The laboratory of Dr. Paul Dent is dedicated to developmental cancer therapeutics. NIH and Department of Defense funded projects have focused on the rational combination of drugs and other molecules to kill tumor cells in vitro and in vivo. Studies have focused on the underlying molecular mechanisms by which our drug combinations synergize to kill tumor cells including modulation of apoptosis, autophagy, colony formation and changes in multiple cell signaling pathways. Specific projects have focused on breast; brain; sarcoma; RCC; NSCLC and liver/pancreatic cancers. Our studies in breast cancer have resulted in a Phase I trial combining pemetrexed and sorafenib which has moved into Phase II evaluation. Our studies in brain cancer have resulted in two Phase I trials. Our phase I trial in pancreatic adenocarcinoma has facilitated tumor mass reduction and Whipple surgeries in multiple patients, including one CR. At present the Dent laboratory has translated 11 oncology therapy concepts into the clinic, with a new trial based on their data also to open in London.

Education

- B.Sc. (1st) 1988, Biochemistry, University of Newcastle upon Tyne, England
- Ph.D. 1992, (with Professor Sir P. Cohen, F.R.S.) Biochemistry, University of Dundee, Scotland

Post-Doc

- Postdoctoral Fellow (with Professor T. W. Sturgill), University of Virginia, Charlottesville, Virginia
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<td>Registration and Poster Presentations (Breakfast will be served.)</td>
<td>All</td>
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<tr>
<td>8:30 – 8:45 am</td>
<td>Opening Remarks and Introduction</td>
<td>Augusto Ochoa, MD</td>
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<td>LSU Health Sciences Center</td>
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<td>Sven Davison</td>
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<td>Louisiana Cancer Research Center</td>
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<td>8:45 – 9:30 am</td>
<td>“Break On Through To The Other Side: Macrophage Contributions To Early Breast Cancer Progression”</td>
<td>Heather Machado, PhD</td>
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<td>Tulane University</td>
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<td>9:30 – 10:15 am</td>
<td>“Rab13 Delivered by Lymph Node Stromal Cell-derived Extracellular Vesicles Effects Colorectal Cancer Growth and Metastasis”</td>
<td>Grace Maresh, PhD</td>
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<td>Ochsner Health System</td>
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<td>10:15 - 11:00 am</td>
<td>“Cancer Bioinformatics: Challenges and Methods”</td>
<td>Kun Zhang, PhD</td>
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<td>Xavier University</td>
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<tr>
<td>11:00 am – 12:30 pm</td>
<td>Poster Viewing and Lunch</td>
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<tr>
<td>12:30 – 1:45 pm</td>
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<td>Keynote Speaker</td>
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<td>1:45 – 2:00 pm</td>
<td>Break</td>
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<tr>
<td>2:00 – 2:45 pm</td>
<td>“Lipid Metabolism And Chronic Inflammation In Cancer – Mechanisms And New Treatment Opportunities”</td>
<td>Augusto Ochoa, MD</td>
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<td>LSU Health Sciences Center</td>
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<tr>
<td>2:45 – 3:00 pm</td>
<td>Closing Remarks and Awards</td>
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<td>Prescott Deininger, PhD</td>
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<td>3:00 – 4:00 pm</td>
<td>Final Poster Viewing</td>
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Section 2-1

Cancer Genetics Abstracts
Polycyclic aromatic hydrocarbons (PAHs) comprise a large family of toxic environmental contaminants that come from natural and anthropogenic sources. Crude oil spills, such as the one from the MC252 (BP) well, introduce large amounts of PAHs into the environment. We used a transgenic yeast-based reporter assay that detects human aryl hydrocarbon receptor (AhR) signaling as a screening platform to identify toxic compounds in fresh crude oil from the MC252 well. Chrysene PAHs were a major component of the bioactivity in the screen for AhR activation. Most toxicity studies have been directed toward identifying the effects of chrysene itself, but less is known about the methylated derivatives that are more abundant in crude oil. We characterized the abilities of the six mono-methyl chrysenes to activate human AhR signaling in comparison to chrysene. 4-methylchrysene was the most potent AhR activator, having more than 6-fold activity than the parent chrysene, but it was not among the methyl chrysenes present in MC 252 oil. The 96-hour exposures to HepG2 cells revealed several of the methyl chrysenes were toxic at 5 μM treatments when assessed using sulforhodamine B dye binding assays. Cytotoxicity and AhR activation potential of the chrysenes were not tightly correlated. Ongoing studies are designed to compare and characterize gene expression changes in HepG2 cells caused by exposures to chrysenes and its alkylated derivatives. (Support: This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org)

Chaperone-mediated Autophagy Promotes Beclin1 Degradation in Persistently Infected HCV Cell Culture
Yucel Aydin1, Christopher M Stephens2, Srinivas Chava2, Zahra Heidari2, Rajesh Panigrahi2, Donkita Danielle Williams3, Kylar Wiltz3, Antoinette Bell4, Wallace Wilson5, Krzysztof Reiss6, and Srikanta Dash7
1-Department of Pathology and Laboratory Medicine, Tulane University Health Sciences Center, New Orleans, Louisiana, USA. 2-Department of Chemical and Biomedical Engineering, Tulane University, New Orleans, Louisiana, USA. 3-School of Medicine, LSU Health Sciences Center, New Orleans, Louisiana, USA

Introduction: We previously demonstrated that the majority of HCC grown in cirrhotic liver undergoes switching from a protective state characterized by high macroautophagy and low chaperone-mediated autophagy (CMA) to an HCC-promoting state characterized by low macroautophagy and high CMA. The molecular and cellular mechanism of this autophagy switching is unknown. Aim: To investigate the molecular and cellular mechanism of autophagy switching during chronic HCV infection. Results: In this study, we found that the mRNA level of the PERK axis of the unfolded protein response (UPR) increases significantly compared to the IRE1α and ATF6 branches in persistently HCV infected HuH-7.5 cells. Activation of the PERK pathway leads to prominent nuclear translocation of Nrf2 in infected culture that increased transcription of the cytoprotective genes Hsc70 and LAMP2A and precipitated the induction of Nrf2 and LAMP2A reduced cell viability, suggesting that autophagy switching is a pro-survival mechanism induced by high endoplasmic stress. Conclusion: We report here a novel mechanism through which sustained activation of the PERK axis of ER-stress during chronic HCV infection triggers oncogenic Nrf2 signaling, that promotes cell survival through autophagy switching.

CCAAT Enhancer Binding Protein β (C/EBPβ) Isoforms and Regulation of DCIS Progression
Caitlin M. Burke1, Sheng Zheng2, Heather L. Machado1
1-Department of Biochemistry and Molecular Biology, Tulane School of Medicine, New Orleans, LA

Ductal carcinoma in situ (DCIS) is defined as hyperplastic intraductal mammary epithelium contained within an intact myoepithelial layer. DCIS lesions often progress to invasive ductal carcinomas (IDC), though no current predictors indicate which DCIS patients will progress to IDC. Some metastatic breast cancers have been shown to overexpress the LIP isoform of C/EBP/Enhancer Binding Protein β (C/EBPβ). C/EBPβ is a transcription factor involved in cell proliferation and differentiation, and its dysregulation has been shown to favor tumor progression in mice and in humans. It functions as a hetero- or homodimer of its possible isoforms: LAP1, LAP2, and LIP. LIP, a transcriptional repressor, can dimerize with the other two C/EBPβ isoforms, LAP1 and LAP2, which are considered potent transcriptional activators. Previous studies have suggested that LIP can also act as a transcriptional activator by binding DNA directly, or by interacting with unknown cofactors to enhance transcription, though a mechanism has yet to be identified. We hypothesize that C/EBPβ isoforms either directly or indirectly bind the Axl promoter, and induce Axl expression, which as been shown to promote breast cancer progression.

To investigate if C/EBPβ isoform regulation promotes DCIS progression, we will first generate C/EBPβ-null cell lines. Our data showed that treatment of C/EBPβ+/- DCIS.com and MCF10AT cell lines with overexpression vectors, pEIZ-LIP, pEIZ-LAP1, and pEIZ-LAP2, induced changes in Axl expression, a receptor tyrosine kinase highly expressed in invasive breast cancer. pEIZ-LAP1 and pEIZ-LAP2 suppressed Axl expression in C/EBPβ+/- DCIS.com cells, while pEIZ-LIP enhances it, suggesting that the ratio of C/EBPβ isoforms influences Axl expression, which is a prognostic indicator in breast cancer. By treating our C/EBPβ null cell lines with these same overexpression vectors and testing them using the MiND model, we hope to recapitulate these results in vivo. Additionally, by
Interactions Among 8q24 Genes Associated With Prostate Cancer Risk In African American Men

Presenter: Catherine Callan

University: LSU Health Sciences Center New Orleans

Collaborators: Hui-Yi Lin and Heng-Yuan Tung

Field of Research: Cancer Genetics

African American (AA) men have a higher risk to develop prostate cancer (PCa) than Whites. While the causes of PCa are not fully understood, genetic variation has a clear impact on disease. Most single nucleotide polymorphism (SNP) association studies are focused on single SNPs, but SNP-SNP interactions are suggested to reveal crosstalk between SNPs (genes) for increasing power to predict disease risk.

Using the 2,253 AA PCa patients and 2,423 AA controls, SNP main effects and SNP-SNP interactions were evaluated among 205 SNPs, passed quality control in chromosomal region 8q24. Region 8q24 has been shown to be an area of great interest due to numerous variants found to be more common in AA men in this region. We randomly selected half of the subjects in the discovery set and other half in the validation set. The promising results identified in the discovery set were evaluated in the validation and combined set. The SNP-SNP interaction analyses were evaluated using SNP interaction pattern identifier (SIPI), which assesses 45 interaction models, by taking non-hierarchical models, inheritance modes, and mode coding direction into consideration. The best model for each SNP pair is chosen based upon Bayesian information criterion (BIC). There were 79 SNP-SNP pairs identified by SIPI associated with PCa (p<0.001 in the combined set).

Among top pairs, there were two super SNPs (rs16902359 and rs9642880) in CASC11, which were involved in 56 and 9 pairs, and 59 pairs involved interactions between CASC11 and PVT1. Both genes have been shown to be associated with PCa risk, but interactions between these two genes have not been reported. Our study has demonstrated that SIPI is a powerful and thorough tool that can be used to detect SNP-SNP interactions because SIPI considers non-hierarchical models, inheritance mode, and 45 different biologically meaningful patterns. Using SIPI, we have identified 79 SNP-SNP pairs of interest in relation to PCa risk of AA men. The interaction of PVT1 and CASC11 provides a great start to future studies in genetic associations where the goal is to build prediction models for clinical use.

Interleukin-17 (II-17) Promotes Metastasis In An Orthotopic Mouse Model Of Prostate Cancer

Cunningham D, Nie C, Zhang Q, Liu S, Lin M, Parajuli K, You Z

1 Department of Structural and Cellular Biology, Tulane University School of Medicine
2 Department of Orthopedics, Tulane University School of Medicine
3 Tulane Cancer Center, Louisiana Cancer Research Consortium
4 Tulane Center for Aging
5 Tulane Center for Stem Cell Research and Regenerative Medicine

Field of Research – Cancer Genetics/Immunology

Morbidity and mortality of prostate cancer (PCa) patients are caused mainly by metastases. Frequent metastatic sites in humans include the regional lymph nodes, bone, lungs, brain, and liver. Current genetically engineered mouse models show a limited metastatic phenotype. Here we describe the use of a mouse prostate adenocarcinoma cell line termed MPC3 that is able to grow in immunocompetent C57BL/6j mice. This study relies on the novel observation that this cell line is able to grow in these mice, providing a unique tool for PCa researchers interested in modeling PCa growth and metastasis in mice with an adaptive immune response. Using a luciferase tracking vector, we show that mice receiving an inoculation of cancer cells in Matrigel™ with 100 ng recombinant mouse IL-17 have larger tumors over the course of time as well as show increased metastatic incidence at endpoint with poorer survival when compared to mice receiving an inoculation loaded with control buffer. This study provides evidence to support the hypothesis that pro-inflammatory immune factors, in this case IL-17, play a part in promoting prostate cancer metastasis. This work was partially supported by Department of Defense Health Program through the Prostate Cancer Research Program (W81XWH-14-1-0050, W81XWH-14-1-0149, and W81XWH-14-1-0458), by National Institutes of Health (P20GM103518, R01CA174714), and by the Developmental Fund of Tulane Cancer Center (TCC) and Louisiana Cancer Research Consortium (LCRC) Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Defense.

Targeting Tumor Microenvironment In Glioblastoma Multiforme, A Novel Treatment


2018 LCRC Annual Retreat Booklet
Suppression of PDHX by miR-27b Deregulates Cell Metabolism And Promotes Growth In Breast Cancer

Steven C. Eastlack, Shengli Dong, and Suresh K. Alahari
Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, New Orleans, LA

Field of Research: Cancer Genetics

Abstract: The disruption of normal gene regulation due to microRNA dysfunction is a frequently cited cause of human disease, particularly in cancer. MicroRNA-27b is an example of such a pro-oncogenic miRNA, and is commonly upregulated in breast cancer. We wished to further characterize the role of miR-27b in breast cancer by identifying additional target transcripts under its control. Our findings indicate Pyruvate Dehydrogenase Protein X (PDHX) is a novel target. PDHX is an essential structural component of the Pyruvate Dehydrogenase complex which catalyzes pyruvate’s conversion into acetyl-CoA—thereby linking glycolysis with downstream oxidative metabolism. By suppressing PDHX, miR-27b effectively uncouples glycolysis from the TCA cycle. Notably, a hallmark of tumor cell metabolism is the propensity to consume glucose heavily, owing to increased flux through glycolysis and
lactate production from glycolytic pyruvate. Accordingly, our experiments showed that elevated miR-27b inhibited activity of the PDH complex, corresponding with increased pyruvate and lactate levels and reduced citrate levels, which requires acetyl-CoA to be produced in the TCA cycle. Additionally, inhibition of PDHX by miR-27b enhanced cell proliferation, arising from the reconfigured metabolism offering more favorable conditions for biosynthesis and growth. We confirmed the effects of miR-27b are due to its explicit targeting of PDHX by replicating these experiments in the context of PDHX-specific knockdown. Thus, we propose a novel mechanism for miR-27b to enhance cancer cell growth—by suppressing PDHX, miR-27b impedes oxidation of glycolytic pyruvate by the PDH complex, leading to accumulation of the products of glycolysis, thereby freeing these metabolites to serve as substrates for the biosynthetic reactions needed to drive cancer cell proliferation. Taken together, our findings reveal a novel association between PDHX and miR-27b in which the tumor-suppressive effects of PDH are mitigated by this oncogenic miRNA.

Characterizing Novel Regions of the L1 Orf2 Protein

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LCRC Scientific Program Assignment: Cancer Genetics

Long Interspersed Element 1 (LINE-1) is a retrotransposon that is present in 500,000 copies in the human genome. Along with Alu and SVA elements, these three retrotransposons account for more than a third of the human genome. These mobile elements are able to copy themselves via an RNA intermediate and integrate back into the genome. LINE-1 encodes two proteins, ORF1p and ORF2p, the latter of which is necessary for retrotransposition of LINE-1, Alu, and SVA. Orf2p drives retrotransposition, leading to insertional mutagenesis, formation of pseudogenes, non-homologous recombination, and double-stranded breaks. This genomic instability has been linked to the initiation and progression of cancer cells. ORF2p contains reverse transcriptase and endonuclease enzymatic activities necessary for integration events to occur. At this point, over 50% of the 150kD protein remains unannotated. Due to the evolutionary constraint of retrotransposon length, it is likely that these regions serve an important function. We have discovered that mutation of certain residues in an unannotated region (termed the Cryptic Domain) inhibits both the reverse transcriptase function and the endonuclease activities of ORF2p. However, the mechanisms underlying these effects are not known. There is a critical need to understand the function of this domain in retrotransposition as it represents a possible target site to inhibit mobilization and other off-target effects. We will use evolutionary guided genetic approaches to determine the mechanism by which the Cryptic Domain modulates the ORF2p function.

Genetic Heterogeneity In Pediatric Rhabdomyosarcoma Tumors

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In the United States, more than 13,000 cases of soft tissue sarcomas are diagnosed per year, of which >5,000 are fatal. Rhabdomyosarcoma is the most common pediatric type of soft tissue sarcoma affecting skeletal muscles. The alveolar subtype of rhabdomyosarcoma (ARMS) typically has a more aggressive clinical course and portends a poorer clinical prognosis than the embryonal subtype (ERMS). ARMS is genetically characterized by the presence of a translocation between chromosomes 2 and 13 at t(2;13)(p35;p14) leading to the production of the PAX3-FOXO1 fusion protein. This oncogenic protein has the potential to enhance the local invasive capacity of the tumor cells and increase metastatic potential. The cytogenetic translocation characteristic of ERMS is the translocation (1;13)(p36;q14), which is responsible for the formation of the oncogenic fusion protein PAX7-FOXO1. Multimodality analyses, such as SNP array and RT-PCR, have previously shown that significant heterogeneity exists within tumor tissue samples from rhabdomyosarcoma patients, leading to a variation in clinical outcome. The aim of this study is to investigate the heterogeneity of pediatric ARMS and ERMS tumor specimens obtained from patients of the Children’s Hospital of New Orleans Hematology/Oncology clinic. We have designed and validated a fluorescence in situ hybridization (FISH) probe in our laboratory which can simultaneously, yet distinctively, tag t(2;13) and t(1;13) translocations. Forty-eight tumor samples from rhabdomyosarcoma patients ranging in age from 15 months to 17 years were analyzed to determine the presence of these clinically significant translocations and the extent of their heterogeneity with respect to clinical outcome. Our analysis confirms that within these rhabdomyosarcoma samples, the cytogenetic markers for the t(2;13) and t(1;13) translocations exhibit significant heterogeneity.

