (Y) 768, 955, and 969 within WT FLT3 recruit the adaptor proteins Grb2, Gab2, and the regulatory subunit of PI3K, p85a, and that mutation of these residues from tyrosine (Y) to phenylalanine (F) results in reduced phospho-Akt levels. Since Shp2 has been shown to participate in protein complexes containing Gab2 and p85a in WT-FLT3 cells, *we hypothesized* that Shp2 may interact with FLT3-ITD via protein complexes at Y768, 955, and/or 969 and that Shp2 and PI3K work cooperatively to promote FLT3-ITD-induced leukemogenesis. To examine this hypothesis, tyrosine to phenylalanine (Y to F) mutations were made at the 768, 955, or 969 residues of N51-FLT3, cloned into the retroviral vector pMSCV, and transfected into the murine cell line 32D. Thymidine incorporation revealed a significant decrease in hyperproliferation induced by N51-FLT3 when the 768 residue was mutated to phenylalanine (N51-FLT3-Y768F), and a moderate decrease in proliferation with mutations at 955 or 969 (N51-FLT3-Y955F or Y969F). Consistently, in immunoblot assays, we observed a significant reduction in p-ERK and p-FLT3 levels in the N51-FLT3 Y768F-expressing cells, and moderate reduction in Y955F and Y969F cells compared to N51-FLT3 expressing cells. Levels of p-STAT5 were unchanged or slightly elevated in the Y768, Y955, and Y969 expressing cells compared to N51-FLT3 expressing cells. A possible mechanistic explanation for selective reduction of p-ERK levels is through decreased recruitment of GRB2, which normally participates in the recruitment of SOS, to activate RAS to its GTP form and follow the RAF/MEK/ERK signaling cascade. Therefore, decreased recruitment of GRB2 could result in decreased levels of p-ERK. Furthermore, pharmacologic inhibition of Shp2 with SH-PT66 or PI3K with GDC-0941 in 32D-N51-FLT3 cells showed decreased proliferation, with an additive effect when used in combination. These data suggest that the Y768 residue plays an important role in p-ERK signaling in N51-FLT3 cells, and that dual pharmacologic therapy with Shp2 and PI3K inhibitors may provide a novel treatment approach for FLT3-ITD positive AML. For future directions, we plan to perform a syngeneic transplant using C3H/HeJ mice, injecting them with either WT-FLT3, N51-FLT3, N51-Y768F, N51-Y955F, or N51-Y969F cells to determine the effect on overall survival. Also, we plan to perform a similar transplant involving WT-FLT3 or N51-FLT3 cells, followed by treatment with Shp2 inhibitor, PI3K inhibitor, or a combination to determine the effect on overall survival.

Basic Science Medical Student

POSTER #52

THE TEMPORAL SEQUENCE OF TELOMERE DYNAMICS IN SPORADIC COLON CANCER

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Defects in telomere maintenance have emerged as having a causative role in carcinogenesis by promoting genomic instability; however, there is little evidence to support this paradigm in human carcinogenesis. In this study, we identified temporal sequence of telomere dynamics by analyzing telomere length, telomerase activity, telomere fusion, and hotspot mutations in oncogenes (*KRAS* or *BRAF*) and a tumor suppressor gene (*TP53*) in tissue samples obtained from 18 colon cancer patients. We show that the extent of mean telomere attrition increases with lymph node invasiveness of tumors, implying that mean telomere shortening correlates with colon cancer progression regardless of telomerase activation. Telomerase activity is relatively higher in most cancer tissues with mutation(s) in *KRAS* or *BRAF* and/or *TP53* compared to those without hotspot mutations, suggesting that telomerase could fully activate at the late stage of colon cancer development. Importantly, our results reveal that both the deficiency of p53 and the shortening of mean telomere length are unnecessary for producing telomere fusions in colon tissue. In addition, BRAF-V600E mutation is rarely associated with telomere fusion in colon cancer, agreeing with the current evidence that the BRAF-V600E mutation is not involved in genomic instability but in high microsatellite instability and/or aberrant DNA CpG island hypermethylation. In some cases, telomere fusion and/or aneuploid DNA was observed even in tissue adjacent to cancerous lesion, suggesting that genomic instability is initiated in pathologically non-cancerous lesions. Interestingly, the majority of telomere fusion junctions in colon cancer appear to be a chromatid-type containing chromosome 7q or 12q. Taken together, this careful correlative study not only supports the concept that telomere fusion presents in the early stages of cancer prior to *TP53/KRAS* mutations, critical mean telomere shortening, and telomerase activation, but also provides additional insights that targeting key telomere fusion junctions may have significant implications for colon cancer diagnostics.

Basic Science Medical Student

POSTER #53

ANOIKIS RESISTANCE IS A CRITICAL FEATURE OF HIGHLY AGGRESSIVE OVARIAN CANCER CELLS

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High-grade serous ovarian cancer (HGS-OvCA) is an aggressive form of epithelial ovarian cancer (EOC), and accounts for the majority of deaths due to EOC. The critical cellular processes and underlying molecular mechanisms that define this malignancy remain poorly understood. Using a syngeneic murine model, we investigated the changes that accompanied the progression to increased aggressiveness induced by *in vivo* passage of mouse EOC cells. We found that enhanced anoikis resistance was a key cellular process associated with greater aggressiveness and tumorigenicity *in vivo*. Biochemical studies revealed that the enhanced anoikis resistance was associated with the activation of the Src/Akt/Erk signaling pathway. A higher rate of metabolism and autophagy were also associated with increased anoikis resistance. Blocking these pathways with specific inhibitors and/or genetic modifications significantly increased anoikis *in vitro* and inhibited tumor development *in vivo*. In addition, we demonstrated that similar signaling pathways were also involved in a human EOC cell line model. Collectively, our data suggest that anoikis resistance represents a critical and a distinguishing feature underlying the aggressiveness of ovarian cancer cells.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #54

DYSREGULATED MIR-205 SIGNALING PROMOTES SYMMETRIC DIVISION OF SELF-RENEWING MAMMARY STEM CELLS AND MAMMARY TUMORIGENESIS

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Epigenetic control plays a key role in regulation of tumorigenesis in response to microenvironmental stimuli; however, the regulatory mechanism involved in the process is largely unclear. We now report that a critical epigenetic regulator miR-205 is repressed by Jagged1, a ligand shown to be secreted from the tumor stroma to promote the cancer stem cell phenotype. Loss of miR-205 in mammary epithelial cells leads to epithelial-mesenchymal transition, disrupted epithelial cell polarity, and enhanced symmetric division to expand the stem cell population. Furthermore, mice deficient in miR-205 spontaneously develop mammary lesions, and activation of miR-205 significantly diminishes the breast cancer stemness. These data provide the first evidence linking the microenvironment and microRNA regulation to the disrupted epithelial polarity and aberrant stem cell division, leading to an expansion of the stem cell population and tumorigenesis. Together, this study elucidates a new role for miR-205 in regulation of stem cell fate, providing a promising therapeutic target for eradicating the genesis of breast cancer.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #55

IDENTIFYING NOVEL THERAPEUTIC TARGETS IN ONCOGENIC FLT3 INDUCED ACUTE MYELOID LEUKEMIA

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Amongst various hematological malignancies, treatment strategies for oncogenic Flt3 (Flt3ITD) induced acute myeloid leukemia (AML) remain inefficient due to patients relapsing with acquired resistant mutations on Flt3ITD. Thus targeting novel signaling molecules in Flt3ITD pathways remain a viable therapeutic option. Amongst various pathways downstream of Flt3ITD, the role of Stat5 has been extensively studied. However, the mechanism behind the translocation of active Stat5 into the nucleus of cells bearing Flt3ITD mutation to induce transformation remains unclear. Here we provide in vitro and in vivo pharmacological, biochemical and genetic evidence to demonstrate that a FAK/Tiam1-Rac1/PAK1 signaling axis plays an essential role in Flt3ITD induced AML. We observed hyperactivation of FAK in Flt3ITD expressing leukemic cells and human AML patient samples, and also activation of Rac1 downstream of FAK that was downregulated upon treatment with FAK inhibitors. We next ascertained the underlying mechanism of FAK mediated activation of Rac1 in Flt3ITD expressing cells and observed RacGEF Tiam1 to be hyperactive in Flt3ITD bearing cells, which was downregulated upon pharmacological inhibition of FAK. More importantly, expression of Flt3ITD in *Rac1*^{-/-} or *FAK*^{-/-} deficient bone marrow cells, showed inhibition of Stat5 activation and its failure to translocate into the nucleus. Similar results were also observed upon shRNA mediated knockdown of Tiam1. To determine a downstream effector from Rac1, we observed an essential role of p21-activated kinase 1 (PAK1), where shRNA mediated knockdown of PAK1 not only inhibited the nuclear translocation of Stat5, it also significantly delayed the onset of AML in vivo, while inhibition of PAK2, showed no such effect. Finally, inhibition of FAK, Tiam1 and PAK1 significantly delayed the onset of AML in the in vivo mouse models along with repression of Stat5 responsive genes involved in survival of leukemic cells. Overall our study indicate an essential role of FAK/Tiam1-Rac1/PAK1 signaling axis in Flt3ITD mediated proliferation, survival and leukemogenesis; and also demonstrates a novel mechanistic role of FAK, Tiam1 and PAK1 in translocating active Stat5 into the nucleus to induce leukemogenic transformation.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #56

REDOX FACTOR 1 (REF-1) SIGNALING IN THE INTERACTION BETWEEN PANCREATIC TUMOR CELLS AND CANCER-ASSOCIATED FIBROBLASTS

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Pancreatic cancer (PDAC) is a deadly disease and is accompanied by a fibrotic phenotype that contributes to the resistance of the disease. Signaling between cancer-associated fibroblasts (CAFs) and tumor are important in the fibrotic phenotype and metastatic spread. Thus, there is a *critical need* to understand the signaling within the cells of the tumor microenvironment (TME) and how it contributes to progression of the disease and resistance to treatment. A novel PDAC microenvironment target, AP endonuclease/Redox factor-1 (Ref-1), is a reduction-oxidation (redox) factor involved in the transcriptional regulation of gene expression. Regulation of transcription factors, HIF-1a, NF-kB, and STAT3, by Ref-1 and are activated in stromal cells as well as pancreatic tumors. The central hypothesis is that Ref-1 redox activity plays a critical role in the signaling between the tumor microenvironment and tumor. The *objective* of this work is to determine the outcome of inhibiting Ref-1 in CAFs and the effects of that inhibition on proliferation and migration in PDAC. We are using several innovative methods to probe the tumor-CAF interaction including: 1) co-culture 2D and 3D models; 2) genetic approach via siRNA to Ref-1; 3) pharmacologic approach via a well-established small molecule inhibitor of Ref-1 redox activity, E3330; and 4) *in vivo* mouse experiments with tumor-CAF co-injection. Our findings indicate that inhibition of Ref-1 is more effective in CAFs than tumor cells with nominal effect on normal fibroblasts. Furthermore, co-cultures of patient derived cells with normal fibroblasts do not show sensitivity to Ref-1 inhibition, in contrast to tumor-CAF co-cultures. Utilizing siRNA to reduce the levels of Ref-1 protein in the CAFs results in a decrease in the size and proliferation of 3D colonies that contain tumor cells plus CAFs that express ~80% reduced levels of Ref-1 protein. These data implicate the redox activity of Ref-1 and its regulation of critical transcription factors as significant in the signaling between the tumor and the CAFs. We are also investigating the effects of targeting STAT3 in the co-cultures based on our published data demonstrating that Ref-1 can activate STAT3 DNA binding and that dual targeting of Ref-1 and STAT3 is synthetic lethal to PDAC cells. Similar to Ref-1 inhibition, targeting of STAT3 is significant in the CAFs compared to normal fibroblasts. However, Gemcitabine treatment does not preferentially kill PDAC cells in co-culture with CAFs in contrast to what we observe with Ref-1 or STAT3 targeting. Due to PDAC's fibrotic nature, targeting the interaction of tumor-stroma through Ref-1 inhibition is a promising avenue for combination treatment. The importance of not only targeting the tumor is clear, therefore, novel approaches that target the TME in addition to signaling pathways within the tumor may offer the most promise against this dreaded disease.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #57

HONOKIOL INHIBITS METASTASIS OF RENAL CELL CARCINOMA BY OVEREXPRESSION OF KISS1 AND KISS1R

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Renal cell carcinoma (RCC) is a common urological cancer worldwide and is known for high risk of recurrence and metastasis. Approximately 70% of patients with RCC will develop recurrence after surgical resection, and 25%-30% of patients will eventually progress to distant metastasis. Honokiol is a small-molecule polyphenol isolated from the genus *Magnolia*, which has been shown to be a potential anticancer agent in multiple facets of signal transduction. We have demonstrated that honokiol inhibited proliferation of RCC cells 786-0 and A498 without affecting cell viability. Here, we found that honokiol also significantly suppressed metastasis (cell migration, invasion and colony formation) of 786-0 cells in a dose-dependent manner. Honokiol regulated expression of many genes related to human tumor metastasis in 786-0 cells based on DNA microarray analysis. Real time PCR analysis confirmed that metastasis suppressor gene *KISS1* and its receptor gene, *KISS1R*, were upregulated by honokiol. In addition, honokiol-induced protein expression of KISS1 and KISS1R in 786-0 cells. Interestingly, the shape changes and excessive formation of actin stress fibers were identified in cells treated with honokiol. This phenomenon disappeared when treated cells with the pharmacological Rho-kinase inhibitor Y-27632 and honokiol. This inhibition can also be identified in 786-0 cells treated with Y-27632 only. Our present results demonstrate that honokiol suppresses metastasis of RCC by inducing expression of KISS1 and KISS1R, which might be associated with activation of Rho and Rho-Associated Kinase (ROCK) pathway. In conclusion, honokiol is a biologically active natural compound which can be considered as an alternative treatment of RCC. The investigation of detailed mechanisms and molecular targets are in progress.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #58

SYNTHESIS AND EVALUATION OF SMALL MOLECULE INHIBITORS OF REPLICATION PROTEIN A

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Cisplatin and carboplatin impart their chemotherapeutic effect by forming Pt-DNA adducts that block DNA replication and transcription, culminating in apoptosis. Repair of those Pt-DNA adducts via nucleotide excision repair (NER) or homologous recombination repair (HRR) can substantially reduce the effectiveness of the Pt therapy, contributing to cellular resistance. Hence, inhibition of these repair pathways holds the potential to sensitize resistant cancer cells to Pt treatment. Unlike most therapies, which are focused on enzyme-substrate interactions, our approach addresses protein-DNA disruption, and it is based on the hypothesis that targeting the NER pathway in HRR deficient cancers in combination with cisplatin therapy will provide increased efficacy with minimal toxicity. Replication protein A (RPA), a single-stranded DNA binding protein that plays a fundamental role in the NER pathway, is the subject of the research herein presented. After having identified a small molecule inhibitor (SMI) of RPA with promising *in vitro* and cellular activity, we synthesized analogs of the lead compound and evaluated their ability to be used in combination therapy. Structure-activity relationship (SAR) studies led us to the selection of an optimized lead, which showed single agent activity in A2780 epithelial ovarian cancer cells and in a xenograft lung cancer mouse model. These data demonstrate the utility of RPA inhibition *in vivo* and the potential in the development of a novel class of anticancer therapeutics that target protein-DNA interactions.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #59

CD1D-MEDIATED ANTIGEN PRESENTATION IN MALIGNANT GLIOMA

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Natural Killer T (NKT) cells are a special subset of T cells that recognize lipid antigens presented by the major histocompatibility complex (MHC) class I-like, cell surface protein CD1d. Upon stimulation by antigen-bound CD1d, NKT cells rapidly secrete cytokines. Thus, CD1d-mediated antigen presentation to NKT cells serves as a bridge between the innate and adaptive immune responses; therefore, it is an important component of the immune system. Several reports suggest that one of the mechanisms for immune evasion by tumors is deregulation of the CD1d-NKT cell axis. Malignant gliomas are a prevalent form of brain cancer that often has a poor prognosis. To better understand immune evasion in malignant gliomas, we studied CD1d-mediated antigen presentation to NKT cells by these tumors. First, immunohistochemical staining of glioblastoma tumors showed infiltrating lymphocytes that include NKT cells. The tumors had CD1d expression, but it was lower than that seen in normal brain tissue. Next, human glioblastoma cell lines as well as tumor cells from patients with malignant glioma were analyzed for CD1d expression and function. Almost all cells analyzed were able to stimulate cytokine production from human NKT cells in a CD1d-dependent manner. However, several of these cell lines required the addition of exogenous lipid antigen. This may be due to the low surface expression of CD1d in many of these cells. An alternative explanation is the presence of endogenous lipids that bind CD1d but fail to activate NKT cells. It has been previously shown that tumor cells can shed lipids as a mechanism to inhibit recognition by NKT cells. We used both pharmacological and shRNA-mediated inhibition of lipid synthesis. We found that inhibition of the biosynthesis of these lipids resulted in increased activation of NKT cells. Our results suggest that malignant gliomas decrease CD1d-mediated antigen presentation as a method of evading immune recognition. Therefore, restoring the CD1d-NKT cell axis may be an approach that can be utilized to develop novel therapeutic strategies for treating these tumors.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #60

LAMP-2C INHIBITION OF TUMOR PROGRESSION

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Autophagy plays a role in many specific physiological and pathological processes such as cell development, immunity, energy homeostasis, cell death, differentiation, and stress responses. Autophagy pathways appear to be tumor suppressive during cancer development but also play a role in tumor survival during cancer progression. Studies have suggested that a selective form of autophagy, known as chaperone-mediated autophagy (CMA), is up-regulated in many solid tumors and promotes tumorigenesis. CMA relies on the lysosome-associated membrane protein (LAMP)-2A to translocate proteins from the cytoplasm into lysosomes. Alternative splicing of the LAMP-2 gene generates 3 highly conserved isoforms LAMP-2A, LAMP-2B, and LAMP-2C. Ectopic expression of LAMP-2C in a human melanoma cell line, DM331, reduced in vitro cell proliferation. Up-regulation of p53, a tumor suppressor protein, and p21, a cell cycle regulator, were observed in addition to a decreased in LAMP-2A protein levels. Furthermore, LAMP-2C overexpression delayed xenograft tumor growth in mice. Thus, LAMP-2C appears to be a negative regulator of CMA and a tumor growth inhibitor. Studies are underway to characterize the role of LAMP-2C as a tumor suppressor. This work was supported by NIH 5T32AI060519, AI079065 and IUCC pilot grant.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #61