Gas6/Axl Signaling Promotes Progression Of Premalignant Mammary Cells

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Tumor cell dissemination often occurs in early stages of disease. Tumor progression is regulated by a complex interplay between neoplastic cells and the tumor microenvironment. Axl is a type I transmembrane receptor tyrosine kinase that is activated by the growth arrest specific gene 6 (Gas6). Gas6/Axl signaling is aberrant in several types of advanced tumors. However, little is known regarding this signaling pathway in early stages of cancer. Using a transplantable p53-null model of early breast cancer progression (PN1a), we found that Gas6 is highly expressed in PN1a mammary lesions, but declines in invasive tumors. We identified that the main source of Gas6 in these lesions are F4/80+CD11b+ macrophages. To determine whether stromal Gas6 regulates early stage mammary tumor progression, we injected primary PN1a cells into wild-type (WT) and Gas6−/− mice. The proliferation status of the lesions was examined by immunostaining for Ki67. The number of proliferating cells was significantly decreased in the lesions from Gas6−/− group as compared to controls. Moreover, tumor formation was decreased in Gas6−/− mice as compared to WT. Using a 3D co-culture system of PN1a cells and bone marrow derived macrophages (BMDMs), Axl activation in PN1a cells was dramatically decreased when co-cultured with Gas6−/− BMDMs as compared to those cultured with WT BMDMs. Co-culture of PN1a cells and WT BMDM showed that STAT3 and AKT are the downstream target activated by Gas6. Finally we analyzed Axl expression in human breast cancer samples, which included ductal carcinoma in situ (DCIS) samples, a non-obligate, preinvasive precursor to invasive ductal carcinoma, as well as invasive carcinoma samples. While Axl expression was low in human hyperplastic breast tissues, we found aberrant Axl expression in some ductal carcinoma in situ (DCIS) lesions. Interestingly, there was a unique pattern of nuclear Axl expression in the epithelium of some DCIS and advanced lesions. Future directions include a comprehensive analysis of Axl expression in tissue microarrays comparing normal breast, DCIS alone, and DCIS with invasive cancer to determine if Axl is a biomarker for DCIS progression. As several Axl blocking agents have been developed and some of them are in clinical trials as cancer therapy, these data have crucial implications for the prevention and treatment of breast cancer.

miRNA Signature Predicts Overall Survival in ccRCC
Jacob Greenberg, Sree Mandava M.D, Jonathan Silberstein M.D, and L Spencer Krane M.D

Introduction:
Kidney cancer makes up almost 4% of all new cancer cases per year, accounting for more than 14,000 deaths annually in the United States. Clear cell renal cell carcinoma (ccRCC) accounts for the vast majority of renal malignancies. Currently, there are no widely adopted biomarkers that predict patient outcomes with ccRCC. The aim of this study is to identify an miRNA signature that could be used to predict patient survival.

Methods:
We pulled miRNA expression level 3 data from the Cancer Genome Atlas (TCGA) repository, an NIH funded open genomic database with 538 patients diagnosed with localized ccRCC (https://portal.gdc.cancer.gov). The expression data was correlated to each patient's metadata containing 528 subjects. We performed regression analysis Kaplan-Meier curves and Heatmap clustering using R packages ComplexHeatmap and Survival. Statistics were performed using R v3.4.4

Results:
From the downloaded TCGA data we were able to single out 4 miRNAs that significantly affected survival and created a score utilizing each miRNA’s weight. There were 101 subjects in the low score group and 427 with a high score. Clustering analysis is illustrated in figure 1a. After the data was normalized, hsa-mir-29b-1 showed increased while hsa-let-7d, hsa-mir-181a-1, and hsa-mir-204 showed decreased expression in the lower survival group. Patients with a low score had decreased survival (P<0.0005) when compared to those with who scored high. (Figure 1b)

Conclusion:
Patients with increased mir-29b-1 expression and decreased let-7d, mir-181a-1, mir-204 have significantly worse survival then other patients with ccRCC. Patients with this miRNA signature may need to be treated more aggressively or with adjuvant therapy to improve survival. Future studies will help determine whether the miRNA identified can be targeted pharmacologically for survival.
The Functional Characterization of Mismatch Repair Missense Variants
Joanna E. Haye Ph.D., LCRC Start up grant, Xavier University of Louisiana.

ABSTRACT

DNA mismatch repair (MMR) functions mainly to correct mis-paired bases that escape the proofreading activity of DNA polymerase during replication. Defects in MMR genes have been linked to compromised genome stability and diseases including Lynch Syndrome (LS) and Constitutional Mismatch Repair Deficiency Syndrome (CMMRD). LS is a common hereditary cancer syndrome resulting in early onset cancers of the colon and ovaries as well as other tissues. CMMRD resulting from homozygous mutations in MMR genes, is characterized by blood, brain and colon cancers early in childhood. The MMR genes mainly affected in Lynch Syndrome families are MSH2 and MLH1 (Lynch et al., 2009). MMR genes primarily affected in CMMRD are PMS2 and MSH6 (Wimmer and Etzler, 2008).

Tumors in LS and CMMRD are difficult to treat, as it gives researchers information about the genes involved in DM formation, and also about the abundance of DM copies, which is important for assessing the expression level of oncogenes found within them. Furthermore, accurate detection of double minutes requires precise reconstruction of their amplicons, which are the highly-amplified gene-carrying contiguous segments that adjoin to form DMs. This work presents AmpliconFinder -- a Hidden-Markov Model-based approach to detect DM amplicons using next-generation DNA sequencing data. To assess its efficacy, AmpliconFinder was used to augment an earlier framework for DM detection (DMFinder), thus improving its robustness to noisy cancer sequence data, and thus improving its sensitivity to detect DMs. Experiments on simulated genomic data have shown that augmenting DMFinder with AmpliconFinder significantly increased the sensitivity of DMFinder on these data. Moreover, DMFinder with AmpliconFinder found all previously reported DMs in three pediatric medulloblastoma datasets, whereas the original DMFinder framework found none.

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Field of research – Cancer Genetics

A Hidden Markov Model-Based Approach To Reconstructing Double Minute Chromosome Amplicons
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Double minute chromosomes (DMs) are circular fragments of extrachromosomal DNA. They are a mechanism for extreme gene amplification in the cells of some malignant tumors. Their existence strongly correlates with malignant tumor cell behavior and drug resistance. Locating DMs is important for informing precision therapy to cancer treatment, as it gives researchers information about the genes involved in DM formation, and also about the abundance of DM copies, which is important for assessing the expression level of oncogenes found within them. Furthermore, accurate detection of double minutes requires precise reconstruction of their amplicons, which are the highly-amplified gene-carrying contiguous segments that adjoin to form DMs. This work presents AmpliconFinder -- a Hidden-Markov Model-based approach to detect DM amplicons using next-generation DNA sequencing data. To assess its efficacy, AmpliconFinder was used to augment an earlier framework for DM detection (DMFinder), thus improving its robustness to noisy cancer sequence data, and thus improving its sensitivity to detect DMs. Experiments on simulated genomic data have shown that augmenting DMFinder with AmpliconFinder significantly increased the sensitivity of DMFinder on these data. Moreover, DMFinder with AmpliconFinder found all previously reported DMs in three pediatric medulloblastoma datasets, whereas the original DMFinder framework found none.

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Selective Killing Of Breast Cancer Cells By Phytochemical-Based Super Cocktail And Its Mechanisms Of Action
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Field Of Research: Cancer Genetics And Translational Research

ABSTRACT

Traditional methods for combating cancers like radiation and chemotherapy often result in undesirable side effects including anemia, weakened immune system, and nausea. Therefore, alternative approaches using natural compounds with anti-cancer properties with few (if any) side effects, have been gaining importance. Majority of such studies have focused on effects of individual natural agents on target cancer cell lines. In contrast, taking advantage of varying action-profiles of known phytochemicals, we have demonstrated that in form of a ‘super cocktail’ of six (indole 3 carbinol, resveratrol, curcumin, quercetin, genestin and C-phycocyanin), they act synergistically at concentrations approaching bioavailable levels (6 - 8 μM) and result in > 90% cell death of breast cancer cells (MCF-7, MDA MB 231 and SUM 149). Importantly, this super cocktail (SC-6) exhibited no toxic effects on the growth and survival of ‘normal’ (non-cancer) control cells (mesenchymal stem, HMEC, normal breast, and 3T3, normal fibroblasts) grown on standard adherent 2-D culture plates. Recently, using SUM149 cell line, which represents the hereditary form of breast cancer (since it carries the BRCA1 mutation); we have demonstrated that when grown under non-adherent conditions, our SC-6 reduces the mammospheres formed by over 90%. Since mammospheres are formed only by cancer cells and strongly correlate with
their tumorigenic potential, these results offer a further (3-D level) evidence for the anti-cancer properties of our SC-6. More recently, we have also obtained strong evidence for the mechanism of action (at cellular level) of our SC-6 in that, at a concentration as low as 6μM; it successfully induces apoptosis within 48 hours (at levels nearing the positive control). In addition, we have recently also obtained statistically significant and reproducible RT-PCR data (using apoptosis and breast cancer PCR arrays) profiling genes that are under and over-expressed (some more than 30 fold). Combined, these results bolster the evidence for translational potential of our SC-6. In the near future, we will conduct in-vivo studies in a mouse xenograft model (BALB/c athymic nude mice) to investigate the ability of our SC-6 to prevent breast cancer (tumor) formation or to slow down the growth of tumors already formed. Results from this study will yield important base-line data on the anti- cancer effects of our SC-6 in whole animals and set the foundation for more advanced translational studies.

Duane Jeansonne – LSU

miR-3189-3p Targets the MYC/NOTCH Axis in Triple Negative Breast Cancer
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LCRC Scientific Program: Cancer Genetics

Triple-negative breast cancer (TNBC) is the most deadly form of breast cancer for which there is no cure. MYC, a regulatory gene involved in cell growth, metabolism, differentiation, and apoptosis, is disproportionately overexpressed in many TNBCs, making it a potential therapeutic target. The NOTCH signaling pathway is an evolutionarily conserved pathway that also regulates numerous cellular functions, including tumor angiogenesis, cancer stem cells maintenance, and tumor immunity. MicroRNAs (miRNAs) are small, non-coding RNA molecules involved in the regulation of gene expression. Although these regulatory molecules have been identified as key players in cancer pathogenesis, the specific miRNAs and pathways involved in TNBC are still largely unknown. Previously, our lab found that miR-3189-3p has anti-tumoral activity through the inhibition of migration and proliferation of cancer cells. Importantly, although MYC is not a predicted gene target for miR-3189-3p, we discovered that MYC protein is downregulated in cancer cells following transfection with miR-3189-3p. This miRNA, however, has no effects on MYC mRNA, suggesting a post-transcriptional regulation.

We additionally found that miR-3189-3p negatively regulates critical NOTCH pathway genes. Finally, expression of miR-3189-3p triggers upregulation of miRNAs predicted to target MYC and that are part of the DLK1-DIO3 cluster of genes.

Therefore, since the MYC-targeting miRNAs along with the NOTCH pathway inhibitor, DLK1, which reside in the DLK1-DIO3 locus, are being silenced in breast cancer we hypothesize that miR-3189-3p targets the MYC/NOTCH axis through direct inhibition of NOTCH-associated genes and through reversing the silencing of the DLK1-DIO3 locus miRNAs which target MYC. Therefore, we are investigating the role of miR-3189-3p in targeting the epigenetic-modulating gene HDAC3, a direct target of the miRNA, and concomitant derepression of the DLK1-DIO3 locus. To better understand the tumor-suppressive activity of miRNAs from the DLK1-DIO3 locus, we aim to determine their effect on the MYC/NOTCH axis.

In conclusion, we have recently identified miR-3189-3p as a miRNA with strong anti-cancer activity. We additionally found that this miRNA indirectly downregulates MYC protein, a molecule highly expressed in TNBC. Among the molecular mechanisms involved in the miR-3189-3p mediated effects, we found upregulation of miRNAs potentially targeting MYC and encoded in the DLK1-DIO3 cluster, a locus often silenced in tumor cells. Therefore, suggesting epigenetic regulator functions for miR-3189-3p. This novel mechanism together with the direct targeting of NOTCH signaling molecules may destabilize MYC protein and compromise tumor growth. This study aims to elucidate the molecular aspects of DLK1-DIO3 cluster expression and resulting effects on the MYC/NOTCH axis, ultimately providing new and more efficient molecular tools toward the development of therapeutics targeting triple negative breast cancer.

Jihoon Jung – Tulane

RNA-Binding Motif Protein 10 Regulates p53 Dependent Apoptosis And Cell Growth Arrest
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RNA-BINDING MOTIF PROTEIN 10 (RBM10), also called S1-1, is an RNA-binding protein frequently deleted or mutated in lung cancer cells. Recent reports indicate that knockdown of RBM10 in human cancer cells enhances growth of mouse tumour xenografts suggesting that RBM10 acts as a tumour suppressor. RBM10 also regulates alternative splicing and control cancer cell proliferation. However, the underlying molecular mechanisms remain unclear. Here, our group for the first-time reports that RBM10 can induce apoptosis and inhibit cell proliferation partially dependent of p53. Overexpression of RBM10 decreased cancer cell proliferation and induced apoptosis through the caspase family proteins. Furthermore, overexpression of RBM10 increased p53 stability and decreased p53 ubiquitination. Inversely, knockdown of RBM10 decreased p53 stability. RBM10 also bound to p53 and MDM2 via the N-terminal domain of MDM2 and inhibited p53-MDM2 interaction. RBM10 interacted with RPL5 and RPL11 and induced p53 expression synergistically with the ribosomal proteins. Interestingly, knocking down either RPL5 or RPL11 abrogates the p53 activation by RBM10. Surprisingly, RBM10 is not required for p53 activation by chemotherapy drugs, such as doxorubicin, actinomycin D, and 5-FU. In addition, RBM10 might prevent cancer progression by inhibiting p53-regulated mitochondrial respiration and migration. Analysis of cancer genomic databases revealed that patients with wild type RBM10 and p53 survive longer than do those with mutated p53 or less RBM10. Together, our results demonstrate that RBM10 plays an anti-oncogenic role by inhibiting cancer cell proliferation and inducing apoptosis in part through the regulation of MDM2 and p53.

This work was supported in part by NIH-NCI grants R01 CA095441, R01CA172468, R01CA127724, and R21 CA190775 to Hua Lu.
**L1 Expression Analysis In Adipocyte Derived Stem Cells**

**Discipline:**
Molecular Genetics

**Authors:**
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**Objectives/Goals:**
Long interspersed element-1s (L1s) are autonomous, mobile elements that are able to copy and insert itself throughout the genome with its own reverse transcriptase and endonuclease. These elements make up 17% of the human genome with over 500,000 copies, though the vast majority of these elements are defective and only a few dozen are potentially responsible for L1 activity. Full-length L1s have the potential to contribute to mutagenesis through random insertion and increased genetic instability. Here we set out to study L1 expression at the specific loci level in bone marrow derived stem cells (bmSCs) and adipocyte stem cells (ASCs) and compare the levels of expression from ASCs from donor patients who are young and lean, obese, and old.

**Methods/Study Population:**
Adipocyte stem cells and bone marrow derived stem cells were isolated from patient donors. The following samples were collected: ASCs from 3 young and lean patients, ASCs from 3 patients over the age of 59, ASCs from 3 patients with BMI>30, and bmSCs from 4 young and lean patients. Cytoplasmic RNA from the cell populations were isolated and sequenced from the cell populations. Using our recently developed bioinformatics pipeline, we set out to quantify L1 expression and identify the few culprit L1s at specific loci that are actively transcribing to RNA in the ASC and bmSC samples.

**Results:**
Here we provide proof of concept with the application of this novel method in characterizing full-length expressed L1s at the specific loci level in ASCs and bmSCs. We identified L1 loci that are commonly expressed in these cell types and observed an increase in L1 expression in the obese and old ASC cells compared to the young, lean ASCs and bmSCs.

**Discussion/Significance of Impact:**
Adipose derived stem cells hold the promise of broad application in the biomedical field including regenerative treatment. There are reports that ASCs cultivated from older and obese donors are less effective in regenerative treatments. By demonstrating an increased expression of the mutagenic L1 element in ASCs from obese and old donors, this study provides further evidence suggesting the preferable use of ASCs from young and lean donors for regenerative therapies.

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**CRISPR/Cas9-Mediated Inhibition of KSHV MicroRNAs**

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Kaposi’s sarcoma-associated herpesvirus (KSHV/HHV8) can establish a latent infection in human cells, and cause several cancers in immunocompromised patients, including Kaposi’s sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD). Current antiviral drug treatments are ineffective when KSHV establishes the latent infection. All KSHV microRNAs expressed during latency play crucial roles in the pathogenesis of the KSHV-associated malignancies. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated gene 9) system is an RNA-guided DNA editing method that has been widely used in gene editing. In this study, we used CRISPR/Cas9 to inhibit KSHV microRNAs expression in latently KSHV-infected PEL cells (BCBL-1 and BCP-1). Our results showed a significant inhibition of two KSHV microRNAs (miR-K12-1 and miR-K12-9) expression by targeting sgRNAs (single-guide RNAs) to corresponding viral genes. KSHV lytic genes reactivation and down-stream targets were all significantly induced in the KSHV-miR-K12-1-knock-out cells. Furthermore, two sgRNAs were simultaneously used to direct a targeted deletion of 230bp in the promoter region of the KSHV microRNA cluster, which encodes 10 different viral microRNAs (miR-K12-1 through -9 and miR-K12-11). The loss of viral microRNAs expression and lytic genes reactivation were verified in the KSHV microRNAs promoter-deleted cells, supporting this promoter as the major regulator of this viral microRNAs cluster. Our studies indicate that the CRISPR/Cas9 system can be effectively targeted to KSHV genomes as a potent therapeutic antiviral strategy that may be used to impair viral replication and clear latent virus infection.
Depletion of Histone Methyltransferase G9a Inhibits Cholangiocarcinoma Development through Hippo Pathway
Wenbo Ma – Tulane
Presenter: Wenbo Ma
PI: Tong Wu
From the Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA

Histone methyltransferase G9a shows increased expression in many cancer types, including cholangiocarcinoma. However, there is little known about the tumorigenic mechanism of G9a. In this study, we report that G9a regulates the growth and metastasis of cholangiocarcinoma through Hippo pathway. The expression of G9a is increased in cholangiocarcinoma samples compared with the normal samples. Depletion of G9a significantly represses the proliferation and migration of cholangiocarcinoma cells. Mechanically, knockdown of G9a decreases the level of dimethylated H3K9, which lead to an upregulated expression of tumor suppressor gene LATS2 and a reduced accumulation of nuclear YAP1. In vivo, we also provide evidence that depletion of G9a significantly suppresses the growth of cholangiocarcinoma. In this study, we demonstrated that the upregulated histone methyltransferase G9a contributes to cholangiocarcinoma development through Hippo pathway. Targeting G9a may be a novel approach for cholangiocarcinoma treatment.

Inactivation Of Ribonuclease (RNase) H2A In Human Colorectal Cancer Cell Line (HCT116) And DNA Polymerase ε Mutants
Vivian Park – Tulane

Vivian Park, Karl Hodel, Zachary Pursell
Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA

DNA polymerase (Pol) ε mutations are common in colon and endometrial cancers. These mutations lead to amino acid substitutions that compromise Pol ε’s exonuclease proofreading activity. We have shown that proofreading-deficient Pol ε is able to insert and extend from ribonucleotides in vitro. However, the extent to which proofreading-deficient Pol ε introduces rNMPs into the genome, and how this might contribute to mutagenesis and tumorigenesis is poorly understood.

In the current study we set out to measure the effect of ribonucleotides incorporated by wild type and mutant Pol ε in human cell lines. The ribonuclease enzyme, RNase H2, is the sole nuclease able to remove single inserted ribonucleotides in the genome and is required for normal development and DNA repair.

We used CRISPR-Cas9 to inactivate RNase H2A, the catalytic subunit of the RNase H2 holoenzyme, in human cell lines expressing either wild type or mutant Pol ε. Initially, we identified genomic knock-out of RNase H2A in the population of Cas9-treated cells and in a subsequent clone. Interestingly, this clone lost the RNase H2A genomic deletion during passaging, raising the possibility that this deletion is incompatible with the absence of functional mismatch repair. Subsequent experiments with multiple guide sequences showed significant knockout efficiencies in the pooled transfected cells; however, no clones were found to be viable. Experiments in RNaseH2A−/− mice from other labs suggested significant activation of the p53 response. To address whether RNase H2A knock-out is biologically incompatible, we attempted to knock-out the enzyme in a p53 null cell line to bypass the p53-dependent DNA damage response pathway and identified several clones. RNase H2A KO was verified via Western Blot.

Molecular Cytogenetic Characterization of RH30 and RH4 Alveolar Rhabdomyosarcoma (ARMS) Cell Lines
Jorge Peñas – LSU

Jorge Peñas1, Katrina Gleditsch1, Danielle Mercer1, Ayesha Umrigar1, Yuwen Liu2, Tian Jan Chen2, Andrew Hollenbach1, Fern Tsien1

Molecular Cytogenetic Characterization of RH30 and RH4 Alveolar Rhabdomyosarcoma (ARMS) Cell Lines

Rhabdomyosarcoma is the most common soft tissue tumor in children. The alveolar subtype of rhabdomyosarcoma (ARMS) typically has an aggressive clinical course and portends a poorer clinical prognosis than the embryonal subtype (ERMS). This diagnosis, while histologically distinct, is also characterized genetically by the presence of balanced translocations at t(2;13) and t(1;13). These translocations lead to the production of fusion proteins PAX3-FOXO1 and PAX7-FOXO1, respectively. Two well-known and commercially available cell lines used in ARMS clinical research are RH30 and RH4. These cell lines were derived from pediatric patients clinically diagnosed with ARMS. Previous studies have cytogenetically characterized the RH30 cell line to gain a better understanding of phenotypic clinical correlations due to these translocations; however, to date the RH4 cell line has not been fully cytogenetically characterized. The specific aim of this project is to determine the significant large scale genetic similarities and differences between RH4 and RH30 cell lines since these cell lines are used in clinical research where cytogenetic variants can affect clinical outcomes. Both RH30 and RH4 were fully analyzed using G banding, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH) and additional spectral karyotyping (SKY) for RH4. Our initial findings suggest that while both cell lines were derived from patients with ARMS they exhibit significant variations which may lead to varying outcomes in the clinical laboratory setting. The analysis of these cell lines may provide future targets for novel therapeutic strategies in ARMS patients.

Hepatocyte Hedgehog Signaling Promotes NAFLD-associated Liver Carcinogenesis Through Inhibiting CD8+ T Cell Mediated Anti-Tumor Immunity
Kyoungsub Song1, Hyunjoo Kwon1, Chang Han1, Tong Wu1

Kyoungsub Song1, Hyunjoo Kwon1, Chang Han1, Tong Wu1
2018 LCRC Annual Retreat Booklet
Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and major risk factor for the development of liver cancer. The activation of Hedgehog (Hh) signaling plays an important role in liver diseases including non-alcoholic steatohepatitis (NASH), fibrosis, and cancer. However, the precise role and regulatory mechanisms of Hh signaling on NAFLD-associated liver cancer is largely unknown. The current study is designed to elucidate the effect and mechanism of hepatocyte Hh signaling on liver carcinogenesis in the setting of NAFLD. Here, we used hepatocyte-specific Smo knock-in mice to activate Hh signaling, and used dimethylbenz[a]anthracene (DMBA) plus high fat diet (HFD) in an NAFLD-associated liver carcinogenesis mouse model. Liver tumor was markedly developed in Smo knock-in mice compared to WT mice treated with DMBA plus HFD at 40 weeks; however, we could not observe significant tumor induction treated with DMBA alone. We observed higher Ki-67 expression (proliferation index) and osteopontin (act as an immune modulator) in the livers of Smo knock-in mice compared to the WT mice. Interestingly, CD8+ T cells were significantly reduced in Smo knock-in mice compared to WT mice in early stages of carcinogenesis (at 20 weeks). We found that recombinant osteopontin promotes the expression of PD-L1 but not CD80 in primary Kupffer cells. Also we also showed that increased expression of IL-10 in Smo knock-in mice treated with DMBA plus HFD compared with WT mice. Treatment of anti-PD-1 or anti-IL-10 results in significantly decreased tumor numbers and sizes. Taken together, our results suggest that activation of Hh signaling promotes liver carcinogenesis in the setting NAFLD, and anti-tumor immune responses may involve in Hh signaling-mediated tumor development.

Kirsten Termine – LSU

Analysis of CNVs in the Hereditary Prostate Cancer Setting Using Next-Generation Sequencing
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Prostate cancer (PCa) is the 2nd leading cause of cancer-specific deaths among American men, and there is a significant racial disparity between African American (AA) and Caucasian (CAU) affected men. So far, somatic copy number variations (CNVs) have been documented in PCa tumors, but germline CNV changes from hereditary PCa (HPC) cases has not been studied extensively. The goal of the present study is to identify germline CNVs associated with HPC through whole-exome sequencing (WES) data analysis. We hypothesize that germline CNVs in actionable genes contribute to higher incidence of PCa within families.

We have WES data on 27 affected HPC cases (16 AA and 11 CAU) and 10 CAU controls. A CNV-specific calling algorithm, CANOES, was used to detect germline CNVs by modeling read counts using a precise distribution, and CNVs were visualized using Integrative Genomics Viewer (IGV). CANOES called 282 germline CNVs on AA cases, 96 of which were IGV-confirmed and 14 of which located within cancer-related genes. CANOES called 466 germline CNVs on CAU cases and controls, 303 of which were IGV-confirmed; 18 were located within cancer-related genes. CNVs in the eleven cancer-associated genes GSDMC, PRMT7, WNT7B, XRN1, DEC1, CT5B, SPAG11A, CADPS, CKMT1B, ASCC1 and CDK11B were found to be only in AA HPC cases and were not present in AA controls nor CAU cases/controls. Four germline CNVs within the cancer-related genes NBPF1, POTEM, MLLT10, C1QTNF9B, ZNF717 and PTGER3 were found only among the CAU HPC cases, but not in controls. Germline CNVs in the seven cancer-related genes GSTM1, DMBT1, GSTT1, POTEM, UGT2B17, MTUS1 and PTGER have been found in both AA and CAU cases, and have also been previously shown to be associated with PCa tumors, deeming them significant genes of interest for our study.

CANOES has proven to be an efficient tool used in successfully calling germline CNVs from WES data on our HPC cases and controls. The results from this study are in concordance with what is being found somatically, suggesting that the germlines of HPC patients may hold valuable information necessary for discovering clinical biomarkers for this disease. In conclusion, the identification of germline CNVs within HPC families will contribute to the impending need of cancer biomarker identification in the era of precision medicine, allowing us to facilitate early diagnosis, screening and disease prevention in HPC families.

Dinh-Van Tran – LSU

Analysis of Copy Number Variations (CNVs) In Hereditary Lung Cancer Families
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Lung cancer is the leading cause of cancer related deaths for both men and women in the United States, with an overall five-year survival rate of roughly 18%. An association between family history and lung cancer risk has reported for years. Research has found that approximately 10% of patients with lung cancer have at least one first-degree relative affected, and 25% of patients have at least one first- or second-degree affected relative. There have been reports of copy number variations (CNVs) in lung cancer cell lines and somatic samples but no germ line specific CNVs have been reported in high-risk lung cancers. To identify germ line genetic variants, we performed targeted whole exome sequencing (WES) on high-risk lung cancer families. The objective of the study is to detect germ line CNVs that might be responsible for increased lung cancer susceptibility in high-risk families. The Genetic Epidemiology of Lung
Cancer Consortium (GELCC) focuses on identification of susceptibility genes in hereditary lung cancer (HLC) families (≥3 cases/family). Previous multipoint linkage analyses of HLC families detected significant linkage to the 6q23-25 region. To identify germ line CNVs, we have analyzed the 6q23-27 targeted sequencing data on nine multigenerational hereditary lung cancer families. The targeted sequencing data of the hereditary families (≥3 LC cases/family) included 25 affected and 55 informative unaffected family members. A CNV-specific algorithm incorporated in two separate programs, CANOES and XHMM, was used to call and identify germline CNVs. To confirm, CNVs from both programs were then independently visualized using Integrative Genomics Viewer (IGV). From comparing the results of both programs, we have identified germline CNVs on several genes of interest, including RGS17, SASH1, ARID1B and PARK2. These genes were previously identified by GELCC as candidates using either linkage analysis or by targeted sequencing analysis of 6q23-25. In the long run, identification and hereditary transmission of these germline CNVs might be useful in conjunction with the identification of other genomic variants in early detection and therapeutic implications of high-risk individuals.

Impact of Alcohol Intake on Prostate Cancer Aggressiveness for Different Genetic Profiles
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Presenter: Xinnan Wang
PI: Hui-Yi Lin
Aims: Alcohol is an uncertain risk factor related to Prostate Cancer aggressiveness (PCa). This may be due to interactions between alcohol intake and inherited genetic variants, Single-nucleotide polymorphisms (SNPs). The major aim is to examine the alcohol-SNP interactions of the 8587 SNPs in the four pathways (angiogenesis, mitochondria, miRNA, and androgen metabolism related pathways), which are related to both alcohol use and PCa aggressiveness.

Methods: We examined 3,306 White PCa patients with valid alcohol information from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) Consortium. The predictors on alcohol intake included the frequency of intake (i.e. moderate alcohol use/MAU, ≤ 2 times/day), the volume of intake (i.e. moderate ethanol level/MEL, ≤ 30g/day) and a binary predictor (i.e. beer daily use/BDU, yes/no). For each SNP, additive, dominant, and recessive modes were considered. The full interaction logistic models were applied.

Results: Although the three alcohol predictors were not significantly associated with PCa aggressiveness (p=0.362 for MAU, 0.802 for MEL and 0.453 for BDU), three SNPs [rs11925452 (ROBO1), rs9907521 (PRKCA), and rs13107662 (CAMK2D)] had a significant interaction with an alcohol predictor (i.e. p-values ≤ 0.01 in discover set, validation set and combined set). The minor allele frequencies for three SNPs were 20.5%, 6.5%, and 34.3%, respectively. Published papers reported these identified genes are related to alcohol dependence, cocaine addiction and pathological gambling. Our findings provide some insights of potential alcohol use impact on PCa aggressiveness of individuals with specific genetic profiles.

HCC-derived IncRNA CRNDE Inhibits T Lymphocyte Activation and Impairs Host Immunity Leading to Immune Evasion and HCC Progression
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Aims: Long noncoding RNAs (lncRNAs) are important players in diverse pathobiological processes, including regulation of immune response and carcinogenesis. In the current study, we performed RNA-seq analysis of hepatocellular carcinoma (HCC) and matched non-cancerous tissues from five HCC patients and showed that the IncRNA CRNDE is one of the most significantly elevated genes in human HCC tissues. This finding was subsequently verified by analysis of publically available microarray dataset E-MTAB-950, which includes gene expression information of 120 HCC and160 non-tumor live tissues. To identify the interactive protein partner of CRNDE, we carried out RNA pull-down and mass spectrometry analysis, which led to identification of CypB as the binding protein of CRNDE. The latter observation was further supported by RNA immunoprecipitation (RIP) and additional RNA pull-down experiments. Given that CypB is the primary target of the well-known immunosuppressive drug Cyclosporine in T lymphocytes, we next assessed the effect of CRNDE on T cell activation. Specifically, we altered the expression levels of CRNDE in Jurkat cells (immortalized human T lymphocytes) via transfection with CRNDE overexpression or shRNA vectors; the cells with forced overexpression or knockdown of CRNDE were then activated through co-stimulation with anti-CD3 and anti-CD28 antibodies. Our data showed that the level of CRNDE was reversely correlated with IL-2, TNFα, and IFNγ mRNA levels in Jurkat cells. Immunofluorescence RNA dye (Exo-RED) staining and RT-PCR analysis showed that CRNDE could be transmitted from HCC cells to Jurkat cells via exosome. Consistent with these observations, our further analyses suggest that the level of CRNDE in HCC tissues may impact T cell response in patients. We carried out immunohistochemistry (IHC) and in-situ hybridization (ISH) analyses of human HCC tissues by using anti-CD8 and CRNDE-specific probes and observed that patients with higher CRNDE levels in the tumors exhibited fewer infiltrating CD8+ cells. By performing gene set enrichment analysis (GSEA) of the 120 HCC patients in the dataset E-MTAB-950, we observed that the gene sets associated with T-cell activation (including IL-2_Stat5, INFγ_response, and INFα_response) were up-regulated in CRNDE-low group (compared to CRNDE-high group). We further analyzed the disease-free survival and tumor CRNDE expression levels in 139 HCC patients available from the TCGA database; this analysis showed that the HCC patients with higher CRNDE levels experienced earlier relapse when compared to patients with lower CRNDE levels (329.0±221.5 vs. 463.8±463.7 days, Log-rank test P = 0.0315). Taken together, our
findings provide novel evidence that HCC-derived lncRNA CRNDE is a central molecule that impairs host immunity in the tumor microenvironment, thus causing immune evasion and promoting HCC progression.