THE ROLE OF APC IN CHEMOTHERAPEUTIC RESPONSIVENESS OF BREAST CANCER

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Breast cancer is the leading cause of cancer-related death in non-smoking women. Despite tremendous amounts of information about the etiology of breast cancer, many questions remain unanswered. It has been demonstrated that specific subtypes of breast cancer respond differently to chemotherapeutic and targeted therapies. The oncogenic events and signaling pathways driving these tumor subtypes are distinct, indicating that increased knowledge of their molecular basis will provide opportunities for novel therapeutic approaches and knowledge of therapeutic responsiveness, to ultimately improve patient outcomes. The Adenomatous Polyposis Coli (APC) tumor suppressor is mutated or hypermethylated in 18-70% of sporadic breast cancers depending on subtype; however, the effect of APC mutation on tumorigenic properties remains unexplored. APC binds both directly and indirectly to microtubules and regulates multiple components of the DNA repair pathway, suggesting it may play a critical role in therapeutic responsiveness. Using the *Apc*^{Min/+} mouse model, we previously identified pre-neoplastic lesions in the breast, alterations in genes critical in therapeutic resistance, and enhanced breast tumorigenesis in the presence of the Polyoma middle T antigen (PyMT) oncogene independent of Wnt/ β -catenin signaling. *Apc* mutation changed the tumor histopathology from solid to squamous adenocarcinomas, resembling the highly aggressive human metaplastic breast cancer. Cell lines derived from PyMT-mediated tumors demonstrated alterations in expression of two ATP-binding cassette transporters, multidrug resistance protein 1 (MDR1) and ATP-binding cassette sub-family G member 2/Breast Cancer Resistance Protein (ABCG2/BCRP), both of which are critical in predicting responsiveness to therapeutic agents. In addition, cells from MMTV-PyMT;*Apc*^{Min/+} mice are more sensitive to paclitaxel compared to their wild-type counterparts. To translate our findings to a human breast cancer cell line, we utilized DU4475 cells, which harbor an APC mutation in the β -catenin binding region. We have generated stable DU4475 cell lines expressing the middle region or C-terminal domain of APC, and showed that these cell lines have increased sensitivity to paclitaxel, cisplatin, and doxorubicin. A third model system, the MDA-MB-157 human metaplastic breast cancer cells, have wild-type APC. Knockdown of APC in the MDA-MB-157 cells results in alterations of the apoptotic response to cisplatin, paclitaxel and doxorubicin. Combined, these data suggest that APC modulates therapeutic resistance in breast cancer cells in a cell- and drug-dependent manner. Future studies will involve the continued studies in the DU4475 and MDA-MB-157 cohorts of cells, and using targeted therapies (based on our molecular characterization studies) in combination with standard chemotherapeutic agents.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #62

NOTCH-DEPENDENT REPRESSION OF MIR-155 IN THE BONE MARROW NICHE REGULATES HEMATOPOIESIS IN A NF-KB DEPENDENT MANNER

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Evidence supporting a non-cell-autonomous role for Notch signaling in the regulation of hematopoiesis has recently emerged; however, the cellular and molecular mechanism(s) by which Notch regulates the integrity of the BM niche are still poorly understood. By using a Notch/RBPJ loss-of-function model we demonstrated that RBPJ functions as a transcriptional repressor of the microRNA *miR-155*, a microRNA involved in inflammation and frequently up-regulated in leukemia cells and solid tumors. Loss of Notch signaling in the bone marrow (BM) niche altered hematopoietic homeostasis and led to a lethal myeloproliferative disease. Loss of Notch/RBPJ-signaling upregulated *miR-155* in BM endothelial and mesenchymal cells, resulting in *miR-155*-dependent inhibition of the NF- κ B inhibitor B-Ras1, followed by NF- κ B activation and increased production of pro-inflammatory cytokines, in particular G-CSF and TNF α . Importantly, deletion of *miR-155* in the stroma of RBPJ^{-/-} mice prevented development of myeloproliferative disease and induction of pro-inflammatory cytokines. Analysis of patient's samples affected by myeloproliferative neoplasia showed elevated levels of *miR155* in the BM. Collectively, these data suggest that Notch/*miR155*/NF- κ B axis may regulate the inflammatory state of the BM niche and may be involved in the development of myeloproliferative disorders.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #63

PRMT5 DIMETHYLATES R30 OF THE P65 SUBUNIT TO ACTIVATE NF- κ B

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The nuclear factor κ B (NF- κ B) plays central roles in immune and inflammatory responses and in tumorigenesis. Complex posttranslational modifications of the p65 subunit (RelA) are a major aspect of the extremely flexible regulation of NF- κ B activity. Although phosphorylation, acetylation, ubiquitination, and lysine methylation of NF- κ B have been well described, arginine methylation has not yet been found. We now report that, in response to interleukin 1(IL-1 α) , the p65 subunit of NF- κ B is dimethylated on arginine 30 (R30) by protein-arginine methyltransferase 5 (PRMT5). Expression of the R30A and R30K mutants of p65 substantially decreased the ability of NF- κ B to bind to κ B elements and to drive gene expression. PRMT5 was the only arginine methyltransferase that co-precipitated with p65, and its overexpression increased NF- κ B activity, whereas PRMT5 knockdown had the opposite effect. Microarray analysis revealed that ~ 85% of the NF- κ B-inducible genes that are down regulated by the R30A mutation are similarly down regulated by knocking PRMT5 down. Many cytokine and chemokine genes are among these, and conditioned media from cells expressing the R30A mutant of p65 had much less NF- κ B-inducing activity than media from cells expressing the wild-type protein. Furthermore, overexpression of PRMT5 promoted colon cancer HT29 cell growth and soft agar colony formation, whereas knockdown of PRMT5 led to the opposite effect. PRMT5 is overexpressed in many types of cancer, often to a striking degree, indicating that high levels of this enzyme may promote tumorigenesis, at least in part by facilitating NF- κ B-induced gene expression.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #64

HYPOXIC CONDITIONS DIFFERENTIALLY REGULATE TAZ AND YAP IN CANCER CELLS

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The Hippo-YAP "Signaling" pathway is altered and implicated as an oncogenic signaling pathway in many human cancers. Hypoxia is an important micro-environmental factor that promotes tumorigenesis. However, the effect of hypoxia on the two most important Hippo-YAP effectors YAP and TAZ have not been reported. In this work, we demonstrate that TAZ was functionally involved in cell proliferation in EOC cells. Hypoxic conditions (1% O₂ or hypoxia mimics) strongly up-regulated levels of S69 phosphorylated and total TAZ in EOC and other cancer type cells, including breast, prostate and colon cancers. In contrast, these conditions induced a modest reduction of YAP phosphorylation and total YAP expression. These data suggest that hypoxic conditions differentially regulated these two closely related Hippo pathway effectors. Up-regulation of TAZ was mainly at the transcriptional level; The canonical YAP/TAZ kinase LATS was not activated under the same conditions. In addition, blockage of Akt, another potential YAP-TAZ kinase did not have any effect on DMOG-induced TAZ up-regulation. Together, our data revealed new regulating mechanisms of TAZ and YAP in cancer cells and suggest that although hypoxia is tumor-promoting in general, it may play negative regulatory roles under certain conditions. This is important since it indicates cautions need to be taken when therapeutic targeting hypoxia.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #65

EFFECT OF MT1-MMP EXPRESSION ON MULTI-CELLULAR AGGREGATES DYNAMICS IN EPITHELIAL OVARIAN CANCER

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The National Cancer Institute reports that there are 22,240 new ovarian cancer cases diagnosed in 2013, resulting in 14,030 American women deaths. Epithelial ovarian cancer (EOC) ranks 5th overall for cancer-related death among women. A woman's lifetime risk of developing invasive ovarian cancer is 1 in 72. Currently, 61% of woman newly diagnosed with ovarian cancer already have distant metastasis, and five year survival rates are only 27%. These statistics highlight urgent needs for both developing early detection strategies and thorough understanding of molecular events that promote metastasis. 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 10⁴ increase in tumorigenicity relative to the same number of parental single cells. The factors that regulate the terminal transition from free-floating MCA to peritoneally anchored metastatic lesion are currently unknown. Matrix metalloproteinases (MMPs) are zinc-dependent proteases which are capable of degrading extracellular matrix proteins. Membrane type 1 MMP (MT1-MMP, MMP-14) is a transmembrane collagenase abundantly expressed in ovarian tumors and correlates with poor survival. Our recent studies demonstrated that acquisition of MT1-MMP expression promotes cellular detachment and MCAs formation. To further assess the effect of MT1-MMP expression on MCAs dynamics in EOC, we generated constructs expressing wild-type MT1-MMP, two cytoplasmic tail mutants: T567E which mimics cytoplasmic tail Thr phosphorylation or T567A which functions as a phosphodeficient mutant, and the catalytically inactive E240A active site mutant. In this study, we evaluated MCAs morphology and dynamics using the cell lines above as well as DOV13 cells that endogenously express high levels of MT1-MMP. MCAs produced using the hanging drop method were evaluated using light and scanning electron microscopy to assess aggregates area and overall morphology. The kinetics of MCAs dispersal on collagen surfaces was also compared between wild type and mutant cell lines. Our results suggest a role for MT1-MMP in regulation of EOC MCAs dynamics.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #66

ACTIVATION OF MTOR PATHWAY IN MYELOID-DERIVED SUPPRESSOR CELLS WITH LYSOSOMAL ACID LIPASE DEFICIENCY STIMULATES CANCER CELL PROLIFERATION AND METASTASIS

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Inflammation plays crucial roles at all stages of tumor development, from tumor initiation to metastatic progression, during which myeloid-derived suppressor cells (MDSCs) are an important participant. Although MDSCs are known to suppress immune surveillance, their roles in directly stimulating cancer cell proliferation and metastasis currently remains unclear. Lysosomal acid lipase (LAL) deficiency causes systemic expansion and infiltration of MDSCs in multiple organs and subsequent inflammation. In the LAL-deficient (*lal*^{-/-}) mouse model, we found that melanoma metastasized massively in allogeneic *lal*^{-/-} mice, which was suppressed in allogeneic *lal*^{+/+} mice due to immune rejection. Therefore, we hypothesized that MDSCs with LAL deficiency directly stimulate cancer cell proliferation and metastasis. Bone marrow-derived MDSCs from *lal*^{-/-} mice directly stimulated B16 melanoma cell proliferation *in vitro* by co-culture analysis and *in vivo* by co-implantation in the Matrigel plugs. These tumor cell-stimulatory effects were diminished when myeloid-specific human LAL (hLAL) was expressed in myeloid cells in *lal*^{-/-} mice. In addition, *lal*^{-/-} MDSCs facilitated B16 melanoma cell metastasis in the lungs of recipient *lal*^{+/+} mice via tail vein injection. Furthermore, the mammalian target of rapamycin (mTOR) and its downstream gene products were significantly up-regulated in *lal*^{-/-} MDSCs. Knockdown of mTOR, Raptor or Rictor in *lal*^{-/-} MDSCs suppressed their stimulation on B16 melanoma cell proliferation, growth and metastasis, indicating the tumor-promoting function of *lal*^{-/-} MDSCs is mediated, at least in part, through over-activation of the mTOR pathway. Finally, *lal*^{-/-} MDSCs stimulated proliferation and growth of Lewis lung carcinoma (LLC), and transgenic mouse prostate cancer (TRAMP-C2) cells, and these effects were impaired after mTOR inhibition. Our results indicate that LAL plays a critical role in regulating MDSCs ability to directly stimulate cancer cell proliferation, and overcome immune rejection of cancer metastasis in allogeneic mice through modulation of the mTOR pathway, which provides a mechanistic basis for targeting MDSCs to reduce the risk of cancer metastasis. Therefore, MDSCs possess dual functions to facilitate cancer metastasis: suppress immune surveillance, and stimulate cancer cell proliferation and growth.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #67

TRANSCRIPTIONAL ACTIVATION OF PRMT5 BY NF-Y IS REQUIRED FOR CELL GROWTH AND NEGATIVELY REGULATED BY THE PKC/C-FOS SIGNALING IN PROSTATE CANCER CELLS

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Protein arginine methyltransferase 5 (PRMT5) symmetrically methylates arginine residues of histones and non-histone protein substrates and regulates a variety of cellular processes through epigenetic control of target gene expression or post-translational modification of signaling molecules. Recent evidence suggests that PRMT5 may function as an oncogene and its overexpression contributes to the development and progression of several human cancers. However, the mechanism underlying the regulation of PRMT5 expression in cancer cells remains largely unknown. In the present study, we have mapped the core promoter of PRMT5 to the -240 bp region and identified nuclear transcription factor (NF-Y) as a critical transcription factor to regulate PRMT5 expression in multiple cancer cell lines. Further, we present evidence that PRMT5 is responsible for cell growth inhibition induced by knockdown of NF-YA, a DNA binding domain-containing subunit that forms a heterotrimeric complex with NF-YB and NF-YC. Significantly, we have found that activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) in LNCaP prostate cancer cells induces c-Fos expression and down-regulates the expression of NF-YA and PRMT5 at transcription level. Given that down-regulation of several PKC isozymes is implicated in the development and progression of several human cancers, our findings suggest that the PKC-c-Fos-NF-Y signaling pathway may be responsible for PRMT5 overexpression in a subset of human cancer patients.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #68

SMALL-MOLECULE INHIBITORS OF MELK FOR NEW THERAPEUTICS IN CANCER

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Maternal embryonic leucine zipper kinase (MELK), an AMP-activated Ser/Thr protein kinase, is up-regulated in a number of tumors including triple-negative breast cancer (TNBC) and glioblastoma multiforme (GBM). MELK has been associated with diverse processes that include cell proliferation, spliceosome assembly, hematopoiesis, stem cell self-renewal, and apoptosis. The levels of MELK are strongly associated with poor prognosis in breast and brain cancer patients. Structure-based computational screening of a compound library led to the discovery of MELK small-molecule inhibitor KIN1. The compound was shown to inhibit MELK enzyme activity with an IC₅₀ of 1 μ M in recombinant protein and in cell culture. Kinome-wide profiling confirmed that MELK is among the top targets of KIN1 and the only serine/threonine kinase. The compound was found to inhibit MDA-MB-231 TNBC and U87 GBM cancer cell proliferation in anchorage-dependent and independent assays. Flow cytometry and cell cycle analysis revealed that the compound caused cell cycle arrest. This is consistent with compound up-regulation of p21, and down-regulation of survivin. Interestingly, the compound inhibited STAT3 phosphorylation and binding to DNA. Our small molecules offer new tools to probe MELK function *in vivo*. The compounds also provide leads for the development of cancer therapeutics for the treatment of TNBC and GBM.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #69

BETA-CATENIN REGULATED ALDH1 IS A TARGET IN OVARIAN CANCER STEM CELLS

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"Spheroids are 3D multi-cell aggregates formed in non-adherent culture conditions." In ovarian cancer (OC), they serve as a vehicle for cancer cell dissemination in the peritoneal cavity and protect cells from extracellular stress-induced anoikis. "We hypothesized that spheroids are enriched in cancer stem cells and investigated pathways activated under these conditions." Affymetrix-based gene expression profiling of OC cells grown as spheroids vs. monolayers identified b-catenin pathway being upregulated in 3D cultures. "Increased expression of b-catenin and target genes (cyclin D1, c-myc) was demonstrated in spheroids vs. monolayers and in successive spheroid generations using OC cell lines and primary cultures." B-catenin function measured as TCF/LEF1 reporter activity was augmented in spheroids vs. monolayers and siRNA mediated b-catenin knock down decreased the number of spheroids ($p < 0.001$). Along with b-catenin, the expression level of ALDH1, an OC stem cell marker, was increased in successive spheroid generations. "The percentage of ALDH1+ cells was significantly higher in spheroids vs. monolayers in IGROV1, A2780, SKOV3, and primary OC cells. b-catenin knock-down decreased ALDH1 expression, suggesting that it is a b-catenin target. The percentage of ALDH1+ cells and the number of spheroids formed were increased in the cell population dissociated from OC xenografts treated with carboplatin compared to control (44% vs. 2.3%), supporting that ALDH1 is a stem cell marker." "A37 is a novel ALDH1a1 enzymatic inhibitor (K_i of 5nM)." A37 decreased cell viability and spheroid formation by IGROV1 cells ($p < 0.001$). A37 also decreased the percentage of ALDH1+ cells under 3D culture conditions. These data support the role of b-catenin regulated ALDH1 in the maintenance of OC spheroids and of a stem cell phenotype and propose new ALDH1a1 inhibitors targeting this cell population. (Funding: US Department of Veterans Affairs and Ovarian Cancer Research Fund)

Basic Science

Post-Doctoral/Medical Fellow

POSTER #70

PHASE I/II TRIAL OF PEGYLATED LIPOSOMAL DOXORUBICIN IN COMBINATION WITH BIBF 1120 (NINTEDANIB) IN PLATINUM-RESISTANT OVARIAN CANCER: HOOSIER ONCOLOGY GROUP GYN10-149

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Background: The triple PDGFRa/b, VEGFR1-3, FGFR1-3 angiokine inhibitor BIBF 1120 (B) is active in ovarian cancer (OC). This phase I/II trial evaluates tolerability and efficacy of B combined with pegylated liposomal doxorubicin Doxil (D) in platinum-resistant OC. **Methods:** The primary endpoints (EPs) are to determine the maximum-tolerated dose (MTD, phase I) and response rate (RR, phase II) to D+B. Secondary EPs were PFS, toxicities and rate of clinical benefit. Translational EPs were treatment effects on circulating hematopoietic stem and progenitor cells (CHSPCs), containing 2 phenotypically distinct populations; pro-angiogenic (p) CHSPCs (ViViD-CD14-glyA-CD34+AC133+CD45dimCD31+ cells) and non-angiogenic (n) CHSPCs (ViViD-CD14-glyA-CD34+AC133-CD45dimCD31+ cells). Eligible pts had measurable OC, primary peritoneal (PP), fallopian tube and uterine (phase I only) cancer, up to 3 prior regimens, ECOG PS of 0-1 and normal end-organ function. B was given orally BID and D was given IV at 40mg/m² every 28 days. 3+3 dose escalation was used, starting with B at 150mg BID. **Results:** Eleven pts were enrolled in phase I. Median age was 59 (range 26-82); 8 pts had OC, 2 uterine and 1 PP cancer. Histologic types were serous (73%), endometrioid (18%) and mucinous (9%) carcinoma. Dose level+1 (B at 150mg BID) was not tolerated due to grade 3 fatigue, and grade 2 diarrhea, causing treatment interruption (DLT). Among 6 pts at dose level-1 (B at 100mg BID), 1 pt with history of chemotherapy induced myelosuppression had grade 4 neutropenia (DLT). Other toxicities were diarrhea (36.4%), fatigue (36.4%), vomiting (27.3%), headache (27.3%), allergic reaction (9.1%) and oral pain (9.1%). Three pts had PRs, 3 SD and 4 disease progression; 1 was not evaluable. A decrease in the pCHSPC/nCHSPC ratio was observed after 1 cycle (1.58, n=8) compared to baseline (1.68, n=10, p=0.4). At treatment discontinuation, the pCHSPC/nCHSPC ratio was 1.97 (n=4). Analyses are ongoing. **Conclusions:** In summary, D+B is tolerated at 40mg/m² and 100mg BID. An expanded cohort using generic liposomal doxorubicin and B at level -1 is planned before initiation of the phase II cohort.