Epigenetically Silenced Candidate Tumor Suppressor Genes In Prostate Cancer: Identified By Modelling The Stratified Promoter Methylation Profiles Over Tumor Samples And Applied To Progression Prediction

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Abstract
Specific aims The genetic etiology of prostate cancer (PCa) substantially varies among individual tumors. Recent studies have showed that epigenetic alterations, especially the hyper-methylated promoters of tumor suppressor genes, contribute to PCa progression and metastasis. In this research, we propose a novel algorithm to identify epigenetically silenced tumor suppressor genes (epi-TSGs) using the data of paired normal::tumor prostate tissue samples.
Research methodology Our method is based on the Gaussian mixture model and is inspired by the perception that the promoter CpG island(s) of a typical epi-TSG has a stratified methylation profile over tumor samples. In other words, we assume that the methylation profile resembles the combination of a binary distribution of a driver mutation and a continuous distribution representing measurement noise and intra-tumor heterogeneity.
Result Status Applying the proposed algorithm and an existing method to the Cancer Genome Atlas (TCGA) prostate adenocarcinomas data, we identify 57 candidate epi-TSGs with the promoters hyper-methylated in 6-67% of tumors. Over one third of these epi-TSGs have been reported to carry potential tumor suppression functions. The negative correlations between the expression levels and methylation levels of these genes are validated on external independent datasets. We further find that the expression profiling of these genes is a robust predictive signature for Gleason scores, with AUC statistic ranging from 0.75 to 0.79. The pinpointed signature also shows prediction strength for tumor progression stages, biochemical recurrences and metastasis events.
Section 2-2

Immunology Abstracts
Targeting Fatty Acid Metabolism in Myeloid Cells to Improve T cell Responses
Matthew Dean, Eliza Zimolag, Liqin Zheng, Amir Al Khami, Dorota Wyczewoska, Augusto Ochoa
Presenter: Matthew Dean
Mentor: Augusto Ochoa
University: LSU Health
Field of Research: Immunology

Although immunotherapy shows great promise in the fight against cancer, it is only effective in certain cancer types, and only in a relatively small number of patients (20-30%). The poor responses seen in many solid tumors and in some patients with generally responsive cancer subtypes is the subject of intense investigation. While this T cell suppressive activity is likely the result of several factors in the tumor microenvironment, one reason certain patients fail to respond is due to the presence of myeloid cells in tumors that suppress T cell-mediated tumor cell killing. Our lab focuses on myeloid derived suppressor cells (MDSCs), and has shown them to be critical in the ineffective T cell responses in experimental animals. It has recently become known that MDSCs are highly dependent on fatty acid metabolic pathways to progress to the suppressive state and reduce T cell activity both in vivo and in vitro. Because T cells are highly reliant on glycolysis, targeting fatty acid pathways presents an ideal target to inhibit MDSCs without negatively impacting T cell activity. Here we show that inhibition of fatty acid metabolism at multiple enzymatic steps reduces the ability of bone marrow cells to differentiate into fully-suppressive MDSCs in vitro, which results in an inability of treated MDSCs to reduce T cell proliferation. This is due in part to a reduction in the protein levels of arginase, which is one of the primary mechanisms through which MDSCs act to inhibit T cell proliferation and activity. Using the same fatty acid metabolism inhibitors in vivo, we have shown a reduction in the size of syngeneic tumors. The tumors from treated animals showed an increase in TUNEL-positive cells and areas of necrosis. In addition, MDSCs extracted from treated tumors expressed lower levels of arginase, and subsequently, a reduced ability to suppress T cell proliferation in a T cell suppression assay. Finally, we have tested the ability of these fatty acid metabolism inhibitors to synergize with conventional chemotherapeutics and immune checkpoint inhibitors. While the fatty acid metabolism inhibitors did not produce any added benefit when combined with chemotherapy, the combination with immune checkpoint inhibitor α-PD-1 has shown promise. We will further test these inhibitors in other syngeneic and spontaneous tumor models, as well as in combination with adoptive cell therapy and α-CTLA-4 and α-OX40 therapies. We hope that by using inhibitors which target MDSCs while sparing T cells in combination with T cell enhancing immunotherapy will result in robust and sustained anti-tumor immune responses that will reduce or eliminate tumors.

Targeting Tumor Microenvironment Inhibits Breast Cancer Stem Cell Tumorigenesis
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The tumor microenvironment (TM) establishes optimal conditions for breast cancer growth and metastasis. TM contains pro-inflammatory mediators downstream of NfκB, which regulate microenvironmental properties and facilitate malignant processes such as neo-vascularization and inflammation. Breast Cancer Stem Cells (CSCs) constitute a small subpopulation within the tumor tissue yet are highly tumorigenic and a significant cause of tumor metastasis, resistance, and recurrence. Previously, we showed that TRAF3IP2, an upstream regulator of NfκB, is a potent regulator of breast cancer-related inflammation as well as a myriad of pro-tumorigenic pathways such as angiogenesis and cell cycle dysregulation. In this study, we are aiming to (1) investigate properties of the malignant TM created by CSC population and (2) to determine the effects of silencing TRAF3IP2 on TM function, specifically inflammatory and other pro-tumorigenic processes. Our in vitro data indicate that silencing TRAF3IP2 significantly affects spheroid formation in breast cancer (MDA-MB231) cells with a 49.95% reduction in sphere diameter after 96h in culture. Flow Cytometry showed a lower percentage of cells with stem cell properties in MDA-MB231 cells in which TRAF3IP2 was silenced (MDATRAF3IP2 KD) compared to MDA-MB231 cells transduced with scrambled shRNA (MDAControl shRNA). Data also indicated a slower growth rate and a reduced spheroid formation ability in MDATRAF3IP2 KD compared to MDAControl shRNA. Transcriptome analysis indicated that silencing TRAF3IP2 significantly reduces expression of NfκB, VEGF and inflammatory cytokines compared to spheroids derived from MDA-MB231Control shRNA cells. We also showed that the CSC subpopulation identified by CD44high CD24low has potential to form spheres. We confirmed that CSCs exhibit significantly more aggressive and metastatic characteristics, compared to the non-cancer stem cell population. Our in vivo methodology was comprised of (1) inducing tumors in immunodeficient mice by intramammary injection of CSC-derived spheres and (2) subjecting these animals to treatment with silencing vector for TRAF3IP2. Notably, the tumor growth was regressed in animals treated with silencing vector for TRAF3IP2.

Our results indicate that silencing TRAF3IP2 can effectively disturb TM organization in CSC-induced tumors. Specifically, inflammatory signaling and sphere-forming ability are significantly reduced in MDATRAF3IP2 KD compared to MDAControl shRNA. As a result, MDATRAF3IP2 KD exhibits notably diminished neoangiogenic and metastatic activity, strongly suggesting perturbation of tumorigenic potential. Based on this, we propose that TRAF3IP2 is a novel regulator of tumorigenesis and malignant transformation in breast cancer. Finally, this innovative study identifies TRAF3IP2 as a promising, effective therapeutic target in breast cancer.
Section 2-3

Molecular Signaling Abstracts
Novel Parathyroid Hormone Antagonist Prevents Breast Cancer Bone Metastasis And Cortical Bone Destruction

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Abstract

The majority of patients (~70%) with advanced breast cancer will develop bone metastases and suffer from severe pain, bone fracture and eventually death. Current palliative treatments have only limited efficacy highlighting an urgent need for development of targeted agents. Bone metastatic breast cancer cells secrete parathyroid hormone related peptide (PTHrP) that promotes autocrine tumor growth, induces bone turnover that releases additional tumor stimulatory factors, and creates cavities in the bone that permit tumor outgrowth. Previous PTH/PTHrP antagonists failed clinically due to short half-life and inadequate targeting to bone. We created a novel bone-targeted PTH antagonist drug, PTH(7-33)-CBD, by fusing an N-terminally truncated analog of PTH (aa 7-33) with the collagen binding domain (CBD) from ColH collagenase (Clostridium histolyticum). Our previous preliminary study demonstrated in vivo efficacy of PTH(7-33)-CBD against tumor burden and cortical bone destruction using an established model of breast cancer bone metastasis by injecting a bone-trophic variant of estrogen receptor-negative MDA-MB-231 breast cancer cells expressing luciferase (MDA-MB-231-BM/luc+) into the tibia marrow of nude mice. PTH(7-33)-CBD treatment did not result in hypocalcemia or reduce animal body weight. We extended this previous study by using increased animal numbers, administration of a control drug, and assessment of apoptosis of breast cancer cell in vitro by PTH(7-33)-CBD. PTH(7-33)-CBD reduced PTH agonist-stimulated cAMP accumulation in SaOs-2 osteosarcoma cells by 71% confirming the PTH antagonist activity of the compound. PTH(7-33)-CBD exhibited a direct cytotoxic effect towards MDA-MB-231 breast cancer cells in vitro by increasing apoptosis as assessed by the Caspase Glow Assay. To assess in vivo efficacy, on Day 0 nude mice were treated IP with either vehicle, 1000 µg/kg control drug PTH-7-34 (without CBD domain), or 1000 µg/kg PTH(7-33)-CBD (N=8/treatment group). On Day 1 mice were injected with 2x10⁶ MDA-MB-231-BM/luc+ cells into the tibia marrow and tumor burden and bone density were assessed weekly by bioluminescence imaging and X-ray imaging, respectively. PTH(7-33)-CBD significantly reduced tumor burden in bone from weeks 4-8 post-tumor cell inoculation compared to vehicle (P<0.05), and from weeks 6-7 compared to PTH-7-34 control drug (P<0.05). PTH(7-33)-CBD reduced cortical bone destruction from weeks 3-7 weeks compared to both vehicle and PTH-7-34 control drug (P<0.05). Taken together, these data demonstrate that PTH(7-33)-CBD reduces both bone metastatic tumor burden and osteolytic lesions, and induces apoptosis of MDA-MB-231 breast cancer cells in vitro.

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The Transcriptional Corepressor TLE1 As A Driver Of Epithelial Mesenchymal Transition (EMT) And Hormonal Therapy Resistance In Breast Cancer

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ABSTRACT

Breast cancer is a highly aggressive disease that exhibits high metastatic potential and resistance to endocrine therapy. In light of increasing importance of epigenetic changes in carcinogenesis, we have previously identified the transcriptional regulatory protein, named Transducin-like Enhancer Split 1 (TLE1), as a “master regulator” in controlling growth and apoptosis resistance in breast cancer cells. Here, we provide evidence in support of the role of TLE1 as an effector of Epithelial Mesenchymal Transition (EMT) and hormonal therapy resistance in ER+ breast cancer cells. Exogenous expression of the TLE1 corepressor in MCF7 cells resulted in heightened EMT phenotypes including enhanced fibroblastoid morphology and increased cell migratory potential with concomitant downregulation of the epithelial marker E-cadherin expression. Conversely, downregulation of endogenous TLE1 expression in these cells further potentiated their epithelial phenotype characterized by a cuboidal-like epithelial cell phenotype, reduced cell motility, and upregulated E-cadherin expression. Importantly, exogenous TLE1 expressing cells exhibited decreased sensitivity to tamoxifen. The TLE1-induced tamoxifen resistance in MCF7 cells was associated with a significantly reduced E-cadherin expression as compared to that of treated control cells. The important role of E-cadherin suppression in tamoxifen resistance is further underscored by our findings that specific knockdown of E-cadherin alone was sufficient to reduce tamoxifen sensitivity in MCF7 cells. Collectively, these data indicate that the TLE1/E-cadherin transcriptional pathway contributes to EMT and tamoxifen resistance in ER+ breast cancer cells.
Molecular Signaling
The RCMI Cell, Molecular and Bioinformatics (CMB) Core performs services and bioassays focused mainly on cancer biology and cell signaling. The CMB core maintains “state of the art” equipment, trains students, lab staff and faculty in the latest molecular techniques and is continually expanding and improving services. Our most recent acquisition is a capillary electrophoresis Western blot system called WES (ProteinSimple). This fully automated system has completely replaced the traditional Western blot workflow in our core. The WES is easy to use and removes variability so results are more reproducible. Since a blotting step is not performed, protein transfer inconsistencies are eliminated, providing more consistent results. The software provided with the system allows quantitative comparison of proteins against a standard curve. WES automates the entire Western blot procedure increasing reproducibility and delivering significant time savings. The entire process from plate loading to data acquisition is roughly 4 hours, compared to two days using traditional Western blotting procedures. In addition, much smaller amounts of total protein are required with the WES. Typically, 0.6 ug of protein lysate is loaded per capillary. Each capillary functions independently, meaning 24 different antibodies can be run simultaneously on a single lysate, 24 independent lysates can be probed with a single antibody, or anywhere in between. Shown are examples of a few of the targets we have successfully probed using the WES technology.

**Understanding the Role of Conformational Changes in Kinesin-5 on Processivity and Inhibition**

Joseph Chaney
Xavier University of Louisiana, LCRC Start-up

Human Kinesin-5, an anticancer drug target, is key to the assembly of the bipolar spindle during mitosis. This research project seeks to define the mechanism of mechanical output by kinesin motor proteins. Our interest is in the neck-linker, a 12-15 residue segment at the N-terminus of kinesin plays an important role in processivity. However, it is not well understood the importance of the conformational change that forms a short β-sheet from a floppy region to its inhibition by anticancer drugs nor its influence on the coil-coil domain, the region necessary for oligomerization. The goal of this project is to determine the effects of substitutions and insertions in the neck-linker of kinesin-5, including human polymorphic variants, on in vitro measurements of catalytic activity and motion and whether the neck-linker controls structural asymmetry and initiation of the coil-coil, necessary for kinesin oligomerization. AIM1: We will insert three residues (DAL) into various places along the neck-linker of dimeric Kinesin-5 construct (Eg5-513) followed by expression and purification of the mutants in a bacterial system. Our rationale is that the introduction of (DAL) at any point of the neck-linker will initiate the start of the coil-coil. We predict that the loss of the neck-linker will start the result in lower processivity of the chimera as the conformational change. Our experimental readout will be MT gliding by the mutant Kinesin-5. We will quantitate MT-gliding velocities for each mutant Kinesin-5 motor as well as WT Kinesin-5 for control. We expect that perturbations to the individual β-strands will prevent the extension of the central β-sheet by the cover neck bundle and reduce the motility of the motor along MT tracks. The anticipated result is that we expect a loss of interaction between β7 and β10 as a result of the glycine mutations. We anticipate that this will lead to total loss of kinesin motility. We will then test the response to inhibition by current anticancer inhibitor STLC. AIM 2: We will generate mutations to the dimeric Kinesin-5 construct (Eg5-513) in β7 (E254G, L255G, V256G, K257G, I258G) by site-directed mutagenesis followed by expression and purification of the mutants in a bacterial system. Our rationale is that the introduction of (DAL) at any point of the neck-linker will initiate the start of the coil-coil. We predict that the loss of the neck-linker will start the result in lower processivity of the chimera as the conformational change. Our experimental readout will be MT gliding by the mutant Kinesin-5. We will quantitate MT-gliding velocities for each mutant Kinesin-5 motor as well as WT Kinesin-5 for control. We expect that perturbations to the individual β-strands will prevent the extension of the central β-sheet by the cover neck bundle and reduce the motility of the motor along MT tracks. The anticipated result is that we expect a loss of interaction between β7 and β10 as a result of the glycine mutations. We anticipate that this will lead to total loss of kinesin motility. We will then test the response to inhibition by current anticancer inhibitor STLC.

**High-Throughput Screening Identified Selective Inhibitors Of Exosome Biogenesis And Secretion: A Drug Repurposing Strategy For Advanced Cancer**

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Field of Research: Molecular Signaling

Targeting exosome biogenesis and release may have potential clinical implications for cancer therapy. Herein, we have optimized a quantitative high throughput screen (qHTS) assay to identify compounds that modulate exosome biogenesis and/or release by aggressive prostate cancer (PCA) CD63-GFP-expressing C4-2B cells. A total of 4,580 compounds were screened from the LOPAC library (a collection of 1,280 pharmacologically active compounds) and the NPC library (NCGC collection of 3,300 compounds)
approved for clinical use). Twenty-two compounds were found to be either potent activators or inhibitors of intracellular GFP signal in the CD63-GFP-expressing C4-2B cells. The activity of lead compounds in modulating the secretion of exosomes was validated by a tunable resistive pulse sensing (TRPS) system (qNano-Izon) and flow cytometry. The mechanism of action of the lead compounds in modulating exosome biogenesis and/or secretion were delineated by immunoblot analysis of protein markers of the endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent pathways.

The lead compounds tipifarnib, neticonazole, climbazole, ketoconazole, and triademenol were validated as potent inhibitors and sitafloxacin, forskolin, SB218795, fenoterol, nitrefazole and pentetrazol as activators of exosome biogenesis and/or secretion in PC cells. Our findings implicate the potential utility of drug-repurposing as novel adjunct therapeutic strategies in advanced cancer.

Nanoparticles As Mid-Infrared Contrast Agents For Cancer Imaging
Department of Chemistry, Xavier University of Louisiana
LCRC Scientific Program Assignment: Molecular Signaling

Abstract
Histological image analysis of cancer in the mid-infrared is a challenge due to complex and overlapping signal from the sample. Thus there is a need for synthesizing infrared imaging agents that are clearly observable against the congested infrared background of the biomass. We hypothesize that the azido group, which has a strong optical signature in the so-called “infrared transparent region” from 1800 to 2400 cm⁻¹, will enable the recognition of these materials in the biological media. Most living organisms do not produce organic azides to support their life cycle. The specific aims of this project are to (a) synthesize azido-based nanomaterials, (b) enhance the azido infrared signal through surface-enhanced infrared absorption (SEIRA) effect of metals, and (c) evaluate the effectiveness of these materials as infrared molecular probes of biomass. To this end, we developed several wet chemistry methods to coated nanoparticles of nickel and platinum with organic azido groups. The metals were chosen for their amenability to surface plasmons in the infrared. Initial experimental evidence shows that the azido-labels on these metal surfaces are stable and environment-sensitive. In the near future, we will demonstrate that these particles can be observed unambiguously in the biomass providing both spatial and chemical information of the sample. This work is significant as it proposes a pathway to circumvent the congested mid-infrared background of biological samples to obtain chemical information. As a result, we will have the ability to accurately recognize cancer at early stages with high levels of precision using the azido vibration which has the potential to revolutionize current histopathological practice.