Basic Science

Post-Doctoral/Medical Fellow

POSTER #71

SUB-COMPLEXES OF DNA REPAIR PROTEINS ASSESSED BY PROXIMITY LIGATION ASSAY

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The repair of damage to chromosomal DNA is orchestrated by a complex array of proteins and their interactions. The nucleotide excision repair (NER) pathway requires over 30 proteins to catalyze the removal of bulky DNA damage including UV-photoproducts and intrastrand DNA lesions induced by platinum-based cancer chemotherapeutics. The non-homologous end joining (NHEJ) pathway can repair a simple DNA double strand break with as few as four proteins. Many of these interactions have been identified and characterized using immuno-precipitation, yeast two-hybrid assays, co-purification and co-localization using immunofluorescence. To interrogate a number of these interactions, we have employed a proximity ligation assay (PLA) which provides excellent resolution and quantification of specific protein-protein associations in individual cells. The XPA-RPA interaction has been extensively characterized and occurs independent of exogenous DNA damage. PLA analysis confirmed this interaction using multiple antibodies to detect the interaction and in numerous human cancer cell lines. Interestingly, the analysis of a number of other NER proteins interactions was detected independent of exogenous DNA damage including XPG with both RPA and XPA and ERCC1-XPA. These data suggest that sub-complexes of NER proteins exist in cells independent of exogenous DNA damage and that assembly of an active NER complex involves pre-formed sub-complexes. We selected the Ku70-DNA-PKcs interactions to monitor the NHEJ pathway interactions. Again, a robust signal was detected independent of exogenously induced DNA DSBs. While the interaction of Ku70 with DNA-PKcs is thought to require a free DNA terminus, we cannot rule out the possibility that the interactions detected are occurring at telomeres, where both proteins have been shown to be localized. Surprisingly, we can also detect interactions with Ku and some of the NER proteins suggesting the possibility of inter-pathway interactions. Overall these results suggest that sub-complexes of DNA repair proteins exist within cells independent of DNA damage and may serve as a reservoir of complexes to be deployed to the sites of damage.

Basic Science Research Technician

POSTER #72

SMALL MOLECULES THAT SELECTIVELY INHIBIT GROWTH OF MYCNARBI+/+ RETINOBLASTOMA CELLS

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Retinoblastoma is the most common pediatric ocular cancer, traditionally thought to be caused by loss of both alleles of the *RB1* tumor suppressor gene. However, recent work showed that amplification of the *MYCN* oncogene can also spur the development of retinoblastoma in a fraction of unilateral patients with normal *RB1* alleles. Our aim was to identify small molecules from a library of known bioactive compounds that show selective growth inhibition of a *MYCN*^A *RB1*^{+/+} retinoblastoma cell line (RB3823) over a *MYCN*^A *RB1*^{-/-} cell line (Y79). Such molecules may be leads for therapy of *MYCN*^A *RB1*^{+/+} retinoblastoma, and will further elucidate the molecular differences between these two subtypes of retinoblastoma. Compounds from the LOPAC1280 library were tested to judge their effect at 10 mM on cell proliferation in both Y79 and RB3823 cell lines using Alamar Blue after a 48 hour incubation in 384 well format. Compounds that reduced cell proliferation by at least 40% in one or both cell lines were tested twice more in both cell lines to confirm efficacy. Dose response testing was conducted using compound concentrations ranging from 100 pM to 1 mM in 384 well format. In initial screening, 95 compounds showed cytotoxicity to one or both cell lines. Secondary screening yielded 9 compounds that reduced RB3823 proliferation by at least 40% compared to untreated cells. Confirmatory screening identified 6 of those 9 compounds that reduced RB3823 proliferation by at least twice that of Y79. In dose-response assays of 5 of these compounds, one compound showed reproducibly higher efficacy in RB3823 than Y79. Dimethoxy-naphthoquinone (DMNQ) had a GI₅₀ in RB3823 at least 3 times lower than that for Y79. DMNQ is an oxidizing quinone that is thought to work by depleting cellular reduction potential leading to oxidation of proteins and DNA, causing cell death. Given our findings, potential may exist for selective treatment options for aggressive, *MYCN*-driven retinoblastoma. DMNQ has not been used in humans, but a DMNQ derivative has shown efficacy in a mouse lung carcinoma xenograft. Further characterization of DMNQ's mode of action in the retinoblastoma context is required, but the door is open to the family of quinones as potential treatment options for *MYCN*^A *RB1*^{+/+} retinoblastoma.

Basic Science

Research Technician

POSTER #73

SUPPORTIVE INTERVENTION FOR ADVANCED CANCER PATIENTS AND THEIR PARTNERS

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Over half a million Americans died from some form of cancer in 2008. Actively working through the associated emotions is associated with less distress at the end of life for the cancer patient and partner. There have been only a handful of empirically tested programs focused on the death-related concerns of dying patients, none of which include a partner. Throughout the cancer progression, and especially at the end of life, relationship issues are of foremost concern for the patient, and the distress of the patient at the time of death affects the partner much as it does at other points of the cancer journey. The objective of this study was to evaluate the feasibility and satisfaction with an intervention to decrease death-related distress in patients and their partners. We also did a preliminary evaluation of efficacy and examined potential mediator variables. Advanced cancer patients and their spouses (n = 12 couples) were recruited through the IUSCC cancer center into a 4 week intervention. The intervention drew from extensive, cross-disciplinary and cross-theoretical research in palliative care and end of life. The result was a meaning-centered supportive intervention that included activities and topics that have demonstrated efficacy across a large population of patients, providers and settings. We saw positive changes in most variables examined, although most were statistically non-significant given our sample size. We were able to recruit 41% of participants referred to the study. All but one couple completed all assessments; one patient passed away mid-intervention, so that couple's participation ended before completion. All participants reported high levels of satisfaction overall with the intervention, with all agreeing that they would recommend the intervention to others facing advanced disease. Our qualitative interviews at the conclusion of the intervention provided rich suggestions for modifications. The pilot study will provide the foundation for a grant proposal to test the intervention in a large-scale study.

Behavioral Faculty

POSTER #74

THE ENIGMA OF THE STIGMA: HAIR LOSS IN CANCER CHEMOTHERAPY PATIENTS

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In both developed and developing countries, breast cancer is now one of the most commonly diagnosed reproductive cancers and a primary cause of death among women. Women treated for breast cancer are likely to receive either radiation or chemotherapy, both of which have secondary effects. Chemotherapeutic treatment produces a range of relatively immediate effects, including pain, nausea, fatigue, mouth sores, depression, problems sleeping, and temporary hair loss. Of these, women across cultures often report that hair loss is one of the more troublesome; it makes them feel unattractive and look like they are sick or dying. Further, they often feel stigmatized by others. Hair, in cultures located around the world, is regarded as important. As we see a response to hair loss across cultures, and the emotional and social responses are so immediate and intense, the evolutionary history of our species may offer us some clues as to why the loss of hair is so often and so widely reported as being traumatic. One of the identifying characteristics of mammals is the presence of hair -- all mammals, and only mammals have hair. One of the first physical traits that distinguished the evolutionary path to modern humans, besides bipedal posture, may have been the loss of much of the body hair, an evolutionary event that may have occurred over a million years ago. For humans, temporary or permanent hair loss, or the excessive shedding of normal human hairs was probably a fairly rare event in our ancestral past. Temporary loss could have been brought about by a variety of stressors including illness and exposure to certain of the parasitic pests with whom we co-evolved. Hair has been seen across cultures as a biological indicator of general health. Symons (1979) observed that because "luxuriant hair" (e.g., thick, shiny, manageable = the so-called "healthy hair") may be universally attractive during our evolutionary history, it has been "reliably associated with health and vigor" and a woman's ability to influence others (p. 187). Hair also is an indicator of illness; among many groups, including the Tonga [Polynesia], Hopi [southwestern United States] and Azande [North Central Africa], the condition of one's hair was used to diagnose illness and communicated that that person should be avoided. In this poster, we look at the cross-cultural patterns of responses to hair loss and examine its possible evolutionary roots. We argue that there is a deep biological basis for these emotions and that, consequently, it is important to develop specific and culturally-tailored interventions to provide support for these women.

Behavioral Faculty

POSTER #75

COLORECTAL CANCER KNOWLEDGE DIFFERENTIATES PEOPLE WHO COMPLETE A SCHEDULED COLONOSCOPY AND THOSE WHO DO NOT

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Up to 50% of people in one safety net hospital in the Midwest who received a recommendation for colonoscopy from their healthcare provider failed to complete the test. Research is needed to understand the complex interplay between individual, social, and health care systems and their influence on colonoscopy completion. The purpose of this study was to compare people who completed a scheduled colonoscopy after receiving a referral with those who did not on demographic and clinical characteristics, CRC knowledge, and health beliefs (perceived risk, perceived benefits, perceived barriers). Quantitative data were collected via telephone interviews from 90 patients; 46 who completed colonoscopy and 44 non-completers. In-depth interviews were conducted with 42 participants to examine perceptions of barriers and facilitators to test completion. Data were analyzed using two-sample t-tests, chi-square tests, Fisher's exact tests and content analysis. People who completed colonoscopy had a higher mean CRC knowledge score ($p=.0008$), and a smaller proportion had hypertension ($p=.03$). No group differences in perceived risk ($p=0.81$), perceived benefits ($p=0.66$), and perceived barriers scores ($p=0.24$) were observed. Non-completers frequently reported that life events interfered with their ability to keep their appointments. While receipt of a provider recommendation is the most important predictor of CRC screening, results showed that receipt of a recommendation with immediate referral to an endoscopist and automatic colonoscopy appointment-making were not sufficient to insure test completion. Education and tailored counseling about the need for and benefits of colonoscopy, along with reminders and tangible assistance to complete the test in the context of competing demands, may be required to increase completion rates.

Behavioral Faculty

POSTER #76

A META-ANALYSIS OF THE RELATIONSHIP BETWEEN SOCIAL CONSTRAINTS AND DISTRESS IN CANCER PATIENTS

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Many cancer patients experience significant distress following a cancer diagnosis. Researchers have focused on the role of social support in cancer patient distress; however, social constraints (SCS; e.g., avoidance, criticism, minimization of patients' concerns) have received less attention, despite work suggesting that they may be more strongly associated with distress than social support. Guided by social cognitive processing theory, we reviewed the relationship between SCS and distress in cancer patients in order to inform future psychosocial intervention development. This meta-analysis: (1) quantified the average strength of the relationships between SCS and general distress (e.g., depressive and anxiety symptoms) and SCS and cancer-specific distress (e.g., avoidance and intrusions); and (2) examined whether age, sex, and time since diagnosis moderated SCS-distress relationships. A total of 30 studies were analyzed, including 26 associations between SCS and general distress and 20 associations between SCS and cancer-specific distress. Correlation coefficients were weighted by sample size and transformed using Fisher's Z-transformation prior to computation of mean effect sizes. We also examined the homogeneity of effect sizes using I² and potential moderators (i.e., age, sex, time since diagnosis). A fail-safe N analysis was conducted for each relationship. Moderate, significant relationships were found between SCS and general distress ($r=.37$) and SCS and cancer-specific distress ($r=.37$). Age and sex did not moderate either relationship. Time since diagnosis moderated the relationship between SCS and cancer-specific distress ($\beta=-.82$, $p=.0001$), but not general distress, such that the relationship between SCS and cancer-specific distress was stronger for samples with a shorter mean time since diagnosis than those with a longer mean time since diagnosis. Findings suggest that SCS may be an important variable to target in interventions to reduce distress in cancer patients. Rebecca Adams is supported by R25 CA117865-06 (V. Champion, PI). Catherine Mosher is supported by K07CA168883.

Behavioral Graduate Student

POSTER #77

GOALS AND PSYCHOLOGICAL WELL-BEING OF CANCER PATIENTS APPROACHING THE END OF LIFE

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Patients with advanced cancer must make healthcare decisions in pursuit of multiple, potentially conflicting goals. To improve the care of patients who may be near the end of life, there is a need to better understand their life and treatment goals, psychological well-being, and how they may change as patients get closer to death. Patients (N=31; median days until death = 145) with incurable advanced lung or gastrointestinal cancers completed a self-report survey, including measures of hope, optimism, and symptoms of anxiety and depression at Time 1 and approximately 3 months later at Time 2. At both timepoints patients also participated in a semi-structured interview where they were asked to freely list their current life goals and cancer treatment goals. Then, they were asked to select five priority goals from among both lists and to rank them in order of importance. Subsequently, these goals were coded using the following categories: Anti-Cancer, Cure, Life Prolongation, Return to Normal Functioning, Symptom Palliation, Life Fulfillment, Social Connectedness, Altruistic, Preparation for Death, Pragmatic, and Spiritual. Patients listed marginally fewer life goals at Time 2 compared to Time 1, $t(30)=1.708$, $p=.098$. There was no significant difference in the frequency or rank of any goal category in priority goals or paramount goals. The number of patients who listed cure in priority goals decreased significantly, $X^2(1) = 8.18$, $p=.004$. Although not significant, there was an increase in the number of Anti-Cancer paramount goals (from 7 to 11). There were no significant changes in hope, optimism, or symptoms of anxiety and depression as patients got closer to the end of life. These results suggest that the valued goals of patients with advanced cancer may not change drastically as they get closer to the end of life. The decrease in Cure goals and increase in Anti-Cancer paramount goals may reflect patients regoaling. Social-Connectedness was the prevailing theme for priority goals at both timepoints, suggesting spending time with loved ones is a valued goal for patients with advanced cancer. Therefore, clinically it may be important to discuss the social implications of treatment options.

Behavioral Graduate Student

POSTER #78

AFRICAN AMERICAN PATIENTS' INTENT TO SCREEN FOR COLORECTAL CANCER: DO CULTURAL FACTORS, HEALTH LITERACY, KNOWLEDGE, AGE AND GENDER MATTER?

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African Americans are at increased risk for colorectal cancer (CRC) and have higher incidence and mortality rates. Research has suggested that CRC screening interventions be targeted to African Americans based upon cultural dimensions. This secondary analysis used baseline data from a large randomized trial to examine: 1) relationships among cultural factors (i.e., provider trust, cancer fatalism, health temporal orientation), health literacy, and CRC knowledge; 2) age and gender differences in cultural variables, health literacy, and CRC knowledge; and 3) relationships among these variables and intention to screen for CRC. African-American primary care patients who were not up-to-date with CRC screening (n=817) completed a telephone interview prior to being randomized to one of two CRC screening interventions. Data were examined using Pearson's correlation, t-test, chi-square tests, and multiple regression analyses. Provider trust was positively correlated with health temporal orientation ($p<.01$). Cancer fatalism was negatively correlated with health literacy ($p<.01$) and CRC knowledge ($p<.01$), whereas health literacy was positively correlated with CRC knowledge ($p<.01$). Age was positively correlated with cancer fatalism ($p<.05$) and negatively correlated with CRC knowledge ($p<.05$) and intention to perform a stool blood test (SBT) ($p<.05$). Men had lower health literacy scores than women ($p<.01$), but reported greater intentions to complete a SBT ($p<.01$) or receive a colonoscopy ($p<.05$). Health literacy significantly predicted intention to receive SBT in multiple regression analyses ($p<.05$). However, neither overall model predicted intention to receive either SBT or colonoscopy in multiple regression analyses. More research is needed to improve understanding of relationships among cultural factors, health literacy, knowledge, and CRC screening intention in order to more effectively increase CRC screening among African Americans.

Behavioral

Graduate Student

POSTER #79

TAXANE-INDUCED MUSCULOSKELETAL PAIN IN WOMEN WITH OVARIAN CANCER: AN INTEGRATED CONCEPTUAL MODEL FOR GUIDING RESEARCH IN ONCOLOGY SYMPTOM MANAGEMENT

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Introduction: An increasing number of individuals are living with serious chronic and life-limiting conditions such as cancer and symptom management has largely become the responsibility of the individual. Oncology nurses play a vital role in the symptom self-management needs of cancer patients. Taxane-induced musculoskeletal pain in women with ovarian cancer is an important symptom requiring further self-management attention. The purpose of this review was to develop an integrated conceptual model to guide future research on taxane-induced musculoskeletal pain in women with ovarian cancer. **Methods:** A review of the literature pertaining to three health behavior theories (i.e. Self-efficacy Theory, the Common Sense Model, and the Transactional model of Stress and Coping) in oncology symptom management research was conducted using the Web of Science database. Potential concepts and relationship statements were identified through the supporting literature and integrated into a new model. **Results:** Primary appraisal, perceived self-efficacy for symptom self-management, symptom self-management, secondary appraisal, and outcomes were concepts from health behavior theory used in the integrated model. To date, no models have assimilated concepts from these three theories to guide oncology symptom management research. **Discussion:** Further research is needed to test the concepts and proposed relationships. Findings from future research may facilitate successful intervention choices, effective symptom management, and improved quality of life in women with ovarian cancer experiencing taxane-induced musculoskeletal pain from chemotherapy treatment.

Behavioral Graduate Student

POSTER #80

LACK OF KNOWLEDGE HAS CONSEQUENCES: HPV VACCINATION AMONG YOUNG MEN WHO HAVE SEX WITH MEN

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Background: Young adult men who have sex with men (YMSM) have high HPV infection rates yet remain significantly under vaccinated. Education increases vaccine acceptance and YMSM tend to have high knowledge regarding HPV transmission, but poor knowledge regarding the consequences of infection. **Objectives:** To examine the association of knowledge on HPV vaccination and intent to get vaccinated among YMSM. **Methods:** 1,449 YMSM who were 18-26 years old (M=22.5, SD=2.4) and members of an online social and sexual networking site completed an online survey covering HPV vaccine history, knowledge, and intention. Intent to get vaccinated was measured on a 3-item scale with response choices ranging from 0-10. The mean score across the items was dichotomized into low (0-5) and high (6-10) intention. Knowledge about HPV transmission/prevention was assessed with 5 true/false items (e.g., Condoms help protect you from HPV). Knowledge about the consequences to males of HPV infection also was assessed with 5 items (e.g., HPV can cause penile cancers). **Results:** 98 (6.8%) reported receiving at least one dose of vaccine. The mean transmission knowledge score (4.1/5) was significantly higher than the mean score for knowledge about consequences (2.0/5; $p<0.001$). Those who were vaccinated had significantly higher knowledge about both transmission (M=4.5/5) and consequences (M=3.0/5) than those who were unvaccinated (M=4.1 & M=1.9, respectively; $p<0.01$). Among the 1,341 unvaccinated YMSM who responded to the intent-to-vaccinate items, 64.3% were in the high intent group, with 35.7% indicating low intent to get vaccinated. Transmission knowledge did not differ between the two groups. However, those with low intent to get vaccinated had significantly lower knowledge scores regarding HPV consequences (M=1.9/5) than those with a high intent to get vaccinated (M=2.1/5; $p<0.01$). **Implications and Impact:** Overall, these YMSM had generally high HPV vaccine acceptability and high knowledge about HPV transmission. However, knowledge regarding the consequences of HPV infection was poor. Moreover, low knowledge of consequences was associated with non-vaccination and lower intent to get vaccinated. Our findings suggest that educational interventions focusing on the consequences of HPV infection for YMSM could help increase vaccination rates.

Behavioral

Graduate Student

POSTER #81

PREDICTORS OF LONG-TERM PHYSICAL ACTIVITY ADHERENCE IN BREAST CANCER SURVIVORS AFTER A LIFESTYLE INTERVENTION PROGRAM

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Background: Physical activity (PA) has an important role in breast cancer (BC) survivorship. However, these effects depend largely on breast cancer survivors' (BCS) PA adherence. The purpose of the study was to determine factors that could predict long-term PA adherence among BCS 6 months after completion of a community-based lifestyle intervention program. **Methods:** Twenty-five BCS who participated in a 6-week lifestyle intervention program, Healthy Eating and Active Living Level 1, were included in this study. Non-modifiable factors, including age, ethnicity, education level, and comorbidity, and baseline stage of readiness to change in PA behavior were assessed. Modifiable factors, including working hours, body mass index, PA adherence, and PA self-efficacy were also assessed at baseline, post-program, and 3 months post-program. Hierarchical linear multiple stepwise regression analyses were used to identify significant predictors of long-term PA adherence at 6 months. **Results:** A majority (60%) of the participants were at stages of action or maintenance in their PA behavior at baseline. The analyses identified two factors that were significant predictors of long-term PA adherence -- baseline PA adherence ($\beta = 0.36$; $p = 0.058$), and post-program PA adherence ($\beta = 0.50$; $p = 0.011$). Both factors accounted for 64.4% of the variance in long-term PA adherence. **Conclusion:** The study findings may have important implications for future designs of community-based lifestyle intervention programs for BCS. PA level before and immediately after intervention appears to be significant determinants for long-term PA adherence, especially in groups that are mostly active or starting to be more active initially.

Behavioral Graduate Student

POSTER #82

BEHAVIORAL CHANGE THROUGH PATIENT ENGAGEMENT USING INFORMATION TECHNOLOGY: A REVIEW OF THE LITERATURE

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Background: Advancements in information technology (IT) and its increasingly ubiquitous nature increase the ability to engage patients in the healthcare process, motivate health behavior change and promote patient-centered care. **Objective:** To systematically review the different types of IT that are being used to change patient behavior, the effectiveness and impact each form of IT has on changing patient behavior among the many divergent disease categories, and the contrasting theories/models used with these technologies to predict patient behavior. **Methods:** PubMed, Web of Science, PsycINFO, and Google Scholar were searched for studies published between February and September 2013, using keywords and medical subject headings (MESH). Potentially relevant studies were retrieved and assessed for eligibility based on pre-determined inclusion criteria. In addition, references to articles identified during the search were also reviewed. **Result:** A total of 133 articles met the inclusion criteria and were reviewed in detail. Overall, 83% of studies showed a positive impact on patient behavior, 11% showed IT as providing no significant value, and 6% showed that patients intend to use technology to modify their behavior. The information technologies used included: Internet-based interventions (48%), mobile-based interventions (27%), telemonitoring (9%), social network sites (11%), video games (3%), and email (2%). Only 48% of the studies used behavioral theories to predict patient behavior. Of those, 78% used cognitive theories, while 22% used behavioral learning theories. **Conclusion:** Although several studies demonstrated that IT has a positive impact on patient behavior, other studies showed no impact. Therefore, further exploration of more comprehensive interventions integrating several technologies to change patient behavior may be warranted. These types of IT intervention need to be based on sound theory and the tenets of engaging patients and incorporating socio-technical design into their development and implementation. In addition, assessing these types of interventions should be conducted using a myriad of measurements, including motivation for behavior change, longstanding adherence, expenditure, satisfaction, and health outcomes.

Behavioral Graduate Student

POSTER #83

ADVANCED PANCREATIC CANCER PATIENTS' END OF LIFE EXPERIENCES: AN INTEGRATIVE LITERATURE REVIEW

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Pancreatic cancer is one of the deadliest types of cancer without a promising treatment and early detection method. At diagnosis, patients generally have low survival rates and short life expectancies. Providing comprehensive and timely end of life (EOL) care is imperative yet challenging for this population. Whereas the majority of studies regarding pancreatic cancer focus on aggressive treatment, there is a pressing need to maximize the pancreatic cancer patients' comfort level at EOL. **Purpose:** The purpose of this integrated review was to explore the advanced pancreatic cancer patients' EOL experiences to provide a foundation for future research, education, and practice. **Methods:** An integrative literature review was conducted to examine studies published between 1980 and 2013 using key words of EOL, palliative or hospice care, and pancreatic cancer. Studies were obtained from the Pubmed, Medline, and CINAHL databases based on inclusion and exclusion criteria. **Results:** Twelve articles were found that met inclusion criteria. More than half were case studies (58.3%) and the remaining manuscripts were quantitative descriptive studies (25%), literature review (8.3%) and phenomenology study (8.3%). All but one study failed to identify an overarching theory and few studies described the EOL needs from patients' or families' perspectives. The top three foci identified from the studies reviewed were patients' physical symptoms and signs (75%), psychosocial aspects (41.7%), and communication and decision making issues (33.3%). Few studies discussed patients' spiritual needs and overall quality of EOL. Studies which focused on physical symptoms identified that fatigue and nutrition related symptoms were the most distressing symptoms and were noted to increase in intensity in the last 8 weeks before death. Severe and poorly controlled pain was also widely reported. With regard to psychosocial aspects, several issues such as worry, financial problems, loss of role function, compromised autonomy, managing other's expectations and relationship with health care providers and family were listed in the studies. For decision making issues, one important example was that patients and families often struggled with deciding the end point of receiving aggressive care. **Discussion:** This review reveals the challenges of providing EOL care for advanced pancreatic cancer patients. The main issues identified in this review can be categorized in issues related to physical symptoms, psychological aspects and communication and decision making. Research is needed that addresses needs and concerns from the patient and family prospective and focuses on difficulties of deciding end points of treatment and initiation of palliative or EOL care. In addition, more exploratory work is needed to identify the gap between patient needs and practice. While symptom management is important in EOL, clinicians should also be aware of other elements such as psychosocial and spiritual needs and the interaction among them.

Behavioral Graduate Student

POSTER #84

ASSOCIATIONS OF HEALTH BEHAVIORS WITH HUMAN PAPILLOMAVIRUS VACCINE RECEIPT AND INTENTIONS AMONG FEMALE COLLEGE STUDENTS

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Objective: To examine associations between health behaviors and human papillomavirus (HPV) vaccine receipt and intentions among female undergraduates. **Participants:** Female undergraduates (N=286) at a Midwestern university completed a survey between September and December of 2012. **Methods:** Pearson's correlations and chi-square analyses were used to examine relationships between health behaviors and HPV vaccine receipt and intentions. **Results:** HPV vaccine receipt was associated with having a regular healthcare provider and having one or more hepatitis B vaccine shots, a flu shot in the past year, a Pap smear, and a dental visit in the past year. Among unvaccinated students (n=121), increased HPV vaccine intentions were associated with receiving a flu shot in the past year and HIV testing. HPV vaccine receipt and intentions were not related to exercise, fruit/vegetable intake, smoking, or alcohol intake. **Conclusions:** Findings suggest that promoting HPV vaccination in conjunction with other preventive medical services might improve vaccine uptake.

Behavioral Graduate Student

POSTER #85

MONITORING AND BLUNTING IN BREAST CANCER PREVENTION CAMPAIGNS: THE IMPACT OF MATCHING MESSAGES TO AN INDIVIDUAL'S PREFERRED COPING STYLE

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Background: According to the C-SHIP model of information processing, tailoring health messages to one's preferred cognitive and emotional processing styles can improve adherence to message recommendations. In this breast cancer prevention study, we examined whether: 1) messages matched to an individual's preferred coping style would increase physical activity; 2) messages matched to an individual's preferred coping style would increase information seeking about breast cancer; 3) whether increased exercise was associated with decreased breast cancer worry and perceived breast cancer risk. **Method:** Five breast cancer prevention flyers were designed to match either monitoring or blunting preferred coping styles. The flyers provided information about the positive effect of current physical activity on future breast cancer risk and attempted to persuade readers to increase exercise. College-aged females ($N = 450$) viewed the flyers and completed psychosocial (cancer worry, perceived breast cancer risk, perceived behavioral control), cognitive (monitoring/blunting), exercise, and information-seeking measures at baseline and four weeks later. The females were categorized as either monitors or blunners, and coded as viewing a matched (1) or an unmatched (0) message. **Results:** Regarding our first question, multinomial logistic regression was conducted to examine the relationship between matched messages and increased physical activity. Results were non-significant. Regarding our second question, binary logistic regression was conducted to predict if psychosocial variables and matched messages led to more information-seeking about breast cancer. A test of the full model was statistically significant ($X^2 (5, N = 439) = 21, p = .001$). Both cancer worry measures (severity and frequency) were significant predictors with the odds of seeking more breast cancer information increasing as both severity (odds ratio [OR] = 1.2, 95% CI, 1.00 to 1.46) and frequency (OR = 1.7, 95% CI, 1.12 to 2.58) increased, as well. Regarding our third question, one-way ANOVA was conducted to examine if increased exercise was related to differences in cancer worry and perceived breast cancer risk. There was no significant difference in cancer worry, but there was a significant difference in perceived breast cancer risk. Individuals who increased their exercise had a significant reduction in their perceived breast cancer risk after four weeks $F (1, 411) = 4.13, p = .04$. **Conclusion:** Messages matched to an individual's preferred coping style neither increased physical activity nor information seeking in our study; however, cancer worry emerged as a significant predictor of information seeking about cancer. This finding supports the literature on the potential motivational qualities of cancer worry. Moreover, increased exercise was associated with decreased perceived breast cancer risk. Whether decreased perceived breast cancer risk leads to an increase or decrease in cancer prevention and early detection behaviors warrants further investigation.

Behavioral Post-Doctoral/Medical Fellow

POSTER #86

CREATING LASTING COMMUNICATION: THE SUPPORTIVE BENEFITS OF LEGACY BUILDING FOR FAMILIES OF ADVANCED CANCER PATIENTS

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Background: Being diagnosed with advanced cancer is a painful reality that impacts patients and their family members. Patients with advanced cancer nearing death face great physical, psychological, social, and spiritual distress. Family members face the challenge of living their lives devoid of their loved one and are left with feelings of loss and sometimes regret from conversations left unspoken. Dignity therapy is an evidence-based intervention that encourages terminally ill patients to reflect upon and communicate their memories, values, and wisdom through the creation of a legacy project. This project succeeds the person in death and is handed down to surviving loved ones, ensuring continuity to the family members who are left behind. Our study qualitatively explores the family members' reactions to the legacy building process and the communication shared in the legacy project to learn about its potential for future bereavement support. **Method:** Brief (20-30 minute) semi-structured phone interviews with family members (n=7) of terminally ill cancer patients were completed approximately one week after receiving the patient's legacy project. Recipients were spouses (n=4), siblings (n=2), or an adult child (n=1). Interviews were coded using conventional content analysis using an open-coding strategy. Themes were recorded as they emerged. **Results:** The following communication themes emerged from the data. Legacy building: (1) facilitates conversation; (2) creates new knowledge and understanding; and (3) leaves lasting communication for future generations. The family members felt that overall communication improved, and they were able to discuss the terminal nature of their loved one's illness. New knowledge was communicated in the legacy project and sometimes this new knowledge led to further understanding of the patient's personal life events. Furthermore, the project was viewed as a family heirloom that would be passed down to future generations, creating lasting communication. All family members stated they would recommend the project to others. **Conclusions:** This study adds to the literature on the supportive benefit legacy building has on families of advanced cancer patients by examining the unique impact of communication. Participants in our study reported deriving meaningful benefit from reading the legacy project, and future research should investigate and quantitatively assess bereavement outcomes over time, as well as the direct impact these outcomes have on family members' quality of life.

Behavioral Post-Doctoral/Medical Fellow

POSTER #87

LUNG CANCER STIGMA AND DELAYED MEDICAL HELP-SEEKING BEHAVIOR

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Background: Lung cancer kills more people than any other cancer because it is often diagnosed at an advanced stage. Lung cancer is thought asymptomatic until advanced; however, many at an early stage experience symptoms but do not seek medical evaluation. Understanding potential barriers to medical help-seeking behavior in lung cancer symptoms is critical; lung cancer stigma is an important barrier. **Purpose:** The purpose was to 1) determine if there was a correlation between perceived lung cancer stigma and delayed medical help-seeking behavior in lung cancer patients, and 2) describe sociodemographic characteristics related to perceived lung cancer stigma. It was hypothesized 1) there would be a positive relationship between lung cancer stigma scores and delayed medical help-seeking behavior, and 2) lung cancer stigma scores would differ by ethnicity, gender, and smoking status. **Methods:** Descriptive, cross-sectional, correlational design (N = 93 patients; diagnosed with non-small cell lung cancer - all stages) using survey methodology to measure perceived lung cancer stigma and an in-person interview to collect demographic and medical characteristic data. **Results:** Pearson correlation revealed a significant relationship between lung cancer stigma scores and delayed medical help-seeking behavior ($r = .27, p = .01$). Independent-samples t-tests revealed no significant difference in lung cancer stigma scores by gender, but a statistically significant difference by ethnicity ($t(91) = -2.57, p = .012$, two-tailed). One-way ANOVA explored the impact of smoking status on lung cancer stigma scores and found no significant difference among never, past, or current smokers ($F(2,90) = .14, p = .872$). **Conclusions:** Lung cancer stigma is a potential barrier to timely medical help-seeking behavior, subsequent diagnosis and treatment in individuals with symptoms suggestive of lung cancer. There may be a cultural component to perceived lung cancer stigma; however, further investigation is needed. Findings indicate a public health need for increasing awareness of the prevalence of perceived lung cancer stigma regardless of smoking status. Future research should target decreasing the stigma associated with lung cancer and increasing early medical help-seeking behavior. Learning Objectives: (1) Report the relationship between lung cancer stigma and delayed help-seeking behavior. (2) Describe sociodemographic characteristics associated with lung cancer stigma. (3) Discuss the public health need of addressing lung cancer stigma in interventions to promote early help-seeking behavior in individuals with symptoms suggestive of lung cancer.

Behavioral Post-Doctoral/Medical Fellow

POSTER #88

ACCEPTABILITY OF THE HPV VACCINE AND REASONS FOR NON-VACCINATION AMONG PARENTS OF ADOLESCENT SONS: RESULTS FROM A NATIONAL SURVEY

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Routine administration of the quadrivalent human papillomavirus (HPV) vaccine has been recommended for 11-12-year-old males since 2011, but coverage remains low. In a U.S. national sample of parents of 11-17-year-old males (N=779), 78.6% of parents reported their sons had not received the HPV vaccine. We explored vaccine acceptability (i.e., willingness to vaccinate) and reasons for non-vaccination among these parents. The most commonly endorsed reason for non-vaccination (61.6%) was My doctor or health-care provider has not recommended it. Non-vaccination due to non-recommendation or other logistical barriers was associated with significantly higher vaccine acceptability than non-vaccination due to attitudinal barriers, such as concerns about vaccine safety or efficacy. In sum, many parents are accepting of the vaccine but have not vaccinated their sons due to lack of access, education, or recommendation from healthcare providers. These findings have important implications for increasing HPV vaccination coverage among adolescent males.