Design and Synthesis of Berberine Analogs as Liver X Receptors
Camri Eaton and Florastina Payton-Stewart
Xavier University of Louisiana
Molecular Signaling or Clinical and Translational Research

Liver X Receptors (LXR) have been proven to show effectiveness in ovarian cancer by suppressing proliferation and inducing cell death. The specific aims for this research include the design of anticancer agents using molecular modeling and literature search targeting the LXR receptor, as well as the synthesis of the designed agents using traditional and microwave chemistry. Our research has shown that a known inverse agonist of the LXR, T0901317, has great affinity for the receptor. The skeleton used to derive a possible new LXR agonist is the photochemical berberine because of its anti-tumorgenic, anti-cancer, and anti-viral properties. We hypothesize that structural modifications of berberine may lead to an even more effective agonist, thus granting us with even more suppressed cell proliferation. We hypothesized that the azido group, which has a strong optical signature in the so-called “infrared transparent region” from 1800 to 2400 cm⁻¹, will enable the recognition of these materials in the biological media. Most living organisms do not produce organic azides to support their life cycle. The specific aims of this project are to (a) synthesize azido-based nanomaterials, (b) enhance the azido infrared signal through surface-enhanced infrared absorption (SEIRA) effect of metals, and (c) evaluate the effectiveness of these materials as infrared molecular probes of biomass. To this end, we developed several wet chemistry methods to coated nanoparticles of nickel and platinum with organic azido groups. The metals were chosen for their amenability to surface plasmons in the infrared. Initial experimental evidence shows that the azido-labels on these metal surfaces are stable and environment-sensitive. In the near future, we will demonstrate that these particles can be observed unambiguously in the biomass providing both spatial and chemical information of the sample. This work is significant as it proposes a pathway to circumvent the congested mid-infrared background of biological samples to obtain chemical information. As a result, we will have the ability to accurately recognize cancer at early stages with high levels of precision using the azido vibration which has the potential to revolutionize current histopathological practice.

Identification of SIK1 as a Potential Therapeutic Target for Desmoplasic Small Round Cell Tumor
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Desmoplasic Small Round Cell Tumor (DSRCT) is a rare and aggressive malignant cancer caused by the chromosomal translocation of t(11;22)(p13;q12) that produces a novel, aberrant transcription factor, EWS-WT1. EWS-WT1 is essential in the formation of DSRCT as well as for maintaining DSRCT cell growth. However, current research has not elucidated how EWS-WT1 leads to the oncogenesis of DSRCT. Through our integrative gene expression data, we identified Salt Inducible Kinase 1 (SIK1) as one of direct target genes of EWS-WT1 in JN-DSRCT-1 cell line. SIK1 is a member of the AMPK related kinases and is involved in gluconeogenesis and lipogenesis regulation, skeletal myocytes development, p53 dependent anolisis, and cell cycle regulation. Through our ChIP analysis, we showed that EWS-WT1 directly binds to a 2kb proximal promoter region of SIK1. Following SIK1 depletion, JN-DSRCT-1 cell proliferation sharply decreased, similar to the growth inhibition observed when EWS-WT1 is depleted. We further showed that cells do not transit to S phase when SIK1 is depleted, suggesting a critical role of SIK1 in cell cycle regulation in DSRCT. Taken together, we have established that EWS-WT1 directly activates SIK1 expression and promotes cell proliferation through SIK1. Therefore, our work identified SIK1 as a new potential therapeutic target in DSRCT.
**Development Of Novel Casein Kinase 16 Inhibitors for Treatment of Alzheimer's Disease**

**Authors:** Vishwajeet Jha, Huyen Duong, Cory Gettridge, Jayalakshmi Sridhar. Department of Chemistry, Xavier University of Louisiana, 1, Drexel Dr., New Orleans, LA 70125.

**Abstract:** Alzheimer’s disease (AD) is a chronic neurodegenerative disorder known to have notable symptoms like short term memory loss. It is the cause of 60% to 70% of cases of dementia. Abnormal hyperphosphorylation (P-tau) of the tau protein results in neurofibrillary tangles inside nerve cell bodies, which in turn causes the disintegration of microtubules through accumulation of neurotoxic peptide amyloid-β (Aβ) leading to ultimate collapses of neuron’s transport system. Therefore reversing tau phosphorylation is a promising therapeutic strategy to prevent progression of AD.

CK1δ (belongs to the Casein Kinase 1 family comprising of eight isozymes) is predominantly expressed in the AD hippocampus >30 fold. CK1δ is thought to play a critical role in AD through phosphorylation of the tau protein (associated with microtubules) which precedes neuritic lesion formation, involving CK1δ in the tau fibrillation reaction pathway. CK1δ has been reported to be associated with pathological accumulation of tau in several neurodegenerative diseases including AD, Down syndrome, progressive supranuclear palsy, and Parkinsonism dementia complex of Guam. Inhibition of CK1δ has been shown to reduce fibrillar lesions and inhibit Aβ production.

Our recent studies on quinones as kinase inhibitors revealed one such compound that pharmacologically inhibited CK1δ and Pim1 kinase preferentially over CK1γ2 and 98 other human protein kinases. Several derivatives of the lead compounds were synthesized and preliminary in-vitro CK1δ kinase inhibition assay have shown a few compounds with good potency. Herein, we describe the synthesis and bioassay results of our study.

**Computational Investigation Of Novel Casein Kinase 1 δ/e Inhibitors For Treatment Of Alzheimer's Disease**

**Authors:** Shruti Vandana Kauloorkar, Vishwajeet Jha, Melyssa Bratton, Cory Gettridge, Huyen Duong, Richard Schroeder, Jayalakshmi Sridhar. Department of Chemistry, Xavier University of Louisiana, 1, Drexel Dr., New Orleans, LA 70125.

**Abstract:** Alzheimer’s disease (AD) is a progressive neurodegenerative disorder known to have notable symptoms like short term memory loss otherwise referred to as dementia. Abnormal hyperphosphorylation (P-tau) of the tau protein leads to the aggregation of amyloid plaques which is the hallmark of AD and several other neurodegenerative disorders.

Casein kinase 1δ (CK1δ) and casein kinase 1ε (CK1ε) are closely related Ser-thr protein kinases belonging to Casein kinase 1 family of eight isozymes. The CK1δ and CK1ε isoforms are expressed in the brain. Overexpression of constitutively active CK1ε leads to an increase of Aβ peptide production. CK1ε inhibitors disrupt the amyloid precursor protein cleavage while an active form is known to augment APP production. This effect is specific for one of the brain CK1 isomers (CK1ε). The upregulation of this particular isoform makes it an attractive target for the treatment of Alzheimer’s disease. CK1δ is thought to play role in neurofibrillary tangle formation, dopamine signaling, neuro transmitter release and cancer.

Our research group has identified quinones as inhibitors of CK1δ and CK1ε kinases. Some of these compounds have shown to be more selective for CK1ε than CK1δ. We have performed docking studies on these compounds with the CK1δ and CK1ε X-ray crystal structures using the MOE modeling software to study the role of residues in selectivity. The results of the docking studies are presented here.

**Molecular And Structural Traits Of IRS-1/LC3 Nuclear Structures – Effects On Autophagy Control And Tumor Cell Survival**

**Presenter’s Name:** Adam Lassak

**PI:** Krzysztof Reiss

**University Affiliation:** Stanley S Scott Cancer Center, Department of Medicine, LSU Health Sciences Center, New Orleans, LA

**LCRC Scientific Program Assignment:** Molecular Signaling

**Collaborators:** Dorota Wyczewieksa, Anna Wilk, Adriana Zapata, Mathew Dean, Luis Del Valle, Francesca Peruzzi, Augusto Ochoa

**Abstract**

Insulin receptor substrate 1 (IRS-1) is a common cytosolic adaptor molecule involved in signal transduction from insulin and IGF-1 receptors. IRS-1 can also be found in the nucleus in tumor cells expressing viral oncprotein, large T-antigen from human polyomavirus JC or from simian polyomavirus SV40. We report here a new finding of unique IRS-1 nuclear structures, which we observed initially in glioblastoma biopsies and glioblastoma xenografts. These nuclear structures can be reproduced in vitro by ectopic expression of IRS-1 DNA cloned in frame with nuclear localization signal (NLS-IRS-1). In these structures, IRS-1 localizes to the periphery while the center harbors a key autophagy protein, LC3. These new nuclear structures are highly dynamic, rapidly exchange and sequester LC3 inside the nucleus as well as outside the nucleus. In tumor cells engineered to express NLS-IRS-1, the IRS-1/LC3 nuclear structures repress autophagy induced both by amino acid starvation or rapamycin treatment. This process, IRS-1 nuclear structures sequester LC3 inside the nucleus, possibly preventing its cytosolic translocation and the formation of new autophagosomes. This novel mechanism provides a quick and irreversible way of inhibiting autophagy, which could counteract autophagy-induced cancer cell death under severe stress including anticancer therapies.

**Obesity Increases the Risk of Both Endogenous and Xenobiotic-Induced Mutagenesis**

**Author line:** Lichtler RC*, Wilson MJ*, Wickliffe JK**

**2018 LCRC Annual Retreat Booklet**
Regulation Of Mammary Gland Development And Breast Cancer Progression By Macrophage-Derived C/EBPβ

Michelle D. Rojo and Heather L. Machado
Department of Biochemistry and Molecular Biology, Tulane School of Medicine - Molecular Signaling

CCAAT enhancer binding protein beta (C/EBPβ) is a transcription factor that regulates the growth and differentiation of macrophages and mammary epithelial cells. C/EBPβ consists of three isoforms (LIP, LAP1, and LAP2) and in normal mammary development, C/EBPβ-LIP is upregulated during stages with enhanced proliferation. Our lab has shown that in breast premalignancy, tumor-promoting macrophages are recruited to pre-invasive lesions where they promote tumor cell invasion. Little is known about how pre-invasive lesions acquire the ability to invade the adjacent stroma, or the role that macrophages play in this process. We hypothesize that C/EBPβ is a key mediator of mammary gland development and C/EBPβ-LIP drives tumor progression. Macrophages are recruited to the mammary gland to help drive ductal elongation during mammary gland development. The mechanisms by which macrophages regulate this process are unclear, but C/EBPβ is an important regulator for macrophage differentiation and function. In vivo studies...
Chronic Ethanol Exposure Of Human Pancreatic Normal Ductal Epithelial Cells Induces Cancer Stem-Like Cell Phenotype Through SATB2

We Yu\textsuperscript{1}, Yiming Ma\textsuperscript{1}, Sharmila Shankar\textsuperscript{1,2}, and Rakesh K. Srivastava\textsuperscript{1,2}

\textsuperscript{1}Kansas City VA Medical Center, Kansas City, MO 66128, USA.
\textsuperscript{2}Department of Genetics, Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA.

The incidence of pancreatic cancer is on the rise. Risk factors for pancreatic cancer include alcohol toxicity and metabolic conditions such as obesity, hypertension, dyslipidemia, insulin resistance, and type 2 diabetes. However, the molecular mechanism by which chronic alcohol consumption contributes to pancreatic cancer is not well understood. The purpose of the study was to demonstrate the effects of long-term chronic ethanol exposure on the transformation of human pancreatic normal ductal epithelial cells. Our data showed that ethanol-transformed HPNE cells were more progressively transformed exhibiting spheroids and colonies, and anchorage-independent growth. These transformed cells contained high levels of reactive oxygen species and induced SATB2 expression. Furthermore, during ethanol-induced cellular transformation, cells gained the phenotypes of cancer stem cells by expressing pluripotency maintaining factors (Oct4, Sox2, cMyc, and KLF4) and stem cell markers (CD24, CD44, and CD133). Ethanol-induced SATB2 can bind to the promoters of KLF4, Oct4, cMyc, Sox2, Bcl-2 and XIAP genes. Suppression of SATB2 expression in ethanol-transformed HPNE cells inhibited cell proliferation, colony formation, and markers of cancer stem cells and pluripotency. These data suggest that chronic alcohol consumption may contribute towards the development of pancreatic cancer by converting HPNE cells to cancer stem-like cells.

Exosome-Hormone Novel Axis Promotes Stem Cell Based Heterogeneous Prostate Cancer Cell Survival Under Hormone-Deprivation Conditions

Sudha Talwar\textsuperscript{1}, Bashir M. Rezk\textsuperscript{1,2}, Hogyoun Kim\textsuperscript{1}, Raju Thomas\textsuperscript{1,3}, Amrita Datça\textsuperscript{1}, Samuel C. Okpechi\textsuperscript{1,3}, Adedoyin Johnson\textsuperscript{4}, Elisa Marie Ledet\textsuperscript{1}, Grimm, Deborah A\textsuperscript{5}, Maghfour Jalal\textsuperscript{1}, Debasis Mondal\textsuperscript{1}, Oliver Sartor\textsuperscript{4}, and Asim B. Abdel-Mageed\textsuperscript{1,4,5}

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\textsuperscript{2}Department of Natural Sciences, Biology Unit, Southern University at New Orleans, New Orleans, LA, USA
\textsuperscript{3}Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA, USA
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ABSTRACT:

An overarching aim of this study, to establish a novel approach to investigate the CRPC metastasis under hormones scarcity:

Presently, a challenging task is to develop a potent therapeutic regimen for the highly heterogeneous metastatic castration-resistant prostate cancer (CRPC). Mechanisms underlying the establishment of a favorable microenvironment for growth and formation of a heterogeneous metastatic niche by the disseminated tumor cells remain elusive. Despite androgen-ablation therapy, androgen receptor (AR) signaling pathway remains viable and plays a central role in survival and growth of the hormone-resistant mCRPC. Current evidence suggests that obesity is a risk factor for disease progression with poor clinical outcome. Recent studies have implicated tumor-derived exosomes in cell-cell communications, tumor growth, and metastases of many human cancers. Here, we report that hormone deprivation conditions (HDC) primed the de novo biosynthesis of androgens (T) and release of aromatase (CYPI9) mRNA containing exosomes by the AR\textsuperscript{1,2} and ER\textsuperscript{2} expressing PC-3 cells. The released T level was sufficient to support the growth and survival of the androgen-dependent AR\textsuperscript{1,2} LNCaP cells under HDC. The uptake of the secretory androgens and exosomes by PC patient’s peri-prostatic fat derived adipose stem cells (pASCs) and primes for de novo synthesis of estradiol (E2), which in turn supported the growth of the AR\textsuperscript{1,2}/ER\textsuperscript{2} expressing PC-3 cells. Our in vitro observations corroborated with detection of high levels of blood-circulating E2 and CYP19 transcript-harboring exosomes as well as tumor ER\textsuperscript{2} protein expression in metastatic CRPC patients samples. Together, in vitro mutual interactions of the triple PC\textsuperscript{1,2}/PC\textsuperscript{1,2}/pASC cell model system suggest that T-E2 novel exosome axis plays a potential role in the formation and survival of heterogeneous metastatic PC niche under HDC. Simultaneous targeting of de
Determined Substrate Specificity of Lysine Deacetylases
Tasha B. Toro and Terry J. Watt*
* PI
Xavier University of Louisiana
Molecular Signaling

Analysis of the human proteome has identified thousands of unique protein sequences that contain acetylated lysine residues in vivo. Lysine deacetylases (KDACs) are enzymes that reverse this post-translational modification, by catalyzing the hydrolysis of ε-N-acetyllysine residues in proteins via a conserved mechanism. Aberrant KDAC activity has been linked to numerous diseases, including several cancers. However, details regarding substrates of particular KDACs and how substrate specificity is determined are lacking. Widely used in vitro systems for studying KDACs involve peptide substrates conjugated to fluorescent dye molecules, such as coumarin. While experimentally convenient, we have demonstrated that these molecules affect the interaction of KDACs with potential substrates in a manner that is neither predictable nor biologically-relevant. To investigate substrate preferences in a more biologically-relevant manner, we have developed a peptide-based KDAC activity assay that does not rely on an attached fluorophore and can be used with any peptide sequence. This assay has allowed us to probe substrate specificity using peptides derived from acetylated proteins. We observed that substrate length impacts specific activity and may not completely mimic activity when compared to a full-length protein. Therefore, substrates must be identified in cells to definitively determine targets of particular KDACs. We are using the CRISPR/Cas9 system to genetically inactivate or enhance the activity of individual KDACs in cells, which will allow us to identify changes in the acetylome that are attributable to particular KDACs. Identifying substrates using this method will lead to a better understanding of how changes in KDAC activity can lead to cancer and other diseases.