Behavioral Post-Doctoral/Medical Fellow

POSTER #89

POSTTRAUMATIC GROWTH AND HOPE IN PARENTS OF CHILDREN WITH CANCER

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Background: Over 13,000 children are diagnosed with cancer each year in the US. For children and their parents, the cancer experience can be transformative and lead to reappraisals in goals. However, the factors that facilitate growth and the mechanisms by which this process occurs are unknown. Hope is the belief that goals can be met, and it consists of two parts: 1) Agency (one's perception that he or she can progress and/or persevere toward goals), and 2) Pathways (the perceived ability to find methods of reaching goals). Dispositional hope is believed to influence people's reactions to life events (e.g., diagnosis of chronic illness) with potential to promote either engagement or disengagement with identified goals. Posttraumatic growth (PTG) is a positive change in major life goals experienced as a result of the struggle with a highly challenging life circumstance. Both hope and PTG are goal-related constructs which can be conceptualized in self-regulation theory. However, few studies have examined how dispositional hope is related to PTG as an outcome. In particular, no studies have examined the relationship between hope and PTG in parents of children newly diagnosed with cancer. **Objective:** To examine the relationship between dispositional hope and PTG among parents of children and adolescents receiving treatment for pediatric cancer. Dispositional hope was hypothesized to be positively related to PTG in this population. **Methods:** Participants were parents (N=85, 82% female) of children (55% female; 2-18 years) with cancer. Parents completed a demographic questionnaire, the Posttraumatic Growth Inventory (PTGI), and Hope Scale (HS). **Results:** Hierarchical regressions were conducted. Following the transactional stress and coping model, demographic and illness covariates were entered on Step 1 of the regressions, and the HS total score was entered on Step 2. Hope was related to PTG in parents ($p=.003$), with higher levels of hope associated with greater PTG. There was a trend for the overall model to be significant ($p=.057$), and it predicted 15% of the variability in PTG. Exploratory analyses were conducted on the subscales of the PTGI. For Relating to Others, hope was related to growth ($p=.002$); the model predicted 17.2% of the variability in relating to others ($p=.028$). For New Possibilities, hope was related to growth ($p=.008$); the model predicted 15.6% of the variability in new possibilities ($p=.048$). For Personal Strength, hope was related to growth ($p=.030$); however, the model was nonsignificant. The model for Spiritual Change was also nonsignificant. For Appreciation of Life, hope was related to growth ($p=.005$); the model predicted 17.1% of the variability in life appreciation ($p=.029$). **Conclusions:** Findings suggest that dispositional hope may facilitate growth in parents of children with cancer. Hope may be an important target for promoting positive goal adjustment in this population.

Behavioral Post-Doctoral/Medical Fellow

POSTER #90

CHLAMYDIA INFECTIONS AND UNPROTECTED SEX INCREASES RISK OF 37 HPV TYPES OF HPV REDETECTION

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Objective: To examine factors associated with the redetection of anogenital HPV types. **Methods:** A longitudinal cohort (N=150) of adolescent women, ages 15 to 17 years at enrollment, with behavioral and sexually transmitted infection (STI) information assessed every 3 months, was used to examine HPV redetection. Cox proportional hazard models were used to assess the influence of sexual behaviors and STIs in the preceding 3 months on the redetection of low risk (LR) and high risk (HR) HPV. **Results:** There were 1248 type specific infections (defined as 2 or more samples positive for the same type) within the cohort; 766 HR and 482 LR-HPV infections. Redetection after a period of non-detection (defined as greater 2 consecutive negative HPV test, approximately 6 months or greater) occurred for 23.9% of HR-HPV (183/766) and 19.3% of LR-HPV (93/242) infections. Periods of non-detection averaged 14.5 months (SD=11.8). Univariate models found chlamydia, more sexual partners, coital frequency, less condom use and use of oral contraceptive pills (ocp) associated with HPV redetection. Multivariate model showed that chlamydia (HR=1.99 [95% CI, 1.15-3.49]), and greater frequency of non-condom use (HR=1.01 [95% CI, 1.00-1.01]) were associated HR-HPV redetection; more sexual partners (HR=1.44 [95%CI, 1.04-1.99]) and ocp use (HR=2.73 [95%CI, 1.52-4.90]) were associated with increased risk of LR-HPV redetection. The multivariate model for combined HR and LR HPVs found that all 4 variables were significantly associated with redetection. Discussion: This study demonstrates a role for chlamydia and sexual behaviors in type-specific HPV redetection. Redetection may represent either reactivation or new infection. However, redetection may also be associated with viral persistence and subsequent progression to cancer.

Population Science/Epidemiology Faculty

POSTER #91

METABOLIC SYNDROME AND TOTAL CANCER MORTALITY RESULTS FROM THE THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES III)

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Objective: Metabolic syndrome (MS) becomes increasingly common primarily due to the epidemic of overweight and obesity in both developed and developing countries. Epidemiologic studies have shown that obesity increases the risk of several cancers. However, it largely remains unclear whether the components of MS are associated with total cancer mortality. Therefore, we sought to evaluate this important question in the Third National Health and Nutrition Examination Survey (NHANES). **Methods:** Data on demographics, socioeconomic, lifestyle factors, and the components of metabolic syndrome were collected among 19,296 men and women aged 18 years or older (pregnant women excluded). Cancer mortality outcomes of these subjects were determined by linkage to the National Death Index (NDI) through 2010. A total of 1,136 cases of all sites of cancer were documented during this period of time. Weighted Cox proportional hazards regression was used to calculate hazard ratios (HR) with 95% confidence intervals (CIs) for total cancer mortality in relation to the components of MS. **Results:** After adjustment for age, sex, race, education, alcohol, and cigarette smoking, subjects in the highest quartile of serum glucose [HR 1.31; 95% CI (1.30-1.31)], waist circumference [HR 1.09; 95%CI (1.09-1.09)] and systolic blood pressure [HR 1.36; 95% CI (1.36-1.37)] experienced an increased risk of total cancer when compared with those in the lowest quartile. However, a reduced risk was observed for those in the highest quartile of HDL cholesterol [HR 0.83; 95% CI (0.83-0.83)] and triglycerides [HR 0.87; 95% CI (0.87-0.87)]. Subjects who were diagnosed with MS (having any 3 of the 5 components abnormal, n=5,940; 31%) had a 20% higher risk of death from total cancer compared to those without the metabolic syndrome [HR 1.20; 95% CI 1.199-1.203]. The risk of total cancer mortality increased with increase in abnormal MS components (ptrend <.0001). Compared with individuals without MS, HRs were 1.12, 1.28, and 1.59 for those with 3, 4, and 5 abnormal components, respectively. **Conclusion:** To our knowledge, the present study is among the first to demonstrate that some components of metabolic syndrome modulate the risk of total cancer among a large representative sample of the US population. Our findings offer novel evidence for the potential role of metabolic syndrome in cancer carcinogenesis and mechanistic data for the associations between obesity and the risk of some cancers.

Population Science/Epidemiology

Graduate Student

POSTER #92

ANALYSIS OF THE INVERSE ASSOCIATION BETWEEN CANCER AND ALZHEIMER'S DISEASE: RESULTS FROM THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE COHORT

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Although a number of studies support a reciprocal inverse association between diagnoses of cancer and Alzheimer's disease (AD), to date there has not been any systemic investigation of the neurobiological impact of or genetic risk factors underlying this effect. To facilitate this goal, this study aimed to replicate the inverse association of cancer and AD using data from the NIA Alzheimer's Disease Neuroimaging Initiative, which includes age-matched cases and controls with information on cancer history, AD progression, neuroimaging, and genomic data. Subjects included individuals with AD (n=234), mild cognitive impairment (MCI, n=542), and healthy controls (HC, n=293). After controlling for sex, education, race/ethnicity, smoking, and apolipoprotein E (*APOE*) e2/3/4 allele groups, cancer history was protective against baseline AD diagnosis (p=0.042), and was associated with later age of AD onset (p=0.001). Cancer history appears to result in a cumulative protective effect; individuals with more than one cancer had a later age of AD onset compared to those with only one cancer (p=0.001). Finally, a protective effect of AD was also observed in individuals who developed incident cancer after enrolling (post-baseline visit); 20 individuals with MCI and 9 HC developed cancer, while no AD patients had subsequent cancer diagnoses (p=0.013). This supports previous research on the inverse association of cancer and AD, and importantly provides novel evidence that this effect appears to be independent of *APOE*, the major known genetic risk factor for AD. Future analyses will investigate the neurobiological and genetic basis of this effect.

Population Science/Epidemiology

Graduate Student

POSTER #93

WITHDRAWN: PHYSICIAN COMMUNICATION ON COST OF CANCER CARE UNDER THE AFFORDABLE HEALTH CARE ACT.

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Background: The steeply rising cost of cancer care in the United States adversely impacts the economy as well as patient emotional well-being and financial stability. The aim of this study was to assess US oncologists' attitudes and perceptions about the cost of cancer care in the wake of ACA implementation. **Methods:** From June through August 2013, a survey instrument was emailed to practicing oncologists in 50 states. Survey items included assessments of self-reported practices concerning communication of cost of therapy as well as influences of the ACA. Other survey items assessed oncologists' perceptions of cost effectiveness data. **Results:** The electronic survey response rate was 16% with respondents from 35 states. Respondents were more likely to strongly or somewhat agree that it was important to discuss out-of-pocket costs [OPC] (89%) and healthcare system costs [HSC] (66%) with patients, $p < 0.0001$. 70% reported that OPC of therapy influence their treatment decisions. 60% agreed that OPC and HSC of cancer treatments were likely or extremely likely to have a larger effect on their decisions regarding which cancer treatments to recommend to patients in the future under the ACA. While 4% agreed the government should play a role in determining the value of a cancer therapy, 53% of respondents thought that government price controls for cancer drugs were needed. A large majority agreed that physician education on the use of cost-effectiveness data (91%) and communicating cost of therapies with patients (85%) was needed. **Conclusion:** US oncologists reported that they desire more cost and comparative effectiveness research as well as more education on how to communicate with patients about costs of therapy. Respondents perceived that both OPC and HSC will play a larger role in their cancer treatment decisions over the next five years, and that they will need to increase their communication with patients about both OPC costs as well as HSC. Respondents appeared divided on the topic of government intervention on pharmaceutical price controls and unified in their resolve to maintain control of decisions on the value of therapy.

Population Science/Epidemiology

Post-Doctoral/Medical Fellow

POSTER #94

DIFFERENCES IN EXPRESSION OF LNCRNAs IN BREAST TUMOR, ADJACENT NORMAL-APPEARING BREAST TISSUE, AND NORMAL BREAST TISSUE FROM HEALTHY DONORS

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Long noncoding RNAs (lncRNAs) are an emerging class of key regulatory RNAs that are greater than 200nt and do not code for protein. They appear to be critical for various biological processes including gene regulation. Increasing evidence has suggested that aberrantly expressed lncRNAs play a key role in the initiation and progression of breast cancer. In most studies of breast cancer, breast tumor tissue was compared to adjacent normal-appearing tissue to identify differentially expressed lncRNAs. However, expression of lncRNAs in normal breast tissue from healthy donors has rarely been studied, and the differences in expression of lncRNAs in breast tumor and normal breast tissue from healthy donors remain largely unknown. Using RNA-sequencing data generated from freshly-frozen breast tissue samples, we examined the expression of lncRNAs in 119 breast tumor, 47 adjacent normal-appearing breast tissue samples, and 23 normal breast tissue samples from healthy donors. Bowtie and RESM algorithms were used to align raw reads and quantify transcript abundance. Differential expression analyses were performed using edgeR software. We assessed the expression of a total of 7,844 mapped lncRNAs using normalized transcript counts. Unsupervised Principal Components Analysis (PCA) demonstrated that the lncRNA expression profile of adjacent normal-appearing tissue was different compared to normal breast tissue from healthy donors, and partially overlapped with the tumor. At various fold change thresholds, more differentially expressed lncRNAs can be detected when the tumor tissue is compared to normal tissue than to adjacent non-tumorous tissue. With a false discovery rate (FDR) < 0.05 and a fold change (FC) of more than ± 2 , we identified 107 differentially expressed lncRNAs for the tumor vs. normal breast tissue, 53 lncRNAs for tumor vs. adjacent normal-appearing tissue, and 20 lncRNAs for adjacent normal-appearing vs. normal breast tissue. In our analyses of tumor vs. adjacent normal-appearing tissue and tumor vs. normal tissue from healthy donors, we identified differentially expressed lncRNAs including HOTAIR, GAS5 and ANRIL/CDKN2B-AS1 that were previously implicated in breast cancer, with considerably higher fold changes in latter comparison. When using normal breast tissue from healthy donors as the baseline, we identified novel putative breast cancer-associated lncRNAs including EMX2OS, TERC, HOTAIRM1, and WT1-AS that are aberrantly expressed in breast tumor. These lncRNAs were not identifiable in a direct comparison of tumor and adjacent normal-appearing tissue. Our results suggest that adjacent normal-appearing tissue undergoes changes in lncRNAs expression similar to those observed in tumor tissue to some extent, and a number of new and potentially important lncRNAs can only be detected using normal tissue from healthy donors as an optimal baseline tissue.

Population Science/Epidemiology

Post-Doctoral/Medical Fellow

POSTER #95

COMBINATORIAL EVALUATION OF DOVITINIB (TKI258) AND EVEROLIMUS IN THYMIC MALIGNANCIES

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Thymomas and thymic carcinomas are rare epithelial tumors derived from the thymic gland located in the anterior mediastinum. All histological types of thymomas can give rise to metastases albeit with different frequencies. Thymic carcinomas have a more aggressive behavior and metastasize earlier and more frequently than thymomas. So far, targeted therapies are not available for either of these cancers. We recently have developed and validated a nine-gene and a ten-gene signature to predict metastatic behavior of thymomas and thymic carcinomas, respectively (Gökmen-Polar et al, *PLOS ONE* 2013; Gökmen-Polar et al, *ASCO* 2013). Pathway analysis of these signatures revealed that PDGFR is significantly associated with metastatic phenotype of both thymomas and thymic carcinomas. In particular, we have demonstrated that platelet-derived growth factor receptor-like (*PDGFRL*) is down-regulated in metastatic phenotype, which acts as a hub for tumor suppressor activity regulating PDGF pathway signaling. Our group also demonstrated the upregulation of mTOR pathway in thymic malignancies. Using IU-TAB-1, a thymoma AB cell line and thymic carcinoma cell line 1889c, our initial efforts focused on the *in vitro* efficacy of dovitinib alone, a tyrosine kinase inhibitor targeting PDGFR and FGFR and evaluate its efficacy in combination of with standard care of therapy in these cancers. Dovitinib alone exhibited inhibition of cell survival at IC₅₀ = 8.39 μM and 6.44 μM (IC₅₀- the concentration that inhibit 50% of cell survival) for IU-TAB-1 and 1889c, respectively. Everolimus alone, a rapalog that inhibits mTOR pathway, was less sensitive for 1889c (IC₅₀ = 12.96 μM) compared to IU-TAB-1 (IC₅₀ = 8.69 μM). Furthermore, combination therapy with dovitinib and everolimus reduced the cell survival synergistically [IU-TAB-1 (IC₅₀ = 1.9 μM; 1889c IC₅₀ = 3.52 μM). However, treatment with everolimus increased the phosphorylation of Akt^{S473} in these cells. Combinatorial therapy did not decrease the high level of p-Akt, suggesting its ineffectiveness to inhibit mTOR pathway. In conclusion, these preclinical models will provide excellent tools to study the relevance and functional role of novel therapeutic agents as well as the rationale for combination therapies, and provide insight for the future treatment strategies of patients with these rare malignancies.

Translational/Clinical Research Faculty

POSTER #96

PREPARATION OF 68GA-DOTA-NOC FOR PET/CT EVALUATION OF NEUROENDOCRINE TUMORS UNDER AN EXPANDED ACCESS IND

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Objective: To provide the somatostatin-receptor-targeting 68Ga-DOTA-NOC radiopharmaceutical for clinical PET/CT evaluation of neuroendocrine cancer patients being considered for multivisceral transplant. **Methods:** An Expanded Access IND was developed for 68Ga-DOTA-NOC, an agent that has had extensive world-wide use in neuroendocrine tumor imaging, but which is not available as an FDA-approved drug product in the United States. The 68Ga3+ precursor was obtained in 1.5 mL 0.1M ultrapure HCL by fractionated elution of a TiO2-based 68Ge/68Ga generator system (Eckert & Ziegler); buffered by addition of ultrapure aqueous NaOAc; and reacted with commercial cGMP DOTA-NOC conjugate (60 µg) at 80°C for 10-minutes. The 68Ga-DOTA-NOC product was then isolated by C18 solid phase extraction; recovered in 0.6 mL 85% ethanol:15% saline; and diluted with additional sterile saline (12 mL) prior to terminal sterilizing filtration into a sterile evacuated vial. Quality control measures, and release criteria, followed the specifications of the published EANMMI Procedure Guidelines for such 68Ga-agents (Eur J Nucl Med Mol Imaging 37:2004-2010; 2010). **Results:** The 68Ga-DOTA-NOC radiopharmaceutical was prepared for PET/CT imaging in 39 patients (administered dose: 4.7 ± 0.6 mCi; 174 ± 22 MBq) during the first year of the IND. Total synthesis time, from generator elution to post-QC release of final sterile product, averaged 46 ± 5 minutes, with a product radiochemical purity of $98.2 \pm 0.7\%$. 68Ge breakthrough in the final product has been at or near the background count rate, averaging $3.0 \pm 3.7 \times 10^{-7}\%$ at the dose expiration time. **Conclusions:** The manual synthesis approach has been effective for 68Ga-DOTA-NOC production, addressing a local clinical need for imaging to assess the location and extent of disease in neuroendocrine cancer patients being considered for surgery.

Translational/Clinical Research Faculty

POSTER #97

A NOVEL HUMAN SUICIDE GENE SYSTEM BASED ON THE HUMAN CYP4B1 ENZYME RE-ENGINEERED TO EFFICIENTLY PROCESS 4-IPOMEANOL

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Adoptive T-cell therapy (ACT) has developed as a hopeful strategy for the treatment of cancer, for example donor lymphocyte infusion (DLI), genetic engineering of T-cells with either T-cell receptors (TCRs) or Chimeric Antigen Receptors (CARs). Therefore, an important strategy to effectively control the toxic side effects would be to equip the T-cells prior to re-infusion with a suicide gene that will enable directed elimination of the engineered T-cells *in vivo* in the patient by inducing apoptosis. The prototype suicide gene, established more than 15 years ago for donor lymphocyte infusions (DLIs) in an allogeneic stem cell transplantation setting, is the thymidine kinase from the herpes simplex virus type 1 (HSV-tk) used in combination with its prodrug, the antiviral substance ganciclovir (GCV). The HSV-tk/GCV system has been successfully employed in clinical phase I/II trials for control of DLI-induced GvHD. However, some groups also reported that the usefulness of this system was limited by the strong immunogenicity of the HSV-tk protein that can lead to the rapid elimination of the genetically modified T-cells, even after stem cell transplantation, and by the fact that ganciclovir is paramount for the treatment of viral infections or reactivations after transplantation.

We developed a novel human suicide gene by systematically optimizing the inactive human cytochrome P450 family 4, subfamily B, polypeptide 1 (CYP4B1) to efficiently process the prodrug 4-Ipomeanol (4-IPO) into a highly toxic DNA alkylating chemotherapeutic agent. Using lentivirus-mediated expression in human liver cells and primary T-cells, we demonstrate that systematically altering single amino acids in the human CYP4B1 protein renders the enzyme as active as the rabbit CYP4B1 protein, the most active isoform of CYP4B1 known in mammals, for processing 4-IPO and thereby inducing cell death within 24 to 72h. Our human suicide gene is especially interesting for use with recent adoptive cell therapy strategies, because a critical advantage for use in T-cell therapies is that the CYP4B1 suicide gene system mediates a proliferation-independent elimination of cells without major bystander effects for the surrounding tissue/cells.

Translational/Clinical Research Faculty

POSTER #98

PRL2 PHOSPHATASE MEDIATES ONCOGENIC KIT AND FLT3 SIGNALING IN ACUTE MYELOID LEUKEMIA

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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 therapeutic targets in solid tumors. While PRL2 is overexpressed in human acute myeloid leukemia (AML), its role in normal and malignant hematopoiesis is largely unknown. Recently, we reported that PRL2 regulates hematopoietic stem cell self-renewal through regulating SCF/KIT signaling (Kobayashi et al., Stem Cells, 2014). To define the role of PRL2 in the pathogenesis of AML, we overexpressed KIT-D814V or FLT3-ITD in *Prl2* null hematopoietic progenitor cells and performed *in vitro* and *in vivo* assays. We discovered that *Prl2* null Lin⁻ cells expressing KITD814V or FLT3-ITD mutant show decreased proliferation compared with wild-type cells in the absence of cytokine. Moreover, PRL2 deficiency significantly delayed the leukemogenesis induced by KITD814V *in vivo*, demonstrating that PRL2 is important for KITD814V and FLT3-ITD-mediated ligand-independent growth of hematopoietic progenitor cells *in vitro* and leukemogenesis *in vivo*. Furthermore, we observed increased level of PRL2 proteins in some AML cell lines and inhibition of PRL2 activity with a PRL2-specific small molecule inhibitor (PRLi) results in decreased proliferation and apoptotic cell death of human AML cell lines expressing PRL2. Importantly, we found that primary human AML cells isolated from patients with FLT3-ITD positive AML and normal karyotype AML are sensitive to PRL2 inhibitor treatment in a dosage-dependent manner, suggesting that human AML cells are dependent on PRL2 activity for proliferation and survival. PRL2 is a dual specificity protein phosphatase. However, the substrates of PRL2 are largely unknown. To identify novel targets of PRL2 activity in AML, we performed protein phosphatase substrate trap assays and identified several potential PRL2 substrates in Kasumi cells, including KIT, SHP2, CBL and PLC-g. We found that the levels of pAKT and pERK1/2 are significantly lower in *Prl2* null Kit⁺ cells compared with those in wild-type cells. When we overexpressed wild-type or the catalytic inactive mutant form of PRL2 (PRL2/C101S) in Lin⁻ cells isolated from *Prl2* null mice, we found that wild-type PRL2, but not the mutant PRL2, augments pAKT and pERK1/2 levels in *Prl2* null Lin⁻ cells, indicating that the ability of PRL2 to enhance cytokine signaling in hematopoietic cells depends on its phosphatase activity. Mechanistically, PRL2 deficiency results in decreased KIT stability and phosphorylation following SCF stimulation, possibly through dephosphorylating CBL. Thus, PRL2 phosphatase is an important mediator of oncogenic signaling in AML and may be a druggable target in myeloproliferative disease (MPD) and acute myeloid leukemia (AML) with oncogenic KIT and FLT3 mutations.

Translational/Clinical Research Faculty

POSTER #99

IMPAIRED B-CELL DEVELOPMENT CAN PREDICT GRAFT-VERSUS-HOST DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELOYDYSPLASTIC SYNDROME

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Background: Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT) and together with immunosuppressive therapy is implicated in the impaired immune reconstitution. Select parameters of immune function and their relation to the clinical outcomes including GVHD have been previously studied. However, only rare reports explore the relationship between early bone marrow (BM) morphologic and immunophenotypic findings and the incidence of GVHD. In this study, we evaluated the histopathologic features of bone marrow biopsies and lymphocyte subpopulations in a cohort of patients post allogeneic HCT, and compared these to the development of acute and chronic GVHD (aGVHD and cGVHD).

Design: Post-HCT BM specimens and blood counts at day +30 and/or day +100 were retrospectively examined in 98 patients who received either myeloablative or nonmyeloablative HCT for acute myeloid leukemia or myelodysplastic syndrome. BM biopsy and aspirate smear examination included the following: % cellularity, % adipocytes, activated osteoblast morphology, paratrabecular fibrosis, trabecular remodeling, new bone formation, the presence of osteoclasts, myeloid:erythroid ratio, % lymphocytes and % hematogones. Bone marrow lymphocyte subsets (CD20+ B-cells, CD3+ T-cells, CD4+ and CD8+ T-cells, NK-cells and stage I hematogones) were quantitated by flow cytometry. These results were correlated with laboratory data, and the presence or absence of aGVHD and cGVHD.

Results: The study included 98 patients (49 males and 49 females; median age 48; age range 17-67 years). There were 32 and 39 patients with aGVHD and cGVHD, respectively. Patients with cGVHD had significantly lower numbers of stage I hematogones ($p=0.002$; 0.49% vs 1.76% for patients with and without cGVHD respectively). cGVHD was also associated with higher BM adiposity (mean 45%, vs 36% without cGVHD; $p=0.02$). There was a trend for lower cellularity in those with cGVHD ($p=0.05$). Other morphologic parameters were not statistically significantly different between cases with and without cGVHD. There was also no correlation between histopathologic parameters and lymphocyte subsets, and the presence of aGVHD.

Conclusion: These findings demonstrate that there is an increased incidence of cGVHD among post-HCT patients with lower numbers of stage I hematogones, increased replacement of the BM space with adipocytes and lower cellularity. Further studies are needed to determine whether monitoring of these parameters can be useful in guiding GVHD prophylaxis.

Translational/Clinical Research Faculty

POSTER #100

ALTERED WORKING MEMORY-RELATED BRAIN ACTIVATION AFTER LEUKEMIA CHEMOTHERAPY AND RELATIONSHIP TO ACADEMIC FUNCTIONING

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Purpose: Children treated with chemotherapy for acute lymphoblastic leukemia (ALL) have been shown to demonstrate cognitive impairment relative to healthy controls (HC), including in executive functions such as working memory (WM). As there has been limited investigation of the neural substrate of these deficits, this study examined WM-related brain activation after ALL chemotherapy using functional MRI (fMRI) and assessed the relationship to academic performance. **Methods:** 23 children >3 years post ALL chemotherapy (mean age 11.7 years, 10 female) and 21 demographically matched HC (mean age 12.8 years, 13 female) completed an fMRI auditory-verbal n-back WM task, neuropsychological (NP) testing, and parent behavioral ratings (CBCL and BRIEF). Data were analyzed using SPM8 and SPSS version 21. **Results:** ALL patients evidenced lower processing speed, executive function, and global NP scores than HC. Parent ratings were elevated for ALL relative to HC only for the BRIEF emotional control scale. Groups had comparable n-back performance, but ALL patients showed greater brain activation than HC during the most challenging WM condition (2-back), particularly in frontal regions. Within the ALL group, increased frontal activation correlated with better task performance. Relative to those without parent-reported academic difficulty, ALL patients with academic difficulty showed lower frontal lobe activation, 2-back performance, verbal memory, and global NP functioning, and higher parental concerns on several CBCL and BRIEF scales. **Conclusion:** In the context of comparable task performance, the finding of greater frontal brain activation in children after ALL chemotherapy relative to HC suggests compensatory recruitment of neural circuitry to support WM processing. This interpretation is supported by a positive correlation between frontal activation and task performance in the ALL group. ALL patients with academic difficulty showed lower frontal activation and greater cognitive and behavioral concerns, demonstrating a putative neural correlate of chemotherapy-related changes, and potential target for treatment or remediation approaches.

Translational/Clinical Research Faculty

POSTER #101

TOBACCO PREVENTION AND TREATMENT (TPT) WORKING GROUP AT INDIANA UNIVERSITY SCHOOL OF MEDICINE

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Tobacco use is the major risk factor for cardiovascular and lung diseases, including lung cancer. The rates of tobacco use in our state are among the highest in the nation, rendering staggering economical and societal cost. The main goal of the TPT working group is to engage a multidisciplinary approach that leverages local talent and resources to prevent the development and improve the outcomes of tobacco-related cancers in Indiana. The working group will address both the societal and individual impact of tobacco exposure and morbidity in Indiana, undertaking epidemiological and biological approaches to study two major areas: Prevention and Early detection as well as Control and Treatment. **Distinct goals** of the TPT working group are: 1) To increase primary prevention of tobacco and similar carcinogens such as biomass, smokeless tobacco (e-cigarettes), or shisha use in Indiana. 2) To increase the rates of cessation of tobacco use in Indiana. 3) To understand the biology of addiction to tobacco. 4) To optimize local lung cancer screening implementation. 5) To develop individualized management approaches to improve outcomes of tobacco-related cancers. 5) To understand individual risk of developing a specific lung cancer phenotype, and individualized responses to therapy. **Our approach** is to identify appropriate stakeholders and engage them in a team approach to formulate action and research plans, and pursue their implementation. The following projects have been identified as *initial areas* of action: For Prevention of smoking initiation, we will focus on pre-teen targeting. We will use educational initiatives targeting church groups, youth groups, and sports teams; we will engage school nurses association, community nurses; and student nurses. To enhance Smoking Cessation, we will pursue educational initiatives and efforts to synergize or integrate current resources on campus, including the Indiana University Health, VA Medical Center, State Health, and Indiana Cancer Consortium. Planned research studies related to this topic are the pharmacogenomics of nicotine cessation drugs; and the impact of implementation of tele-health in remote rural areas to enhance smoking cessation. Another major area of attention will be the assessment and integration of existent Lung Cancer Screening Initiatives and pursue research related to appropriate implementation. The **short term milestones** of the TPT working group will be the implementation of productive working teams of investigators; successful internal and extramural grant applications; recruitment of junior investigators to the working group; and increased visibility on campus as a useful local resource for tobacco-related policies and initiatives. **Long term milestones** are measurable decreases in rates of tobacco use by the general population and high school students in particular which will likely translate in decreased rates of lung cancer diagnosis and lung cancer mortality in Indiana.

Translational/Clinical Research Faculty

POSTER #102

A LARGE MICRORNA CLUSTER ON CHROMOSOME 19 IS A TRANSCRIPTIONAL HALLMARK OF WHO TYPE A & A/B THYMOMAS

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Purpose: Thymomas are one of the most rarely diagnosed malignancies. The relationship of current histological subtyping with prognosis is poorly defined, supporting the need for a deeper understanding of transcriptional biology to develop targeted therapies. To address this, we performed next-generation RNA sequencing to examine the transcriptional landscape of this disease. **Experimental Design:** RNA was sequenced from 13 thymic malignancies and 3 normal thymus glands. Validation of microRNA expression was performed on a separate set of 35 thymomas using qPCR. For cell based studies, a thymoma AB cell line (IU-TAB1) was used. **Results:** Hierarchical clustering revealed 100% concordance between gene expression clusters and WHO subtype. A subsequent clustering of 705 precursor-microRNAs also showed substantial concordance between clusters and subtype. By analyzing the dendrograms, A & AB tumors were significantly different from other thymomas. A substantial differentiator was a large microRNA cluster on chr19q13.42 that was significantly over-expressed in all A & AB tumors and whose expression was virtually absent in the other thymomas and normal tissues. Over-expression of this microRNA cluster, which is normally silent in adult tissues, hyperactivates the PI3K/AKT/mTOR pathway. Treatment of IU-TAB1 cells, the only known thymoma AB cell line, with a panel of PI3K/AKT/mTOR inhibitors resulted in marked reduction of cell viability suggesting sensitivity to these agents. **Conclusions:** A large microRNA cluster on chr19q13.42 is a transcriptional hallmark of type A & AB thymomas. Furthermore, this cluster activates the PI3K pathway, suggesting the possible exploration of PI3K inhibitors in patients with these subtypes of tumor.

Translational/Clinical Research

Faculty

POSTER #103

THE SENSITIVITY OF SENTINEL LYMPH NODES IDENTIFIED WITH ROBOTIC FLUORESCENCE IMAGING FOR DETECTING METASTATIC ENDOMETRIAL CANCER: INTERIM RESULTS FROM THE FIRES TRIAL

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Objectives: Sentinel lymph node (SLN) mapping for endometrial cancer has been described as an alternative staging technique, but definitive data regarding its diagnostic accuracy are lacking. Fluorescence imaging of indocyanine green (ICG) dye is a novel feasible modality for SLN mapping. The FIRES trial (fluorescence imaging for robotic endometrial cancer sentinel node mapping) is a prospective, multicenter cohort study of comprehensively staged women with endometrial cancer. The primary objective of the trial is to estimate the sensitivity and negative predictive value (NPV) of SLNs for detecting metastatic disease. **Methods:** Patients with clinical stage I endometrial cancer undergoing robotic staging received cervical injection of ICG (1 mg) and both SLN mapping and pelvic para-aortic (PA) lymphadenectomy. All histologies and grades were eligible. All lymph nodes were evaluated with hematoxylin and eosin sectioning. SLNs were ultrastaged with immunohistochemistry for cytokeratin. This study has ongoing recruitment at seven United States centers. It is registered with Clinical Trials.gov. **Results:** 109 eligible patients have undergone attempted SLN mapping. The mean body mass index of the cohort is 35.2. Pelvic lymphadenectomy was performed in 106 patients (97%) and PA lymphadenectomy in 73 patients (69%). Three patients had aborted mapping and no nodes removed due to inability to perform lymphadenectomy; 96 patients (90.5%) had successful mapping (at least one SLN identified). Bilateral SLNs were identified in 66 of these patients (69%). The median number of SLNs was 4 (range, 0-15). A median number of 23 (range, 2-61) total LNs were removed per patient. Thirteen (12%) patients had stage IIIC disease, 11 of whom mapped a SLN. Among those who mapped, nodal metastases were correctly identified in the SLNs in all cases, yielding a sensitivity and NPV of 100%. Isolated PA metastatic disease was identified in SLNs of 2 patients (2%). Sixty-three percent (7) of nodal metastases were identified with ultrastaging pathology techniques. **Conclusions:** The interim results of the FIRES trial show a high degree of diagnostic accuracy for SLNs identified with robotic fluorescence imaging in women with endometrial cancer. There were no false-negative SLNs in patients who mapped. Replacement of complete lymphadenectomy for SLN biopsy is not advocated until the study's statistical endpoints have been met.

Translational/Clinical Research

Faculty

POSTER #104

PET/CT AND BIOMARKER ASSESSMENT OF HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) remains a global health problem with unique diagnostic and therapeutic challenges, including difficulties in identifying the highest risk patients. Previous work from our lab has established the murine multidrug resistance-2 mouse (MDR2) model of HCC as a reasonable preclinical model that parallels the changes seen in human inflammatory associated HCC (ref). **Methods:** ¹⁸F-FDG and ¹¹C-acetate PET/CT was performed on 12m MDR2^{-/-} mice with HCC and 12m MDR2^{+/+} control mice without HCC. In addition, serum alpha-fetoprotein (AFP), lysophosphatidic acid (LPA), cAMP and hepatic tumor necrosis factor alpha (TNFa) were quantified in 3-12m MDR2^{-/-} mice using commercially available ELISA kits. **Results:** Hepatic ¹⁸F-FDG metabolism was not significantly increased in MDR2^{-/-} mice. In contrast, hepatic ¹¹C-acetate metabolism was significantly increased in MDR2^{-/-} mice when compared to MDR2^{+/+} controls. Serum AFP and LPA levels increased in MDR2^{-/-} mice contemporaneous with the emergence of HCC on imaging. This was accompanied by a significant decrease in serum cAMP levels and an increase in hepatic TNFa. **Conclusion:** HCC imaging by ¹¹C-acetate PET/CT in MDR2^{-/-} mouse model tracks well with the clinical characteristics of human HCC where we have seen serologic markers and C11 acetate uptake with the emergence of liver cancer. It is reasonable to consider using ¹¹C-acetate PET/CT in evaluating extent of disease burden as well as tumor response after intervention.