Targeting Notch One Notch Above
Deniz A. Ucar-Bilyeu1, Margarite D. Motossian2, Van T. Hoang-Barnes1, Fokhrul M. Hossain1, Mohit Gupta4, Hope E. Burks2, Thomas D. Wright1, Jane Cavanaugh4, Patrick Flaherty1, Matthew E. Burrow1, Lucio Miele1. 1Louisiana Cancer Research Center, Department of Genetics, New Orleans LA 2 Tulane University School of Medicine, Department of Medicine, New Orleans LA 3 National Cancer Institute, Laboratory of Cell and Developmental Signaling, Frederick, MD 4 Duquesne University, Mylan School of Pharmacy, Department of Pharmacology, Pittsburgh, PA

Triple negative breast cancer (TNBC) is a molecularly heterogeneous, clinically aggressive disease group that is highly prevalent among African-Americans and younger patients. Standard chemo/radiotherapy often produces clinical responses, but recurrence and metastasis are unfortunately common. Metastatic disease is generally incurable. Chemo/radiotherapy has been shown to induce EMT and enrich a chemo-resistant cancer stem cell-like (CSC) population in TNBC. CSCs are thought to drive disease recurrence. Notch signaling, particularly Notch1, is critical for maintenance of TNBC CSC. Expression of Notch1 and its ligand Jagged1 are correlated with poor prognosis. Efforts to pharmacologically target Notch directly have been impaired by the systemic toxicity of the Gamma Secretase Inhibitors (GSI), used, and the fact that Notch1 also plays a key role in anti-tumor adaptive immunity. Therapeutic agents that target Notch signaling in breast cancer cells indirectly and selectively are a potentially attractive strategy. However, no such target has been identified to date. We have found that the MAPK5-ERK5 kinase pathway, which contains at least two druggable targets, functions as a master regulator of Notch signaling in TNBC cells. ERK5 knockout TNBC cells have dramatically decreased expression of Notch receptors, ligands and targets. In vivo, these cells form barely detectable tumors that do not metastasize and express lower levels of Notch1 and its ligand Jagged1. Using in silico screening method, we have identified a small molecule compound that targets MAP2K5 (MEK5) and decreases phosphorylation of MAPK7 (ERK5). Consistent with ERK5KO cells, suppression of ERK5 phosphorylation decreased the amount of Notch1 and Jagged1 protein and mRNAs. More importantly, without suppressing the T-cell proliferation, a selective MEK5 inhibitor, SC-181, reversed EMT and reduced the CD44hi/CD24lo CSC population in TNBC cells. Treatment with nM concentration of this compound decreased the number and size of mammospheres in a dose dependent manner. Our preliminary results suggest that targeting the MEK5-ERK5 pathway is a promising strategy to targeting Notch signaling of CSC in TNBC to overcome metastasize and resistance.

Circadian Melatonin Disruption By dLAN Activates IL6/STAT3/DNMT1 Pathway To Epigenetically Inhibit The Tumor Suppressor ARH1 And Drive Paclitaxel Resistance In Breast Cancer
Shulin Xiang, Robert T. Dauchy, Aaron E. Hoffman, David Pointer, Lin Yuan, Tripp Frasch, David E. Blask, and Steven M. Hill
Department of Structural and Cellular Biology, Tulane University School of Medicine, Tulane New Orleans, LA 70112

Abstract
Chemotherapeutic resistance represents a major impediment to the successful treatment of breast cancer. Disruption of circadian time structure and suppression of circadian nocturnal melatonin (MLT) production by exposure to dim light at night (dLAN), as occurs with shift work, and/or disturbed sleep-wake cycles, is associated with a significantly increased risk of breast cancer and resistance to tamoxifen and doxorubicin. Melatonin inhibition of human breast cancer growth and chemo-resistance involves mechanisms including suppression of tumor metabolism and inhibition of various kinases, and transcription factors which are often activated in TNBC. Determining Substrate Specificity of Lysine Deacetylases
Tasha B. Toro – Xavier

Determining Substrate Specificity of Lysine Deacetylases
Tasha B. Toro and Terry J. Watt*
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Molecular Signaling

Analysis of the human proteome has identified thousands of unique protein sequences that contain acetylated lysine residues in vivo. Lysine deacetylases (KDACs) are enzymes that reverse this post-translational modification, by catalyzing the hydrolysis of ε-N-acetyllysine residues in proteins via a conserved mechanism. Aberrant KDAC activity has been linked to numerous diseases, including several cancers. However, details regarding substrates of particular KDACs and how substrate specificity is determined are lacking. Widely used in vitro systems for studying KDACs involve peptide substrates conjugated to fluorescent dye molecules, such as coumarin. While experimentally convenient, we have demonstrated that these molecules affect the interaction of KDACs with potential substrates in a manner that is neither predictable nor biologically-relevant. To investigate substrate preferences in a more biologically-relevant manner, we have developed a peptide-based KDAC activity assay that does not rely on an attached fluorophore and can be used with any peptide sequence. This assay has allowed us to probe substrate specificity using peptides derived from acetylated proteins. We observed that substrate length impacts specific activity and may not completely mimic activity when compared to a full-length protein. Therefore, substrates must be identified in cells to definitively determine targets of particular KDACs. We are using the CRISPR/Cas9 system to genetically inactivate or enhance the activity of individual KDACs in cells, which will allow us to identify changes in the acetylome that are attributable to particular KDACs. Identifying substrates using this method will lead to a better understanding of how changes in KDAC activity can lead to cancer and other diseases.

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Abstract
Chemotherapeutic resistance represents a major impediment to the successful treatment of breast cancer. Disruption of circadian time structure and suppression of circadian nocturnal melatonin (MLT) production by exposure to dim light at night (dLAN), as occurs with shift work, and/or disturbed sleep-wake cycles, is associated with a significantly increased risk of breast cancer and resistance to tamoxifen and doxorubicin. Melatonin inhibition of human breast cancer growth and chemo-resistance involves mechanisms including suppression of tumor metabolism and inhibition of various kinases, and transcription factors which are often activated in TNBC to overcome metastasize and resistance.
drug-resistant breast cancer. Signal Transducer and Activator of Transcription 3 (STAT3) is frequently overexpressed and activated in Paclitaxel (PTX)-resistant breast cancer and can promote the expression of DNA methyltransferase one (DNMT1) to epigenetically suppresses the transcription of the tumor suppressor Aplesia Ras homolog one (ARHI). ARHI inhibits PTX-resistance by binding and sequestering STAT3 in the cytoplasm. Our data demonstrate that breast tumor xenographs in rats that are circadian MLT disrupted by exposure to dLAN express elevated levels of various oncogenes including the phospho-activated and acetylated STAT3, increased DNMT1, but reduced Sirtuin 1 (SIRT1) and ARHI. This work further demonstrates that in vitro administration of MLT and/or SIRT1 can block/reverse Interleukin 6 (IL-6) induced acetylation of STAT3 and its methylation of ARH1 to increase ARH1 mRNA expression in MCF-7 breast cancer cells. Finally, our analyses of the I-SPY 1 trial found that elevated levels of the MT1 receptor is significantly correlated with pathologic complete response following neo-adjuvant therapy in breast cancer patients. Melatonin acts as both a metabolic inhibitor and circadian-regulated kinase inhibitor in breast cancer to reestablish the sensitivity of breast tumors to PTX and drive tumor regression. This is the first study to demonstrate that circadian disruption of nocturnal MLT by dLAN drives intrinsic resistance to PTX chemotherapy via epigenetic mechanism.

Field of Research: Molecular Signaling

Inhibition Of Colon Cancer Cell Invasion And Metastasis With A Non-Cyclooxygenase Inhibitory Activity Of Sulindac By Suppressing miR-17/QKI Axis
Bin Yi, Hongyou Zhao, Zhipin Liang, Ruixia Ma, Burthia E. Booker, Che'sque M. Phillips, Huike Yang, Xiaolin Liu, Yaguang Xi.
Department of Genetics and Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac, have been reported for striking chemopreventive activity in various types of human cancer, including colon cancer. We recently reported that sulindac sulfide (SS) could potently inhibit the invasion of human colon cells at non-toxic concentrations without affecting tumor cell proliferation. The underlying mechanism involves the inhibition of NF-κB transcriptional activity to suppress specific miRNAs without COX inhibition. MiR-17, a well-documented oncogenic miRNA which promotes tumor cell invasion and metastasis, is of particular interest. Using a bioinformatics approach, we found all six miRNAs within the miR-17-92 cluster target the RNA-binding protein, protein quaking (QKI) which has been reported as a tumor suppressor in colon cancer. In this study, we focus on investigating the role of the miR-17/QKI axis in mediating the anti-invasive activity of sulindac in vitro and in vivo. Firstly, our results show that miR-17 is significantly upregulated in colon cancer cells and tissues compared with normal control. Moreover, QKI is significantly downregulated in colon cancer tissues compared with normal control. Furthermore, SS can both downregulate the mRNA expression of miR-17 and induce QKI in HCT116 and LIM2405 colon cancer cells. In addition, we determined that miR-17 directly targets QKI using dual luciferase and western blotting assays. Importantly, QKI overexpression enhances the anti-invasive activity of SS and QKI knockdown attenuates the anti-invasive activity of SS in HCT116 and LIM2405 cells. As expected, COX inhibition is not required for SS inhibition of Lim2405 colon cancer cell invasion. Furthermore, miR-17 facilitates the formation of lung metastasis nodules, whereas miR-17 knockdown shows the inverse phenotypes in vivo. Finally, miR-17 knockdown increases the anti-metastatic activity of SS in vivo. In conclusion, these observations suggest that the miR-17/QKI axis is responsible for the inhibitory effect of SS on colon cancer cell invasion and metastasis in vitro and in vivo.

Keywords: sulindac, colon cancer, invasion, metastasis, microRNA, QKI

Age-Associated Alteration In Th17 Cell Response Is Related To Prostate Carcinogenesis
Zhang Qiuyang, Liu S', You Z', Rowan BG, Jazwinski SM, Myers L', Wang AR5, Sartor O2,6
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Abstract

T-helper 17 (Th17) cells produce interleukin-17 (IL-17) that plays an important role in many autoimmune and inflammatory diseases. IL-17 promotes prostate cancer formation and growth in mouse models have been demonstrated in our recent studies. However, little is known whether aging affects the functions of Th17 cells. The aim of this study was to investigate the role of Th17 cells in the aging process in prostate carcinogenesis. Initial investigation of mouse prostate tissues from wild-type C57BL/6J mice showed that aged (>80-week-old) mouse prostates had significantly increased inflammatory cell infiltration, increased protein and mRNA levels of pro-inflammatory mediators and Th17 cytokines and activated NF-κB and ERK1/2 signaling compared to young (12-20-week-old) mouse prostates. To gain a mechanistic understanding of how the Th17 cells promote prostate carcinogenesis in the aging process, we isolated splenic T cells from young and aged mice. Naïve CD4+ T cells isolated from the young and aged mice were differentiated in vitro for 48 and 72 hours and harvested for subsequent experiments. Results indicated that Th17 cells, Th17 cytokines and Th17/Treg ratio were significantly increased in aged mice compared to young mice. The human prostate cancer cell lines (LNCaP, DU-145 and PC3) and mouse prostate cancer cell line (PTEN-CaP8) was cultured in their suitable medium. These cell lines were treated with the above mentioned conditioned media for 48 and 72 hours and harvested for subsequent experiments.
experiments. When human and mouse prostate cancer cell lines were exposed to the aged Th17 conditioned media, cell proliferation, migration and invasion were significantly increased, and the pro-inflammatory NF-κB pathway in PCA cell lines was activated compared to cells exposed to young ones. In summary, these results collectively indicate that Th17 cell responses are elevated in mice in the aging process, and this age-related elevated Th17 cell responses may play an important role in prostate carcinogenesis.

This work was supported by the NIH-National Institute of General Medical Sciences COBRE (2P20GM103629-06, PI: SMJ; Pilot Project PI: QZ).

Sulindac Inhibition of Colon Tumor Cell Growth through miR-182/FOXO3a/CyclinG2 Signaling
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Nonsteroidal anti-inflammatory drugs (NSAIDs) display promising antineoplastic activity in many human cancers including colorectal cancer. Previous studies reported that sulindac sulfide (SS) inhibits the growth of tumor cells through cyclooxygenase-2 (COX-2) dependent or independent pathways. As we know that COX-2 independent pathways lessen toxicity and support the clinical potential for using sulindac as a chemoprevention drug. However, the molecular mechanisms responsible for COX-2 independent pathways have not been completely elucidated. In this study, we found for the first time that CyclinG2 plays a role in the cell cycle arrest induced by SS. By using the loss-of-function strategy, we found that FOXO3a is responsible for the SS induction of CyclinG2. After screening of miRNAs that potentially target FOXO3a, our results show that the expression of miR-182 was inversely correlated with the expression of FOXO3a in colon tumor cells treated by SS. By using a luciferase assay, we validated that miR-182 directly binds to the 3'-UTR of FOXO3a. When miR-182 was knocked down, SS could neither efficiently upregulate the expression of FOXO3a nor lead to cell cycle arrest as it did in wild type cells. Finally, we used real-time PCR to examine the expression of miR-182/FOXO3a/CyclinG2 in paired clinical samples; the results suggest that the expression of miR-182 is inversely correlated with the expression of FOXO3a and CyclinG2 in tumor tissues. Therefore, our results support a pathway consisting of miR-182/FOXO3a/CyclinG2 as a novel non-COX inhibitory mechanism involved in SS anticancer activity in colon cancer cells.

Key Words: Sulindac, Cell cycle arrest, miR-182, FOXO3a/CyclinG2
Section 2-4

Population Sciences Abstracts
Fostering Cancer Education And Participation In Research Among Latinos And African Americans In Louisiana

Presenter's Name: Margarita Echeverri, MSc, PhD, Associate Professor, mechever@xula.edu, 504-520-6719
Field of research: Population sciences
Collaborators: Anna Napoles, PhD, MPH, Professor, University of California San Francisco; Elizabeth Yanez, BS, Xavier University of Louisiana.

Background: Louisiana has one of the highest cancer mortality rates among African Americans and cancer is the leading cause of death among Latinos in the U.S. Our previous studies measuring the Cancer Health Literacy (CHL) among these two populations (N=1,000) found that 21.8% scored in the lower level of CHL (0-10), 49.8% scored in the medium level (11-20), and 28.4% in the higher one (21-30), and recommended developing culturally appropriated educational interventions to address cancer misconceptions and lack of knowledge among the two populations.

Objectives: To develop an educational intervention in cancer screening, research, and bio-banking among African Americans and Latinos in Louisiana, field-test the preliminary comparative effectiveness of the intervention through three different delivery methods (print, video-based, and face-to-face), and assess which delivery method produces the best results.

Methods: Results of the assessment of cancer literacy were used to identify misconceptions and knowledge gaps regarding cancer risks, screening and research. Focus groups assessed the applicability of educational materials selected from the literature review and that addressed the gaps identified. The training “Cancer 101: A Cancer Education and Training Program” (Briant, et al., 2012), available in English and Spanish, was selected, adapted and pilot-tested with 10 Latinos and 10 African Americans from different age-ranges and educational backgrounds. The resulting trainings “Cancer 101 for Latinos” (in Spanish) and “Cancer 101 for African Americans” (in English) were implemented using the three different delivery options (print, video-based, and face-to-face). A field-test of the preliminary comparative effectiveness of the three interventions was conducted with a stratified sample (by gender, age-range and education) of 54 Latinos and 54 African Americans, who were randomly assigned to the different arms (18 in each arm). All participants completed the same pre-posttests before and after the intervention and also completed evaluation of the training.

Results: For the field tests of the three interventions, 54 Latinos Spanish-speaking (24 men, 30 women) and 54 African Americans (16 men, 38 women) completed the study. In general, the preliminary comparative effectiveness of the three delivery methods of the intervention for both groups of participants resulted in a 13.3% increased knowledge (95% CI:10.6%, 16.0%, p<0.000). However, African Americans had higher increase in knowledge (14.2%) than the Latinos (12.4%). Although there were no significant pre-posttest differences by age, gender, or intervention type, there was a significant interaction of educational level in the posttest scores (p<0.022).

Conclusion: Addressing cancer literacy in minorities is becoming critical for increasing their participation in cancer screening and research. Overall participants learned more about cancer after receiving the targeted intervention, regardless of delivery type. Results show that educational interventions may be the best approach to address lack of knowledge and trust about cancer research. However, considering that no intervention method (print, video-based or face-to-face) produced higher knowledge changes, we recommend the use of hybrid educational methods to address the learning needs of Latinos and African Americans with low cancer literacy levels.

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Tyra Gross – Xavier

WIC Participants and Tobacco Use: The Current Research and Opportunities

Presenter: Tyra T. Gross, Ph.D., MPH
PI: Michael D. Celestin Jr., MA
Collaborators: Tung-Sung Tseng, DrPH, Sarah Moody-Thomas, Ph.D.
Program Assignment: Population Sciences

Department of Public Health Sciences, Xavier University of Louisiana
School of Public Health, Louisiana State University Health Sciences Center

Background: Tobacco use before, during, and after pregnancy threatens maternal and child health. Many women spontaneously quit smoking upon becoming pregnant, but one in ten women report smoking during the last three months of pregnancy. The USDA Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) provides nutrition assistance for low-income pregnant and postpartum women and their children from birth to age five. The Louisiana Tobacco Control Initiative (TCI) provides evidence-based cessation services to health systems and is in the formative phase of developing a smoking cessation intervention for pregnant women served by WIC clinics. Purpose: The present study reviewed research literature relative to smoking and cessation interventions among WIC participants. Methods: In February 2018, we searched the PubMed, PsycINFO, and CINAHL databases using keywords ‘WIC’ AND (‘smoking’ OR ‘tobacco’). Criteria for including studies in this review were that they had to be published in English language and the past ten years. Results: Twelve studies met review criteria of which four discussed smoking behaviors, five evaluated cessation interventions (i.e., SMART, Babies Living Safe and Smoke-Free, Baby & Me Tobacco-Free, 5 A’s clinical protocol), and three examined environmental changes (i.e., smoke-free home rules, tobacco advertisements). Conclusion: This review highlights the variety of approaches used to educate and empower WIC participants to quit smoking. As a social service provider, WIC clinics can be an important public health partner to promote smoking cessation to pregnant and postpartum women.
Social Determinants of Triple Negative Breast Cancer Disparities in Louisiana

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²LSUHSC, School of Public Health

Field of Research: Population Sciences

Abstract:
Triple Negative Breast Cancer (TNBC) is an aggressive, heterogeneous subtype of breast cancer. TNBC patients have generally high risk of recurrence and metastasis, and treatment options remain limited, as there are no effective targeted therapies available. In USA, TNBC is diagnosed disproportionately more frequently in African American (AA) women than in European American (EA) women. We set out to investigate the role of social determinants in racial disparities in TNBC.