Translational/Clinical Research

Faculty

POSTER #105

EXPAND YOUR RESEARCH: NEXT-GEN SEQUENCING, GENOTYPING, GENE EXPRESSION, AND EPIGENETICS AT THE CENTER FOR MEDICAL GENOMICS AT IUSM

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The Center for Medical Genomics (CMG) provides Indiana researchers with next-generation sequencing, SNP genotyping, gene expression and epigenetics. We provide expertise in experimental design, carry out the procedures, and assist with analyses and interpretation. These state-of-the-art technologies have enabled a large number of grants to be funded over the years, and have resulted in a very large number of publications. Our next-generation sequencing technology includes SOLiD5500xl, Ion Proton and Ion Torrent PGM (Personal Genome Machine). This set of instruments covers a wide range of next-generation sequencing capabilities from small bacterial genomes to the whole human genome, transcriptome (total RNA), small RNA, targeted DNA fragments, exome, ChIP-seq, and methyl-seq. We have generated sequencing data for 52 projects over the past two years. Our SNP genotyping facility, using the Sequenom MassArray platform, specializes in targeted genotyping of 20-30 SNPs per assay and is an excellent choice for candidate gene studies and for following up results from GWAS and next-generation sequencing. It has been a central part of several large, multi-site collaborative genetic studies, including Genetics of Alcoholism (COGA), bipolar disorder, osteoporosis and hypertension, as well as many smaller projects; it is most efficient for sets of approximately 370 samples. We have produced more than 20 million targeted SNP genotypes to date. This platform is also capable of measuring allele-specific gene expression, and targeted quantitative DNA methylation for epigenetics study. For many projects, microarrays offer a good alternative to next-generation sequencing for measuring gene expression. We use Affymetrix GeneChip microarrays, capable of measuring expression of nearly all genes in humans (and all exons within them), rats, mice and most model organisms, and can measure expression of miRNAs. We can also use RNA extracted from FFPE samples. We have carried out more than 6,700 GeneChip hybridizations to date in support of many different projects. The CMG partners with the Center for Computational Biology and Bioinformatics for data analysis. We are recognized as a Core Facility of the Indiana CTSI and available to faculty not only from IU and IUPUI, but also from Purdue and Notre Dame Universities.

Translational/Clinical Research Faculty

POSTER #106

EFFECTS OF DEVELOPMENTAL MICRORNA REGULATION IN LIVER ON DRUG DISPOSITION AND RESPONSE

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Many chemotherapy drugs such as tamoxifen and paclitaxel undergo hepatic metabolism, so alterations in drug metabolism and disposition protein expression in the liver due to aging can alter the efficacy and safety of these drugs. These changes can be characterized as either an increased or decreased expression due to aging, that translate to increased or decreased function in order to have an impact on patient response to chemotherapy. Although some of these changes in drug metabolizing enzyme expression have been previously observed, the mechanism responsible for these changes is unknown. In order to determine the cause of these changes in drug disposition, we focused on developmentally changing miRNAs in the liver, the primary site of drug metabolism. These miRNAs, through imperfect complimentary binding to the 3' UTR of the targeted mRNA, cause translational repression or degradation. We measured expression of 533 miRNAs in 90 human liver samples (30 fetal, 30 pediatric, 30 adult) using TaqMan Open Arrays. After correction for multiple testing using false discovery rate, 121 miRNAs were upregulated and 119 were downregulated from fetal to pediatric/adult populations and 2 and 3 from pediatric to adult. Next, using Ingenuity Program Analysis to predict miRNA targets among regulatory genes (HNF4A, PXR, CAR) and the top 32 ADME (absorption, distribution, metabolism, excretion) genes determined from PharmaADME, there were 132 miRNA-mRNA interactions either predicted or experimentally validated involving 85 miRNAs and 28 genes. These targeted mRNAs included many of the Phase I enzymes (CYP450s), Phase II enzymes (GSTs, NATs, SULTs, TPMT, UGTs), transporters (ABCs, SLCs, SLCOs), and regulatory proteins (HNF4A, PXR). Previously, hsa-miR-34a, which was upregulated 2-fold from pediatric to adult, was confirmed to target HNF4A, a transcriptional regulator of drug disposition genes. Furthermore, a SNP in the miRNA binding site of HNF4A was shown to reduce miRNA negative regulation by hsa-miR-34a, therefore causing an increase in HNF4A and its downstream targets such as CYP2D6, responsible in activation of tamoxifen, first line treatment for breast cancer. We retrospectively genotyped a cohort previously probed for CYP2D6 activity using dextromethorphan and showed that this SNP in HNF4A abolished the miRNA binding site and resulted in increased CYP2D6 activity. Collectively, this data suggests that age-related changes in miRNA expression have the ability to regulate drug metabolism and disposition by targeting enzymes and transporters directly or indirectly through upstream regulatory genes. Furthermore, SNPs in the miRNA binding sites within the 3' UTR of targeted enzymes, transporters, and regulatory genes mRNA, can also contribute to variable drug response in chemotherapy drugs metabolized in the liver. With the knowledge of validated miRNA-mRNA interactions and characterization of SNPs involved in creating or abolishing miRNA binding sites, personalized medicine across the age spectrum can be further advanced.

Translational/Clinical Research

Graduate Student

POSTER #107

PD0332991 INDUCES EPITHELIAL TO MESENCHYMAL TRANSITION IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) remains the fourth leading cause of cancer-related death among both men and women in the United States. Its overall survival rate of 6% underscores the need for improved therapies. Cancer cell proliferation and cell-cycle dysregulation is a major target in cancer treatment. Cyclin dependent kinase (CDK) and cyclin inhibitors act as anti-proliferative agents that target upregulated cell-cycle progression proteins in various tumors. The efficacy of PD0332991, a CDK4/6 inhibitor, is currently being investigated in clinical trials in patients with retinoblastoma protein (Rb)-positive breast, ovarian, and non-small-cell lung cancers but has not yet been examined for treatment in PDAC. We have recently shown that PD0332991 inhibits cell proliferation in PDAC cell lines but initiates epithelial to mesenchymal transition (EMT) and induces cancer cell invasion in some of these cells. Using human PDAC tissues and tissues from genetically engineered mouse models (GEMMs) of PDAC, we also recently showed that loss of RB function is common in PDAC. To further assess the therapeutic potential of PD0332991 in PDAC, we now compared its effects in PANC-1 human PDAC cells (wild-type *RB1* gene) and in murine PDAC cells devoid of Rb that were derived from a GEMM expressing mutant *KRAS*^{G12D} and devoid of RB (KRC mice). In PANC-1 cells, PD0332991 (1 μ M) inhibited proliferation and induced EMT. By contrast, in KRC 1022-4 murine PDAC cells, the same concentration of PD0332991 induced EMT without altering proliferation. Moreover, removal of PD0332991 from the incubation medium reversed its growth inhibitory actions in PANC-1 cells, but did not reverse its effects on EMT in either PANC-1 or KRC 1022-4 cells. Taken together, these findings suggest that PD0332991 may exert long term deleterious effects in patients with PDAC limiting its potential therapeutic use in this deadly cancer, except in conjunction with agents that target EMT. This study was supported, in part, by NCI grant R37-075059 to MK.

Translational/Clinical Research

Graduate Student

POSTER #108

CHOLESTERYL ESTER-DEPLETING NANOMEDICINE FOR CANCER SELECTIVE CHEMOTHERAPY

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Chemotherapy has been used to treat cancer for several decades but undesirable side effects remain a significant challenge. Here we introduce a new strategy that exploits cancer cells own cholesterol against them. Altered lipid metabolism is increasingly recognized as a hallmark of cancer cells. We found aberrant cholesteryl ester accumulation in a wide range of cancer cells by acyl-CoA cholesterol acyltransferase-1 (ACAT-1)-mediated cholesterol esterification. To safely and efficiently target the cholesterol esterification in cancer cells, we developed a cholesteryl ester-depleting nanomedicine called Avasimin, consisting of Avasimibe (ACAT-1 inhibitor) and human serum albumin. Using label-free Raman spectromicroscopy, we demonstrated that Avasimin significantly reduced cholesteryl esters in lipid droplets while elevating intracellular free cholesterol levels, which led to apoptosis and suppression of proliferation in a wide range of human cancer cell lines. Furthermore, with improvement of the water-solubility of Avasimibe by up to 40 times, Avasimin can be intravenously injected at high concentration, which increases the bioavailability of Avasimibe in blood and tumor compared to standard oral administration. Systemic treatment of Avasimin notably suppressed tumor growth and also extended the length of survival time in mouse models of human prostate and colon cancers. No adverse effects of Avasimin treatment to normal cells, blood components, and organs were observed. Thus, this study shows a new clinically viable strategy for cancer selective chemotherapy by targeting altered cholesterol metabolism of cancer cells.

Translational/Clinical Research

Graduate Student

POSTER #109

A NOVEL TH17-PRONE CD146+CCR5+ T CELL POPULATION AS AN EARLY MARKER OF INTESTINAL GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease (GVHD) of the gastrointestinal (GI) tract is a major limitation of allogeneic hematopoietic stem cell transplantation (HSCT). We performed proteomic analysis to identify biomarkers using plasma taken 14 days prior to clinical manifestations of GI-GVHD. We selected candidates that were increased at least 1.5 fold in plasma taken from GI-GVHD patients compared to HSCT patients without GVHD. The chemokine motif ligand 14 (CCL14) and CD146 were the two lead candidates. CCL14 binds to the chemokine receptor CCR5 on T cells. CD146 is expressed by activated T cells and endothelial cells. We analyzed peripheral blood cells from 214 HSCT patients (71 GI-GVHD, 48 No GVHD, 33 non-GVHD enteritis, 22 skin first GVHD, 40 isolated skin GVHD) at onset of symptoms. The frequency of CD146⁺CCR5⁺T cells was significantly increased in GI-GVHD patients compared to patients without GVHD ($p<0.001$), non-GVHD enteritis ($p<0.001$), or with isolated skin GVHD ($p=0.007$), but not with skin first and then GI-GVHD ($p=0.28$). We then classified patients into low and high-risk groups according to the median CD146⁺CCR5⁺ frequency in GI-GVHD patients (2.3%). A high frequency of CD146⁺CCR5⁺T cells predicted higher 6-month non-relapse mortality in patients who eventually developed GI-GVHD (42% vs. 20%, $p=0.02$). We then further characterized this population. In patients, CD146⁺CCR5⁺T cells had a Th1 and Th17 phenotype (ROR γ ⁺T-bet⁺) compared to CD146⁺CCR5⁺T cells. They also expressed a higher level of the activation marker ICOS ($p=0.01$). In vitro, naive T cells differentiated with both Th17 inducing cytokines and ICOS stimulation increased the percentage of CD146⁺CCR5⁺T cells (expressed hereafter as % mean \pm SEM) compared to naive T cells differentiated with Th1 and CD28 (4.9% \pm 0.8%, $p<0.05$), Th17 and CD28 (4.9% \pm 0.6%, $p<0.05$), or Th1 and ICOS (4.9% \pm 0.9%, $p<0.05$). Naive T cells differentiated with both Th17 and ICOS also co-expressed more Th1 and Th17 cytokines (IL-17A⁺IFN γ ⁺) than those differentiated with Th1 and CD28 (1.8% \pm 0.3%, $p<0.0001$), Th17 and CD28 (1.8% \pm 0.5%, $p<0.0001$), or Th1 and ICOS (1.8% \pm 0.4%, $p<0.0001$). Th17 polarization and ICOS stimulation also increased other Th17 markers (IL-22, CD161, CXCR6). Compared to CD146⁺T cells, CD146⁺T cells co-expressed more Th1 and Th17 cytokines (IL-17A⁺IFN γ ⁺) (15.9% \pm 4.8% vs. 1.8% \pm 0.9%, $p<0.05$) and pathogenic Th17 markers (GM-CSF, BATF, IL-23R). In addition, no matter the culture condition (Th1, Th17, CD28, or ICOS), sorted CD146⁺ T cells always co-expressed more Th1 and Th17 cytokines as compared to sorted CD146⁺ T cells, further suggesting that the CD146⁺ T cell subset is Th17 prone. We conclude that CD146⁺CCR5⁺T cell frequency is a cellular biomarker of GI-GVHD with prognostic value. ICOS is critical for the development of this CD146⁺CCR5⁺T cell population. This subset is Th17 biased. CCR5, ROR γ , and ICOS are drug-targetable and may represent new avenues to treat GVHD.

Translational/Clinical Research

Graduate Student

POSTER #110

DUPA CONJUGATION OF CYTOTOXIC INDENOISOQUINOLINE TOPOISOMERASE I INHIBITORS AS A METHOD FOR SELECTIVELY TARGETING PROSTATE CANCER CELLS

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Prostate cancer is the second-leading cause of cancer death of men in the United States. Current treatments are compromised by adverse side effects. Fortunately, most prostate cancer cells overexpress the prostate-specific membrane antigen (PSMA), whereas the receptor is present at low or undetectable levels in normal cells. This difference could be taken advantage of in order to selectively deliver non-specific cytotoxic drugs to these pathogenic cells while sparing normal cells that lack PSMA, thus improving potencies and reducing toxicities. PSMA is a very attractive therapeutic target that has high affinity for the ligand 2-[3-(1,3-Dicarboxypropyl)-Ureido]Pentanedioic Acid (DUPA) ($K_i = 8$ nM). After binding to a DUPA-drug conjugate, PSMA internalizes, unloads the conjugate, and returns to the surface. A release mechanism that would facilitate the intracellular cleavage of indenoisoquinoline topoisomerase I inhibitors from their DUPA conjugates was investigated in the present study. A suitable peptide linker was added as a spacer between the drug and the DUPA ligand in order to ensure the binding of PSMA and its ligand. In order to provide preliminary support for this methodology, two indenoisoquinoline topoisomerase I inhibitors and their DUPA conjugates were synthesized and tested in both LNCaP and 22RV1 cell cultures, both of which overexpress PSMA. All of the compounds exhibited GI_{50} values in the low nanomolar range. The efficacy in an animal model was determined by administering one of the two DUPA-drug conjugates to 22RV1 tumor xenograft-bearing mice, and the results showed a complete cessation of tumor growth and no toxicity (loss of body weight or death of mice) during the treatment period. The results demonstrate that it is possible to selectively target cytotoxic indenoisoquinoline topoisomerase I inhibitors to prostate cancer cells while reducing adverse side effects to normal tissues.

Translational/Clinical Research

Graduate Student

POSTER #111

NERVOUS SYSTEM SEQUELAE OF CHEMOTHERAPY TREATMENT: ASSOCIATIONS AND PROPOSED MECHANISMS

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Background Breast cancer treatment with chemotherapy often results in neurotoxic sequelae including chemotherapy-induced peripheral neuropathy (CIPN) and cognitive dysfunction. Although CIPN and cognitive dysfunction are often viewed as separate phenomena, we hypothesized that these effects may be related. **Methods** Breast cancer patients treated with (Ctx+, n=27) and without (Ctx-, n=26) chemotherapy and healthy controls (HC, n=26) were assayed post-surgery, pre-treatment and one month post-treatment. All but one Ctx+ treatment included paclitaxel or docetaxel. Peripheral neuropathy symptom severity (PNS) was evaluated with the sum of the self-reported FACT/GOG-Ntx 11-item scale, cerebral gray matter density (GMD) and perfusion were assessed using MRI, and cognitive complaints, depression, and fatigue were assessed with the Multiple Ability Self-Report Questionnaire (MASQ), the Center for Epidemiologic Studies Depression scale, and the Fatigue Symptom Inventory. **Results:** Chemotherapy treatment was associated with increased PNS and cognitive complaints ($p<0.01$), which remained significant after adjusting for fatigue and depression. PNS was associated with increased cerebral perfusion in the left anterior cingulate (LAC) gyrus, a region associated with pain processing. Interestingly, a previously published Ctx+ frontal GMD decrease in this cohort was correlated with the LAC perfusion change ($p<0.01$) and PNS change ($p<0.01$). Individuals with GMD decrease showed lower LAC perfusion and fewer PNS, while increased PNS were correlated with increased cognitive complaints ($p<0.05$) and depression and fatigue (both $p<0.01$). **Conclusions:** This is the first study to identify cerebral perfusion change associated with peripheral neuropathy symptoms in a breast cancer cohort. Our findings suggest that chemotherapy-associated gray matter decrease may also reduce LAC perfusion and associated pain perception. This finding and the observed relationships between CIPN symptoms, depression, fatigue, and cognitive complaints highlight the importance of further research on the causal pathways underlying these effects, and of developing therapies targeting this symptom cluster.

Translational/Clinical Research

Graduate Student

POSTER #112

EPIGENETIC TARGETING OF OVARIAN CANCER STEM CELLS

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In ovarian and other cancers, emerging data indicate that cancer stem cells contribute to chemoresistance and that their persistence alters clinical outcome. As epigenetic regulators play a major role in the control of normal stem cell differentiation, epigenetics may play a critical role in targeting this subpopulation. Epigenetic aberrations, especially DNA methylation, result in silencing of tumor suppressor and differentiation-associated genes and regulate ovarian cancer stem cell (OCSCs) survival. To test the hypothesis that DNA hypomethylating agents "reset" OCSCs towards differentiation, we investigated the effect of the new DNA methyltransferase inhibitor SGI-110 on OCSCs, defined as aldehyde dehydrogenase 1 (ALDH)(+) cells. We demonstrated that ALDH(+) OC cells possess multiple stem cell characteristics, were highly chemoresistant, and were enriched in xenografts residual after platinum therapy. Low dose SGI-110 reduced the stemness properties of ALDH(+) cells, including their tumor initiating capacity, resensitized these OCSCs to platinum, and induced re-expression of differentiation-associated genes. Maintenance treatment with SGI-110 after carboplatin inhibited OCSCs, caused profound global tumor hypomethylation, and delayed tumor progression, supporting epigenomic targeting as a novel strategy after platinum-based therapy in OC. Collectively, our data suggest that a strategy targeting DNA methylation in OC exerts potent anti-tumor activity by allowing elimination of OCSCs enriched in residual, platinum resistant tumors. Our study provides the first evidence that epigenome targeting strategies delay tumor progression by targeting and reprogramming residual cancer stem cells, suggesting that SGI-110 in combination with platinum has the potential to prevent recurrent and chemoresistant OC.

Translational/Clinical Research

Graduate Student

POSTER #113

PROBING FIBROBLAST GENE EXPRESSION TO DEVELOP STRATEGIES FOR NIPPLE REGENERATION.

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Mastectomy still is a common outcome for breast cancer patients, occurring in ~1/3 of the 1.5 million cases worldwide. While breast reconstruction is becoming more routine, approaches for replacement of the nipple still lag behind. The nipple is the aesthetic focal point of the breast, and similar to lip, palm and sole, it is an example of specialized skin. Sites of specialized skin are characterized by epidermal layers with reduced numbers of appendages, distinct stratification and unique keratin expression. In addition, skin of the nipple exhibits extracellular matrix with higher carbohydrate content and smaller collagen bundles, a denser capillary network, increased numbers of nerve fibers, monocytes, mast cells, as well as dermal melanocytes. Since all of these characteristics are dependent on the inductive and supportive capacity of the connective tissue fibroblasts, we hypothesized that these cells would express a unique subset of growth factors relative to dermis. To examine this, we used the nipple-like ventral skin of the keratin 14 promoter driven parathyroid hormone related protein transgenic mouse (K14-PTHrP) as a source to probe gene expression from these unique fibroblasts. Fibroblasts were isolated from the K14-PTHrP mice and the ventral skin of wild-type littermates using fluorescent activated cell sorting for CD140. RNA was purified and subject to microarray with the Mouse Gene 1.0 ST chip. Analysis of the gene expression profile revealed more than 500 genes that were differentially regulated greater than 2-fold in nipple fibroblasts as compared to the wild type, including extracellular matrix genes, growth factors, cytokines and several hormone receptors. As anticipated, nipple stromal cells express a profile of genes consistent with reduced canonical Wnt signaling, as has been reported for fibroblasts from the palm and the sole. Interestingly, tumor growth factor-beta 1 (TGF β) levels were reduced and a key regulated transcript hepatocyte growth factor (HGF) was up-regulated in nipple fibroblasts. We confirmed that HGF is highly expressed in nipple and nipple-like ventral skin of K14-PTHrP mouse *in vitro* and *in vivo* but not in keratinocytes. Moreover, HGF is further up-regulated in the nipple during growth of the structure associated with pregnancy and lactation. HGF has a broad spectrum of effects on tissue serving as a key epithelial morphogen, altering wound healing properties and influencing pigmentation. We have therefore focused on the TGF/HGF ratio for tissue engineering approaches to nipple regeneration.

Translational/Clinical Research

Graduate Student

POSTER #114

DETECTION OF HUMAN TELOMERASE IN PLASMA: A POTENTIAL SIMPLE BLOOD TEST FOR CANCER DIAGNOSIS

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Telomerase is a unique ribonucleoprotein enzyme that maintains chromosomal telomere length. Telomerase activity has been shown to be very low or absent in human non-malignant somatic cells, however, is activated in nearly 90% of human cancers. Therefore, telomerase has been proposed to be a useful diagnostic marker for cancer. In this study, we investigated whether the presence of cancer correlates with telomerase activity in the corresponding plasma, thus representing a surrogate serological marker for cancer patients. Plasma telomerase activity was measured by the TRAP (Telomeric Repeat Amplification Protocol) assay in 5 patients with colon cancer, 6 patients with breast cancer, and 7 healthy control subjects. The TRAP is a PCR-based method and takes only a few hours to obtain the results. Our preliminary data show a trend that telomerase activity in plasma distinguishes between healthy subjects and cancer patients. Thus, our finding provides an idea that a useful blood test for cancer diagnosis may be developed using the TRAP assay in plasma. Further case-control studies are necessary to assess the current outcome.

Translational/Clinical Research

Medical Student

POSTER #115

6 MONTH DOSE-SPECIFIC TUMOR RESPONSE FOLLOWING UNILOBAR YTTRIUM-90 RADIOEMBOLIZATION FOR HEPATOCELLULAR CARCINOMA.

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Purpose: To determine individual lesion dose-specific multi detector computed tomography (MDCT) response 6 months following unilobar radioembolization (Y90) for hepatocellular carcinoma (HCC). **Materials and Methods:** Immediate-post therapy MDCT images of patients who underwent unilobar Y90 for HCC between February 2011 and July 2012 and had 6 month follow up MDCT scans were evaluated. Individual tumors within the treated volumes were measured using WHO, RECIST, mRECIST, and 3D EASL criteria. Y90 activity on corresponding immediate-post-Y90 PET images was measured, converted into dose maps using voxel based S value MIRD methodology, and individual lesion dose was calculated using both mean lesion and voxel threshold dose schemes. 6 month follow up triple phase MDCT imaging was evaluated using the same measurement criteria and each lesion was labeled as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). **Results:** 34 patients with 71 HCC lesions underwent unilobar Y90 therapy with acceptable post procedural PET/CT. 29 patients with 61 lesions were included. 61 included lesions with an average follow up of 222 days (SE \pm 61.7 days) received a mean dose of 206 Gy (SE \pm 172 Gy) with minimum and maximum lesion doses of 30 and 1091 Gy. We observed a significant dose-reponse relationship for volume, mRECIST and 3D EASL measurements (all p values <0.05). 3D EASL: CR will be achieved with 70% and 90% probability when 31%, [25%] and 77%, [65%] of lesion voxels receive 175 Gy and [200 Gy] respectively. The fractional response sharply improved beyond 150 Gy. **Conclusion:** Lesion PR and CR are achievable through unilobar radioembolization with increasing dose. 3D EASL and mRECIST suggest lower doses are necessary to achieve 90% likelihood of CR when compared to RECIST, WHO, and volume measurements. 3D EASL suggests 90% CR is achievable when 77% of a lesion receives 175 Gy. Voxel dose based evaluations suggest individual internal lesion variability, which likely alters response.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #116

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/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #117

PROTON RADIOTHERAPY FOR MIDLINE CNS LESIONS: A CLASS SOLUTION

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Objective: Midline and central lesions of the brain requiring conventional radiation therapy (RT) present complex difficulties in dose avoidance to organs at risk (OAR). In either definitive or adjuvant settings, proper RT coverage of these lesions involves unnecessary treatment of large volumes of normal brain. We propose a class solution which has not been previously described for these lesions using proton therapy (PrT). **Materials/Methods:** The records of the IU Health Proton Therapy Center were reviewed for patients presenting between 1 January 2005 and 1 October 2013 with midline central nervous system (CNS) lesions. Twenty-four patients were identified. After institutional review board approval was granted, their dosimetry was reviewed for target volume doses and OAR dose avoidance. **Results:** Most of these cases were meningiomas (8 cases); the next most prevalent were craniopharyngiomas (6 cases). The others were various different deep mid-line brain tumors (10 cases). In all cases, fields formed by vertex and anterior and/or posterior superior oblique PrT beams along the mid-sagittal plane were used to provide coverage with minimal dose to the brainstem deep, or to the cerebral hemispheres. The median prescribed dose to target PTV for treating these patients was 54.0 Gy RBE (range 48.6 to 62.5 Gy RBE) with a mean dose of 53.5 Gy RBE. The average of the mean doses to the brainstems using these fields in the twenty-four plans was 17.3 Gy RBE (range 0.0 Gy to 44.7 Gy RBE). Similarly, the average of the mean doses to the hippocampi was 15.8 Gy RBE (range 0.0 Gy to 52.6 Gy RBE). **Conclusions:** We consider these patients to be optimally treated with proton radiotherapy (PrT) and preferentially refer patients whenever possible. The use of modified mid-sagittal PrT schemas allows for treatment of midline CNS lesions with sparing of most of the uninvolved brain.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #118

DECITABINE REACTIVATED PATHWAYS IN PLATINUM RESISTANT OVARIAN CANCER

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Combination therapy with decitabine, a DNA methyltransferase inhibitor and carboplatin resensitized chemoresistant ovarian cancer (OC) to platinum inducing promising clinical activity. We investigated gene expression profiles in tumor biopsies to identify decitabine-reactivated pathways associated with clinical response. Gene expression profiling was performed using RNA isolated from frozen paired tumor biopsies before and 8 days after decitabine treatment from 17 patients with platinum resistant OC. Bioinformatic analysis included unsupervised hierarchical clustering and pathway and gene set enrichment analyses (GSEA) distinguishing profiles of "responders" (progression-free survival, PFS > 6 months) and "non-responders" (PFS < 6 months). Functional validation of selected results was performed in OC cells and tumors. Pre-treatment tumors from responders expressed genes associated with enhanced glycosphingolipid biosynthesis, translational misregulation, decreased ATP-binding cassette (ABC) transporter expression, transforming growth factor beta (TGF- β) signaling, and numerous metabolic pathways. Analysis of post-treatment biopsies from responders revealed overexpression of genes associated with reduced Hedgehog (Hh) pathway signaling, reduced DNA repair/replication, and cancer-associated metabolism. Gene Ontology and GSEA analyses revealed upregulation of genes associated with glycosaminoglycan binding, cell-matrix adhesion, and cell-substrate adhesion. Computational findings were substantiated by experimental validation of expression of key genes involved in two critical pathways affected by decitabine (TGF- β and Hh). Gene expression profiling identified specific pathways altered by decitabine and associated with platinum resensitization and clinical benefit in OC. Our data could influence patient stratification for future studies using epigenetic therapies

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #119

HEPATIC OLIGOMETASTASES TREATED WITH STEREOTACTIC BODY RADIATION THERAPY: UPDATED 10 YEAR ANALYSIS OF THE INDIANA UNIVERSITY EXPERIENCE

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Purpose: Stereotactic body radiation therapy (SBRT) is a non-invasive, effective technique in the treatment of a limited number of liver metastases from solid tumors. We present the update of our single institution SBRT experience outcomes and toxicity. This is thought to be the largest single institution experience present in the literature. **Patients and Methods:** We treated 81 patients 89 different times, for a total of 106 lesions. Inclusion criteria were patients with 1-3 liver metastases without evidence of extra-hepatic progression, and at least 700 cc of liver (minus the GTV) receiving less than 1500 cGy. The majority of patients had colorectal primary cancers. Other diagnoses included: Non-colorectal GI, breast, ovarian, NSCLC, and others. Among the lesions treated the majority received prior chemotherapy. **Results:** The median overall survival was 31 months. Kaplan Meier survival estimates at 12, 24, 36, and 48 months were 85%, 61%, 34%, and 18%. The local control rate was 94% with Kaplan Meier estimates at 12, 24, 36, and 48 months being 95%, 90%, 90% and 90%. The observed toxicities noted among the 106 treatments included mostly CTC grade 1-2 toxicity with only two grade 4 and one grade 5 toxicity. There was no difference in the toxicity based on the primary site. The majority of patients had grade 1 to 2 non-hepatic GI toxicity, grade 1-2 fatigue or grade 1-2 chest wall pain. The most severe toxicity noted was 1 patient with grade 5 hepatic toxicity and 2 patients with grade 4 hepatic toxicity. In addition, two patients developed grade 1 pleural effusions thought to be secondary to treatment. Parameters affecting toxicity were evaluated based on grade 4-5 hepatic toxicity. Generalized estimating equation models were fit to test for an association between each categorical factor and grade 4-5 hepatic toxicity. Linear mixed models were used to test for an association between each continuous factor and grade 4-5 hepatic toxicity. The only patient with grade 5 toxicity was treated three different times to a total of four lesions. **Conclusion:** Stereotactic body radiation therapy is a safe and effective treatment for patients with 1-3 liver metastases with a limited toxicity profile.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #120

AGE AND GENDER PATTERNS IN THE USE OF ANESTHESIA FOR CHILDREN RECEIVING RADIOTHERAPY

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Objective: Complications in pediatric patients receiving anesthesia-assisted radiation therapy (AART) are rare, but the procedure is time, space and resource consuming. Influencing the decision to use AART may include ill-defined patient functional or psychological factors as well as parent/physician discretion. We analyzed our experience with AART for identifiable patterns regarding age and gender in children receiving daily proton radiation therapy. **Materials and Methods:** After Institutional Review Board approval, we reviewed our records from the Indiana University Health Proton Therapy Center for patients requiring AART between January 9, 2004, and June 30, 2013, with respect to age and gender in our pediatric patients (defined as patients <18 years of age). **Results:** A total of 390 pediatric patients were treated in this era. Of these, 182 were girls and 208 were boys. The median age at start of treatment for pediatric patients treated with AART was 4 years vs. 13 years for those not requiring AART. Similarly, the median age at start of treatment for pediatric boys and girls treated with AART compared to those not requiring AART was 4 years vs. 13 years and 3 years vs. 12 years, respectively. Overall, the likelihood of requiring AART decreased with age after 3 years. All children <3 years of age, 50% of children <7-8 years of age, and 10% of children 12 years of age required AART. There was no significant difference in any age group by gender. **Conclusion:** While children aged <3 invariably require AART in our experience, not surprisingly, the need for AART decreases with increasing age. A small cadre of older children have functional or other issues that require them to receive AART for daily radiation treatment. There is no difference in AART requirement by gender. This pattern of care data may assist centers in pre-planning needs for pediatric radiation therapy cases referred from distant referral sites. Additionally, it establishes a baseline curve for AART requirements in the pediatric population from which future studies can build upon.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #121

ENGRAFTMENT OF HUMAN PERIPHERAL BLOOD STEM CELLS IN NOD/SCID/IL-2R γ NULL MICE FOLLOWING LONG-TERM CRYOPRESERVATION

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Background: Peripheral blood stem cells (PBSC) are usually cryopreserved awaiting clinical use. Data is limited regarding how long PBSC can be cryopreserved and retain abilities to engraft. Existing data suggests that PBSC units can reconstitute patients up to 11 years after cryopreservation, however, decreased colony forming activity has been found in PBSC units stored longer than 10 years. No data on transplantation of cryopreserved PBSC into immunodeficient mice has been published to date. Hypothesis: Long-term cryopreservation does not negatively impact recovery of PBSC nor ability to engraft in NOD/SCID/IL2R γ null (NSG) mice. **Design:** Ten discarded PBSC units were previously collected and cryopreserved at our institution for clinical use. Following standard clinical thaw procedures, hematopoietic stem cell recovery was measured by the following: post-thaw cellular counts and viability as compared to pre-freeze, in vitro colony forming assays for CFU-GM and in vivo transplantation into 25 NSG mice Prior to transplantation into mice, PBSC units were thawed, washed once with PBS buffer (phosphate-buffered saline supplemented with 1 mM EDTA) and CD34 selected via magnetic microbeads (Miltenyi). Twenty-five immunodeficient mice were conditioned with 300-cGy total-body irradiation, and $\sim 2.5 \times 10^5$ CD34+ cells/mouse were transplanted. **Results:** *PBSC Unit Characteristics:* 10 PBSC units with mean of 17 years in cryopreservation (range 13.6-18.3 years). Mean donor age at time of collection was 47 years (range 24-66 years).

PBSC Cell Recovery:	mean \pm SD (range)	Colony Forming Activity:
% Total cell recovery:	88 \pm 12% (68-110%)	BFU-E growth in 9 of 10 units post-thaw.
% Post-thaw viability:	69 \pm 17% (34-86%)	CFU-GM growth in 7 of 10 units post-thaw.

Mouse Engraftment Data: All mice demonstrated long-term engraftment at 12 weeks with mean 34 \pm 24% human CD45+ cells. All mice showed evidence of differentiation with presence of human CD19, CD3 and CD33+ cells. Harvested bone marrow from all mice demonstrated growth of erythroid and myeloid colonies. **Conclusion:** We demonstrated highly efficient total cell recovery of 88% in all ten thawed PBSC units and cell viability of 62 to 86% in eight of the ten units. Most units showed growth of myeloid and erythroid colonies with exception of those with lowest post-thaw viability. More importantly, engraftment (both short- and long-term) was demonstrated in NSG mice with differentiation into multilineage phenotypes. PBSC can be cryopreserved for 17+ years and retain colony forming ability, engraft into NSG mice and likely lead to successful clinical transplantation.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #122

MODULATION OF TELOMERASE IN HUMAN NORMAL BREAST LUMINAL PROGENITOR CELLS

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Telomeres are essential for genomic integrity and their dysfunction has been broadly implicated in tumorigenesis. Epithelial tissues, including the mammary gland of humans as well as mice, undergo extensive cell turnover, and recent studies indicate that this involves an organized hierarchical differentiation process. To date, analysis of telomere length regulation in normal mammary epithelial cells has been limited to reports of shorter telomeres in luminal cells in histological sections. However, the presence of telomere fusions has been noted in primary mammary epithelial cells passaged in vitro, and we have recently reported that telomere dysfunction-specific chromosomal fusions are common in early-stage breast cancers. Here we show that phenotypically separable compartments of normal human mammary epithelial cell subpopulations, isolated from normal tissue samples, have markedly different telomere lengths and telomerase activity, along with differential expression of a large set of telomere and DNA damage response genes. These subset-specific telomere biological alterations point to a significant change in telomere maintenance during normal human mammary cell differentiation. Interestingly, a subset that is highly enriched in luminal progenitors (LPs) is uniquely characterized by critically short telomeres but possessed high telomerase. Here we study the modulation mechanism of telomerase control in LPs, which may act as a tumor suppressive pathway. Previous studies showed that telomerase needs to be associated with Cajal bodies in order to be recruited to telomere. Our preliminary data show that the telomerase in LP does not form complex with Cajal bodies. In addition, the CST complex is known to bind telomeric ends to inhibit telomerase action. Our immunofluorescence data suggests that telomerase in LP cells co-localizes with the CST complex. Thus, we are beginning to understand how telomerase is controlled in normal LPs and how this process may be altered during tumorigenesis.

Translational/Clinical Research

Post-Doctoral/Medical Fellow

POSTER #123

BIOMARKERS FOR ACUTE GRAFT-VERSUS-HOST-DISEASE FOLLOWING NONMYELOABLATIVE ALLOTRANSPLANTATION

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Five candidate plasma biomarkers (ST2, REG3 α , elafin, TNFR1, sIL2R α) were measured at specific time-points following cyclophosphamide/fludaraine-based nonmyeloablative allotransplantation (NMAT) in patients who did or did not develop acute graft-versus-host-disease (aGVHD). Plasma samples from 34 patients were analyzed at days +7, +14, +21 and +30. At a median follow-up of 358 days, 17 patients had experienced aGVHD with a median time to onset of day +36. Risk of aGVHD was associated with elevated plasma ST2 concentrations at day +7 (c-stat=0.72, p=0.03), day +14 (c-stat=0.74, p=0.04), and day +21 (c-stat=0.75, p=0.02); elevated plasma REG3 α concentrations at day +14 (c-stat=0.73, p=0.03), day +21 (c-stat=0.76, p=0.01) and day +30 (c-stat=0.73, p=0.03); and elevated elafin at day +14 (c-stat=0.71, p=0.04). Plasma concentrations of TNFR1 and sIL2R α were not associated with aGVHD risk at any of the time-points studied. This study identified ST2, REG3 α and elafin as promising prognostic biomarkers to evaluate risk of aGVHD following Cy/Flu-based NMAT. These results need to be confirmed in an independent validation cohort.

Translational/Clinical Research

Post-Doctoral/Medical Fellow