Methods: TNBC patients diagnosed in Louisiana from 2010-2012 were identified from the Louisiana Tumor Registry. Patients were geocoded to census tract of residence at time of diagnosis. Census tract population and socioeconomic measures were obtained from the US Census American Community Survey. We used multilevel statistical models to analyze the role of neighborhood concentrated disadvantage index (CDI), a robust measure of physical and social environment, in racial disparities in TNBC incidence, stage at diagnosis, and stage-specific survival for the study population. CDI scores were calculated according to the PhenX Toolkit protocol.

Results: We identified 1,216 women with TNBC for the study. Controlling for age, we found that AA women had a 2.21 (1.96, 2.48) fold risk of TNBC compared to EA in Louisiana. Results from multivariate analyses indicated that the incidence of TNBC was independent of neighborhood CDI, as was the racial disparity. However, CDI did explain existing racial disparities in both stage at diagnosis and stage-specific survival.

Overall, our results suggest that the excess risk of developing TNBC in AA women is due to biological risk, while social determinants appear to have a role in TNBC disparities associated with stage and survival in Louisiana. Further research is needed to determine the mechanisms through which social determinants affect the promotion and progression of this disease and guide efforts to improve overall survival.

The Relationship Between Smoking And Quality Of Life: A Hospital-Based Survey In Louisiana

Presenter's Name: Yu-Hsiang Kao
PI: Tung-Sung Tseng
University Affiliation: LSUHSC, School of Public Health
LCRC Scientific Program Assignment: Population Science
Names of collaborators: Michael D. Celestin, Qingzhao Yu, Sarah Moody-Thomas

Summarize specific aims
Smoking is a major risk behavior to provoke cancers. Studies also found smokers tend to have a lower health-related quality of life (HRQoL). However, the relationship between smoking status and EuroQoL 5-dimensions (EQ-5D) especially in which dimension is not well understood. The specific aim of this study is to investigate: 1. the relationship between smoking behavior and EuroQol visual analogue scale (EQ-VAS); 2. the relationship between smoking and the EQ-5D; and 3. The relationship between smoking and EQ-5D index score.

Research methodology
This study applied the cross-sectional study design and used the 2017 LSU Tobacco Control Initiative (TCI) patient survey that conducted in the eight public hospitals located in population centers across the Louisiana. Patient’s smoking status was assessed by two questions: “Have you ever smoked at least 100 cigarettes in your lifetime?” and “Do you now smoke every day, some days, or not at all?” EQ-VAS is a 20 cm vertical scale with end points of 0 and 100 to ask interviewee’s health status on the day of the interview. EQ-5D is composed of five questions on mobility, self-care, pain/discomfort, anxiety/depression, and usual activities with five possible answers for each item. EQ-5D index score, range from 0 to 1, was calculated by the U.S. population-based preference weights. According to different interesting outcomes, we adopted multiple linear regressions to estimate the coefficient for EQ-VAS and EQ-5D index scores. For EQ-5D, we used multiple logistic regressions to estimate the odds ratio for each dimension across patient’s smoking behavior.

Result status
Current smokers (35%) reported the lowest EQ-VAS (62.99) and EQ-5D index score (0.76) than former and never smoker. A statistically significant decline trend was found between smoking status and both EQ-VAS and EQ-5D index score. After controlling other covariables, current smoker had not only a lower EQ-VAS (coefficient=-5.23) and EQ-5D index score (coefficient=-0.03) but also a higher likelihood of having either pain/discomfort or anxiety/depression (adjusted odds ratio=1.66 and 1.90, respectively) than never smokers. Our study found that current smokers had a worse overall HRQoL and had higher probabilities of getting problems in pain/discomfort and anxiety/depression as well. The findings revealed that smoking might be associated with lower HRQoL and more 2018 LCRC Annual Retreat Booklet
The Effects Of Smoking Cessation On HbA1c In African-American Diabetic Smokers

Mirandy Li1,2, Michael Celestin1, Yu Hsiang Kao1, Sarah Moody-Thomas1, Qingzhao Yu1, Tung Sung Tseng1
LSUHSC School of Medicine, 1 LSUHSC School of Public Health

Objective: In the United States today, smoking continues to be a major public health issue, especially among diabetic African-American smokers. HbA1c is a marker for diabetes management, with HbA1c levels ≥6.5 mmol/mol being indicative of poorer control of diabetes. Because prior smoking cessation research has focused almost exclusively on non-Hispanic Whites with diabetes, the precise relationship between smoking cessation and HbA1c in African-American diabetics remains unclear.

Hypothesis: Compared to diabetic African-Americans who continue to smoke, diabetic African-American quitters have an average lower HbA1c level one year after baseline.

Methodology: Using a retrospective, cohort study design, we analyzed electronic health record data collected between 2009 and 2012 for outpatients receiving care at seven LSU Health Care Services Division state hospitals. The effect of smoking cessation on HbA1c levels was assessed between baseline and one year follow-up. HbA1c levels were categorized as “normal” or “high” based on national guidelines. McNemar tests assessed changes in HbA1c for continuing smokers and sustained quitters, and logistic regressions determined the relationship between cessation and HbA1c after adjusting for age, gender, financial class, BMI, and use of glucose medications.

Results: The sample included 464 smokers, of which 431 (93%) were continuing smokers at follow-up. After one year, continuing smokers showed a significant increase in developing high HbA1c levels (p=0.0017) compared to baseline. No significant change in HbA1c was observed among sustained quitters. After controlling for all risk factors, sustained quitters were significantly less likely than continuing smokers to develop high HbA1c levels at one year follow-up (OR 0.611, 95% confidence interval 0.493-0.760, p=0.0001).

Conclusion: This study suggests that in this African-American diabetic smoking population, smoking cessation may be beneficial for diabetes management.

The National Health and Nutrition Examination Survey (NHANES) 2009-2014

Does Income Affect the Association Between Smoking Status and Periodontitis?

Mirandy Li1, Michael Celestin1, Yu Hsiang Kao1, Sarah Moody-Thomas1, Qingzhao Yu1, Tung Sung Tseng1
1 LSUHSC School of Medicine, 1 LSUHSC School of Public Health

Field of Research: Population Sciences

Objective: The National Health and Nutrition Examination Survey (NHANES) 2009-2014

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Methodology: Using a retrospective, cohort study design, we analyzed electronic health record data collected between 2009 and 2012 for outpatients receiving care at seven LSU Health Care Services Division state hospitals. The effect of smoking cessation on HbA1c levels was assessed between baseline and one year follow-up. HbA1c levels were categorized as “normal” or “high” based on national guidelines. McNemar tests assessed changes in HbA1c for continuing smokers and sustained quitters, and logistic regressions determined the relationship between cessation and HbA1c after adjusting for age, gender, financial class, BMI, and use of glucose medications.

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Conclusion: This study suggests that in this African-American diabetic smoking population, smoking cessation may be beneficial for diabetes management.
Section 2-5

Clinical and Translational Research Abstracts
Lymph Node Stromal Cells Enhanced Human Renal Cell Carcinoma Epithelial Mesenchymal Transition In A Patient-Derived Orthotopic Xenograft Model Detected Using Multiplex Immunofluorescence Staining

**Introduction:** Renal cell carcinoma (RCC) incidence is increasing, and incurable metastases affect up to 25% of RCC patients. Epithelial to mesenchymal transition (EMT) have been shown to play a role in the tumorigenic process. Previously, using our unique patient-derived orthotopic xenograft (PDOX) model that mimics metastatic RCC, we showed that lymph node stromal cells (LNSC) enhanced tumorigenicity and metastases of certain RCC cell lines and patient tumor specimens. The objective of this study was to use 4-color immunofluorescence (IF) staining to investigate potential molecular mechanisms that promote RCC metastasis.

**Methods:** Luciferase-tagged human RCC cell lines (A498 and CAKI-1) and one patient tumor (KiCaPt58) cells were implanted by subcapsular kidney injection into NOD/SCID mice with or without LNSC (HK) cells for tumor growth and metastases. Upon necropsy, paraffin embedded primary tumors were examined with 4-color IF staining with antibodies against human EMT markers (vimentin and E-cadherin), and tumor markers CD44 and claudin-2. Photoshop software was used to digitally quantify expression levels of EMT markers in CD44+ tumor cells and Student t tests were used to determine significant differences.

**Results:** Co-injection of HK cells enhanced vimentin expression of CD44+ cancer cells in both A498 (p<0.05) and CAKI-1 (p<0.005) tumors, and a decreased expression of E-cadherin in A498 and a KiCaPt58 patient tumors. However, claudin-2 staining showed less affinity and more variability compared with CD44 staining. KiCaPt58, due to its sarcomatoid phenotype, which is a more aggressive sub-form of RCC, showed minimal vimentin upregulation. This could explain its reduced dependency on HK cells and tendency to form tumors without HK cells.

**Conclusion:** The ability to digitally quantify EMT markers via multiplex immunofluorescence staining on a single tumor slice has the potential to increase the reliability and specificity of data collection in various RCC tumor specimens, assisting the discovery of treatment targets.

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Regulation Of Myeloid Cells Function By Mirnas In HIV-Infected Patients

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**LSU Health Sciences Center, Stanley S. Scott Cancer Center**

**LCRC Scientific Program Assignment: Clinical and Translational Research**

Human Immunodeficiency Virus 1 (HIV-1) replication in myeloid cells highly contribute to the chronic inflammation observed in HIV+ patients and serve as viral reservoirs. Persistence of immune activation could lead to T cell dysfunction, immune exhaustion, HIV progression and increased risk of developing HIV-associated malignancies. Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells and have the strong ability to suppress immune cell function.

MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression by inhibition or degradation of target mRNAs. Mounting evidence suggest the involvement of miRNAs in HIV pathogenesis and immune function. However, it is largely unknown how the HIV-mediated changes in miRNA expression affect immune responses and promote viral persistence.

We have profiled eight miRNAs based on their involvement in HIV pathogenesis and myeloid cell function in the following cell types obtained from HIV+ subjects on ART and healthy controls: monocytes, monocytic MDSCs (M-MDSCs), granulocytes and granulocytic MDSCs (G-MDSCs). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of HIV+ and controls. Cells were then sorted by FACs into pure populations of the cell types mentioned above. MiRNAs -101-3p, -146a-5p, -155-5p, -16-5p, -17-5p, -20a-5p, -21-5p and -223-3p were shown by qPCR to be differentially regulated among HIV+ patients when compared to healthy controls in both monocytes and granulocytes. Most notably miR-101-3p expression increased over 43 times in monocytes of HIV+ vs controls. We also evaluated miRNA expression among M-MDSCs vs monocytes and G-MDSCs vs granulocytes in HIV+ patients.

Currently, we are investigating the effects of these eight miRNA on the expansion and function of HIV-derived monocytes. This is...
NGFR Expression in Primary and Metastatic Human CRC as a Potential Target in Advanced Disease

**Authors:** Diaz, A. F.¹, MS; Sullivan, R.², MD; Kemp, M.¹, MD; Maresh, G.¹, PhD; Green, H.³, MS; Halat, S.³, MD; Margolin, D. A.², MD; Li, L.¹, MD, PhD

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**LCRC Scientific Program:** Clinical and Translational Research

**Background:** Nerve Growth Factor Receptor (NGFR) is a transmembrane glycoprotein that is ubiquitously expressed in both neuronal and non-neuronal tissues. NGFR’s role as a tumor suppressor and oncogene is currently being explored in many cancers (urogenital, breast, and prostate). In colorectal cancer (CRC), NGFR has been suggested to play a role in tumorigenesis and metastasis, but there is limited data available concerning this relationship. We aim to elucidate this relationship and hypothesize that NGFR is a possible target in advanced disease.

**Methods:** Twenty primary CRC samples and matched normal colon controls were acquired from the Department of Colon and Rectal Surgery at Ochsner Medical Center. Tissues were formalin fixed, paraffin embedded, and sectioned for staining. Tissue microarrays of 28 matched primary and metastatic CRC patient samples were constructed for H&E staining and for NGFR via immunohistochemistry (IHC). Aperio Image Scope software was used to digitally quantify NGFR staining positivity. Total percent positivity was compared between samples.

**Results:** NGFR protein expression digitally measured by total percent positivity of IHC staining was higher in human CRC samples compared to control samples with an average of 6 fold change (P=0.0018, n=20). Matched metastatic CRC tumors showed a higher NGFR total percent positivity compared to primary tumors (P=0.026, n=28).

**Conclusion:** In CRC, we show that NGFR expression was significantly higher in tumor samples compared to normal controls. Furthermore, NGFR levels were higher in metastatic tumors compared to matched primary tumors. This increased expression suggests that NGFR plays a potential tumorigenic role in CRC. Moreover, the difference between primary and metastatic tumors alludes to NGFR involvement in metastasis and could serve as a potential target for metastatic CRC. Additional work utilizing our metastatic orthotopic CRC murine models could further elucidate the role of NGFR in CRC tumor progression.

Shengli Dong – LSU

Combination Treatment Of Androgen Receptor Inhibitor And Curcumin For Triple-Negative Breast Cancer

Shengli Dong¹ and Suresh K. Alahari ¹,²

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2018 LCRC Annual Retreat Booklet
Abstract

**Background:** Triple-negative breast cancers (TNBCs) do not express estrogen (ER), progesterone (PR) receptors and the HER2 protein on cell surface. TNBCs account for approximately 15–20% of breast cancers patients. TNBC is clinically characterized as more aggressive and has a poor prognosis. By virtue of TNBC lacking ER, PR and HER2 expression, there are no targeted biological therapies available for these cancers. Given the limited available treatment options for TNBC, the top research priority is to find potential therapeutic targets. Androgen receptor (AR) has been detected in 80–90% of all breast cancers, including up to 55% of ERα-negative breast cancers overall and up to 12%–35% of TNBC. AR stimulated growth and survival in TNBC cells. Inhibition of AR signaling resulted in growth inhibition of TNBC cells both *in vitro* and *in vivo*. Treatment with bicalutamide, an AR antagonist, resulted in a 19% clinical benefit rate in AR+ TNBC patients.

**Methods:** AR+ TNBC cells were exposed to various concentration of curcumin, AR antagonist bicalutamide or combination of curcumin and bicalutamide. The effects of drug treatments were determined by MTT assay, flow cytometry analysis and western blot assay.

**Results:** Curcumin dramatically suppressed WNT signaling pathway in AR+ TNBC cells. Curcumin treatment inhibited AR protein expression in AR+ TNBC cells. Curcumin or bicalutamide treatment causes apoptosis and exerts inhibitory effects on the TNBC cells growth. Furthermore, the combination treatment of curcumin and bicalutamide could kill AR+ TNBC cells more effectively *in vitro*.

**Conclusions:** Our study suggests that AR inhibition is a potential therapeutic approach for AR+ TNBCs. The combination treatment of curcumin and bicalutamide could synergistically kill AR+ TNBC cells.

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**Novel Linkers For Advanced Magnetic Materials To Be Used In Medical Theranostics**

Clinical and Translational Research

Galina Goloverda, Vladimir Kolesnichenko, Ezinne Agwaramgbo, Melyssa Bratton, Elena Skripnikova and Thomas Wiese

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Nanotechnology offers new pathways for the development of hybrid inorganic/organic materials with broad range of applications in medicine. Nanoparticulate materials, containing magnetic core, can be used as imaging agents for the cell and biomolecule labeling, contrast agents for MRI, or therapies as delivery or hyperthermia agents. Performance of these materials as imaging or therapeutic agents relies heavily on the organic shell which helps to stabilize them in colloidal form, alleviates toxicity, facilitates bioconjugation and provides the desired pharmacokinetic properties. The component of a hybrid material, which helps with its assembling, is the linker which is usually an organic molecule with several reactive functionalities and tunable geometry. One side of the linker molecule is responsible for the binding to inorganic core such as metal oxide, usually contains multiple oxygen, nitrogen or sulfur donor atoms. Another side of it has a reactive group responsible for bioconjugation with the targeting vector, drug or dye molecules. Novel linkers based on 2-hydroxyisophthalic acid with demonstrated high affinity to inorganic surfaces and colloid stabilizing efficiency, have been developed. The following acid derivatives with X = OH, NH2, NO2, N2aryl, Br, OCH2CO2H, OC2H4OH, OC3H5(OH)2, OC3H5(OH)OC3H5, OC3H5(OH)Cl, OC3H5(OH)OC3H5, OC3H5(OH)NH2, OC3H5(OH)OC3H5 and PEG600 have been synthesized and fully characterized. The linkers are redox stable, easily derivatized and offer an excellent linkage with various components, including biomolecules. Maghemite (Fe2O3) 5 nm nanoparticles coated with 5-(PEG600)-2-hydroxyisophthalic acid form stable colloids at least in the pH range of 4.4-8.7 and withstand brief heating at 100°C (at pH 7). HeLa cell viability assay on X = OC3H5(OH)2 and OC3H5(OH)OC3H5 derivatives showed no toxicity. The described findings open the avenue for the application of 2-hydroxyisophthalic acid-based linkers as theranostic agents for cancer detection and treatment.

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**Generation Of Calculated Panel Reactive Antibody Values Using A Reference Panel Of Bone Marrow Donors**

Loren Gragert – Tulane

Evan Kransdorf1, Loren Gragert2,4, Marcelo J Pando3, Navcheta Kaur2, Jignesh K. Patel2, Irene K Kim1, Xiaohai Zhang1, Martin Maiers4, Jon A Kobashigawa4

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**LCRC Scientific Program Assignment:** Population Sciences and Prevention

Calculated panel reactive antibody is the official metric of sensitization used by the United Network for Organ Sharing (UNOS) for kidney allocation. The reference panel for CPRA is derived from low resolution HLA typing of 14,282 kidney donors. This panel may be undersized, especially for minority ethnic groups. We sought to compare CPRA values generated from the UNOS reference panel to values generated from a reference panel derived from high resolution HLA typing of 6.6 million bone marrow registry donors. A dataset of high resolution HLA A~B~C~DQB1~DRB1 haplotype frequencies were obtained from the National Marrow Donor Program (NMDP). Frequencies for high resolution HLA alleles were summed to UNOS antigen equivalents. CPRA was calculated using both NMDP haplotype frequencies (CPRA_{NMDP}) and standard UNOS frequencies (CPRA_{UNOS}) for a cohort of 17,053 sensitized kidney transplant candidates added to waiting list between 2009-10-01 and 2014-12-03. The panels were compared using a Cox proportional hazard model with CPRA as a time varying predictor for time to transplant.

Antigen frequencies were similar between the NMDP and UNOS panels, but linkage disequilibrium between HLA-C or DQB1 and other HLA locus was lower in the UNOS panel. As a result, there were 6200 candidates (36%) whose CPRA_{NMDP} was ≥ 1% different than their CPRA_{UNOS}. For candidates with CPRA ≥ 80%, CPRA_{NMDP} (p<0.001), but not CPRA_{UNOS} (p=0.06), predicted a decreased likelihood of transplant.
kidney transplant. Applying the current priority points in the UNOS kidney allocation system to this cohort, 3,203 candidates would move points groups based on CPRA\textsubscript{NMDP} as compared to CPRA\textsubscript{UNOS}.

Using NMDP haplotype frequencies as the reference panel for generating CPRA values is feasible and may improve equity in organ allocation by providing a more accurate estimate of haplotype frequencies in the population. NMDP reference panel also enables to measure CPRA when presented with any combinations of unacceptable UNOS antigens and high-resolution HLA alleles.

Enrollment, Retention and Outcomes of Two Anal Dysplasia Studies

Clinical and Translational Research
Presenter: Michael Hagensee
University Affiliation: LSUHSC LCRC Program: Tumor Virology
Collaborators: Joel Palefsky, University of California, San Francisco
Michael Hagensee, Olivia Edwards, and Joel Palefsky

Objective:
Anal cancer is increasing in the US and worldwide in part due to the HIV epidemic. In the HIV+ individual, anal cancer is 5-100 fold increased as compared to HIV-negative individuals. Currently it is unclear if detection and removal of precancerous lesions will lead to prevention of anal cancer. Thus, the triage on HIV+ men and women with high-grade anal lesions is not known. LSUHSC has opened and is currently enrolling into two national studies that will determine if treatment of high-grade lesions will prevent anal cancer. These are the AIDS Malignancy Consortium AMC-088 protocol and the Anal Cancer HSIL Outcomes Research Study, ANCHOR. The goal of AMC-088 is to enroll 150 HIV+ individuals with extensive anal high-grade disease. The larger ANCHOR study plan to enroll over 5,000 HIV+ individuals with high-grade anal disease.

Methods:
HIV+ men and women will be screened for high-grade lesions of their anus by high resolution anoscopy and biopsy of suspicious lesions. For AMC-088, those with high-grade lesions will be randomized to no treatment, versus treatment with topical Aldara (imiquimod) or Effudex (5-FU). Patient will be followed at 6-month intervals for 1-year to determine outcomes. For ANCHOR, those with high-grade lesions will be randomized to no treatment, versus treatment by any modality as determined by the provider and the patient. The subjects will then be followed at 6-month intervals for at least 5 years. To date, 4 individuals have been enrolled and randomized into AMC-088 and 36 have been screened for ANCHOR with 10 randomized for the longitudinal phase.

Results:
For AMC—088, one subject was in the observation arm, the lesion did not improve and is now receiving imiquimod. Two are in the imiquimod arm, one had no improvement and the other is still in follow-up. One treated with Effudex seems to be responding. For ANCHOR, 5 subjects have been randomized to active monitoring and 5 to treatment. All treatments have been local ablation with hyfercation. These subjects are all in the follow up phase with biopsies occurring at 1 year interval. No follow up exams to date have been concerning for the development of anal cancer.

Conclusions:
High rates of anal cancer exist in HIV+ populations. The AMC-088 and ANCHOR trials will help determine the optimal way to prevent anal cancer. Increased enrollment for both studies is critical, as the sample size is too small for comparisons. We are actively recruiting subjects from UMC, New Orleans as well as at our contributing sites in Baton Rouge, Lafayette, Lake Charles and Monroe. The outcomes of these studies will help determine clinical care for at-risk populations.

Generating an In-Vitro Tumor Organoid Bank for Research Studies
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Field of Research: Clinical and Translational Research

Background: Subcutaneous xenograft of human tumor tissues in animals is a popular method to preserve samples for in-vivo and pre-clinical research. However, these samples are subject to the animal’s ailments, infection, and the introduction of animal tissues to the xenografts. Traditional two-dimensional cell culture may offer a more controlled, contaminant-free environment for study, but by nature it does not exhibit the heterogeneity of tissues in living organisms. Recent advances in three-dimensional (3-D) culture have
allowed primary cells to grow in-vitro while retaining their key structures and functions. Also, they can be stored indefinitely for lower cost. We evaluated and compared the tumor growth capacity and histological characteristics of the tumors generated from 3-D culture (organoids) with the xenografts from the same patient.

Methods: Colorectal cancer (CRC) samples were received from consented patients. To compare organoids and xenografts side-by-side, the samples were washed, mechanically minced, and for xenografts, injected subcutaneously in mouse flanks. For organoid culture, the minced pieces were digested, passed through a 100µm filter, and the cells suspended in Matrigel in serum-free defined culture medium. For immunohistochemistry, original tissues, subsequent organoids, and xenografts were selected for sectioning and staining.

Results: Of 27 CRC samples received from 2017-2018, 11 succeeded as xenografts (40.7%) and 2 out of 3 attempts at organoid culture succeeded (66.7%). These two tumor tissues that grew in 3-D culture did not engraft successfully. Sample CoCa501 tumor produced organoids that were then able to produce xenografts. The cultured cells share phenotypic characteristics with its xenograft and original tumor counterparts. The other, sample CoCa528 tumor, has produced organoids that are currently growing well. All patient and xenograft-derived organoids have been stored cryogenically for future use as a patient tumor bank, and retained viability when revived from storage.

Conclusions: We have successfully established and will continue to grow a viable tumor bank via organoid cultures. These patient-derived organoids could be used to study human cancer pathology without the limitations of small animal usages, which holds greater relevance to clinical therapy applications.

Inhibition of the Chemo-Resistant Breast Cancer Tumor Growth in the Nude Mice on Treatment with Ceramide Analog

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Specific Aim: To assess in vivo efficacy of the most potent ceramide analog 315 on orthotopic xenograft nude mice model (achieved by assessing the tumor growth)

Research Methodology: We have synthesized several ceramide analogs with a greater efficacy and specificity than endogenous ceramide. In this study, Analog 315 was tested for inhibition of chemo-resistant breast cancer tumor growth in nude mice. Chemoresistant breast cancer MCF-7/TN-R cells were cultured and 5x10^6 million cells were injected subcutaneously into the right and left mammary fat pads of female nu/nu mice (Charles River Laboratories). After three weeks of tumor development, they were randomized into two groups of five mice and injected intraperitoneal (i.p.) with 50 µl of DMSO or ceramide (25mg/kg/day) for five days. Tumor sizes were measured using calipers and calculated as: volume = (width squared X length)/2

Results: Treatment of immunocompromised nude mice bearing tumors with 25 mg/kg per day of analog 315 for 5 days showed a 20% reduction in tumor weight and 40% decrease in tumor volume when compared to control mice. Furthermore, Analog 315 showed potent anti-tumor activity without causing significant body weight loss or toxicity. Therefore, this compound or similar analogs may be promising therapeutic anti-cancer drug targets.

Conclusions: We have successfully established and will continue to grow a viable tumor bank via organoid cultures. These patient-derived organoids could be used to study human cancer pathology without the limitations of small animal usages, which holds greater relevance to clinical therapy applications.

Informatics Tools For Mapping Molecular HLA Typing Data To UNOS Antigen Equivalencies

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United Network for Organ Sharing (UNOS) operates Organ Procurement and Transplantation Network (OPTN) in United States for equitable organ allocation from deceased donors. For histocompatibility evaluation between donor and recipient, molecular human leukocyte antigen (HLA) typing of the donors must be converted to antigen equivalencies for entry into the UNOS database, UNet. Maintaining an up-to-date mapping table for this purpose of converting alleles to antigens has so far been deemed infeasible by UNOS, though OPTN provides general guidelines for antigen assignment. To automate this conversion process for histocompatibility laboratories and researchers, we aimed to develop a mapping table and supporting informatics tools that would handle both unambiguous and ambiguous molecular typings.

Data sources referred for mapping the antigens to HLA alleles included an OPTN policy document for UNOS antigen mapping and equivalency tables, an IMGT/HLA antigen mapping table maintained by WHO and the World Marrow Donor Association (WMDA) and the 2008 HLA Dictionary. Mapping of population-specific ambiguous HLA typings was done using allele frequencies from 26 US populations obtained from National Marrow Donor Program (NMDP). Validation of antigen mapping was done by mapping NMDP high resolution frequencies to antigens then comparing with UNOS calculated panel reactive antigen (CPRA) antigen frequencies using
We present a UNOS antigen mapping table for 16093 HLA alleles of the A, B, C, DRB1, DRB3/4/5, DQA1, and DQB1 loci from the v.3.30.0 release of the IMGT/HLA database. A web application and microservices to map HLA typing results were also developed for use by histocompatibility labs (Available at http://www.transplanttoolbox.org). An example mapping of allele codes to UNOS antigens by the conversion tool is illustrated in the figure below. Similarity score between the NMDP reference panel and the UNOS CPRA reference panel for A, C, B, DR and DQ antigen frequencies for 4 US broad population categories (Caucasians, Hispanics, African Americans and Asian/Pacific Islanders) ranged from 0.85 to 0.97, indicating that the alleles were correctly mapped to antigens. The table provides a standardized reference for antigen mapping that can be automatically updated to incorporate new alleles added to IMGT/HLA. Informatics tools for managing molecular HLA typing data may improve accuracy and reduce the manual effort needed to enter data into UNet.

Figure 1: Illustration of mapping of HLA typing data in the form of multiple allele codes to respective UNOS antigen equivalencies by the conversion tool.

Genetic Testing For African-American Women With Breast/Ovarian Cancer: Results And Barriers
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SPECIFIC AIMS: To determine the frequency of genetic mutations predisposing to breast and ovarian cancer (BRCA) in an African-American population of great genetic diversity. Also to determine possible barriers to testing in that community.

METHODS: As part of an ongoing Quality Improvement study, the records of women self-identified as African-American and diagnosed with and treated for breast and/or ovarian cancer from 2014-17 were examined to determine: 1) whether BRCA 1/2 testing was offered and/or accepted, 2) presence of deleterious mutations, 3) presence of variants of undetermined significance, 4) the timing of and reasons for testing in relation to treatment, 5) demographics.

RESULTS: 56 women (42-Breast, 12-Ovary, 2-Breast and Ovary) were identified as being diagnosed with breast and/or ovarian cancer in the study period, and had testing performed. 10/56 (17.8%) including 6 with breast cancer, 2 with ovarian cancer, and 2 with Breast and Ovarian cancer had deleterious mutations. 4/56(7.1%) (all breast) had variants of undetermined significance. No subjects who were offered testing declined to be tested. 27/56 subjects were diagnosed in 2014-2015, and of these, 3/27(11%) underwent testing prior to/during initial treatment. 29 subjects were diagnosed in 2016-17, and 27/29(93%) underwent testing prior to/during initial treatment. Reasons for deferring testing included lack of awareness and/or inability to pay for the test.

CONCLUSIONS: Hereditary predisposition to breast/ovarian cancer, as determined by BRCA 1-2 genetic testing, is more common than anticipated in this African-American population of high genetic diversity. The use of genetic testing in this population has been limited by lack of awareness and/or inability to pay for the test. Standard screening for and counseling regarding genetic cancer risk and testing should be performed in this population.

Discovering Extracellular Vesicle Carried miRNA From Lymph Node Stromal Cells That May Alter Disease Progression In Colorectal Cancer

Purpose/Background: Outcomes from colorectal cancer (CRC), the third most common malignancy worldwide, have been shown to be dependent on depth of tumor invasion, lymph node (LN) involvement, and the presence of extra-nodal metastasis. Previous studies suggest the LN stromal microenvironment plays a role in metastasis via extracellular vesicle (EV) mediated communication. These vesicles carry various molecules including micro RNAs (miRNAs), small non-coding RNAs that can alter gene expression by targeting mRNA. Here we aim to identify specific miRNAs carried by LN stromal cell (LNSC) EVs that alter local progression and distant metastatic spread in colorectal cancer.

Methods/Interventions: HK (a LNSC line) cells and HK-EVs, mesenteric LNSCs and LNCS-EVs, and 5 well studied colon cancer (CoCa) cell lines were analyzed for expression of 2822 known human miRNAs using Next Generation Sequencing (NGS). The miRNAs expressed in higher quantities in HK cells, LNSCs and their EVs than in CRC cell lines were selected and further analyzed using in silico prediction models (DIANA-miRPath v2.0). Ten miRNAs, with higher expression in EVs than CoCa cell lines and predicted to have roles in cancer specific Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, were selected for confirmation by qRT-PCR (Table 1). Briefly, custom plates were obtained with primers for these ten miRNAs and for miR-191-5p, a previously proven reference RNA. Then, using a commercially available kit cDNA was synthesized from CoCa cells, HK cells and EVs, and LNSCs and EVs. miRNA
expression was then confirmed by qRT-PCR.

**Results/Outcomes:** 493 miRNAs were found to be expressed higher in EVs from both HK cells and mesenteric LNSCs than in the CRC cell lines. *In silico* analysis revealed those miRNAs with roles in relevant KEGG pathways including "colorectal cancer" and "microRNAs in cancer." The PCR threshold cycles (Ct's) of the miRNAs of interest were subtracted from the Ct of our reference miRNA. This confirmed our NGS findings of higher expression of our selected miRNAs in LNSC- and HK-EVs than in CoCa cell lines.

**Conclusion/Discussion:** We have previously found that LNSCs and their EVs assist in CRC tumor growth and spread. Here we identify miRNAs that may be introduced to colorectal tumors by EVs and are predicted to play roles in well studied cancer pathways. Using commercially available miRNA mimics and inhibitors we will next analyze the roles of these selected miRNAs *in vitro* and then *in vivo* using our patient-derived orthotopic mouse model.

**Field of Research:** Clinical and Translational Research
**Presenter:** Aaron Klinger MD

**PI:** Li Li MD

**Collaborating Authors:** Grace Maresh PhD, Xin Zhang, MD PhD, Linh Hellmers BS, Carlos Salomon Gallo PhD, David Margolin MD

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**Targeted Delivery of Doxorubicin to Cancer Cells by Silencing P-gp**

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**PURPOSE:** The MDR (multi-drug resistance) of metastatic breast cancer cells is accompanied with the overexpression of P-gp transporter. This study has focused to determine whether silencing the expression of P-gp by aptamer-labeled siRNA nanoparticles could enhance the delivery of doxorubicin into breast cancer cells. The expression and targeted knockdown of P-gp have been assessed by western blotting and immunofluorescence analysis. The doxorubicin accumulation into the cells has also been observed before and after the knockdown of P-gp by immunofluorescence and FACS analysis. **RESULTS:** This study has shown that the uptake of Dox by Dox-resistant 4T1-R is significantly less than Dox-sensitive 4T1-S which is partly contributed to the higher expression of drug-efflux pump P-gp on the surface of the resistant cells. The targeted knockdown of P-gp has been enhanced when the particles carrying P-gp siRNA was labeled with aptamer. Concurrently, the uptake of Dox into the Dox-resistant 4T1-R breast cancer cells has increased significantly when the P-gp was silenced by P-gp siRNA-encapsulated aptamer-labeled nanoparticles. **CONCLUSIONS:** This preliminary study concludes that downregulating P-gp expression by targeted delivery of P-gp siRNA using aptamer-labeled lipid based hybrid nanoparticles could effectively increase the intracellular trafficking of doxorubicin in Dox-resistant mouse breast cancer cells. **GRANT SUPPORT:** This work is funded in part by the Louisiana Cancer Research Consortium, NIMHD grant number TL4GM118968 and T34GM007716, NIGMS grant number UL1GM118967 and R25GM060926, CUR from Xavier University of Louisiana, LBRN Pilot and NSF.

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**Comprehensive Analysis Of AR Alterations In Cell Free DNA From Prostate Cancer Patients**

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**Background:** Somatic alterations identified in cfDNA may be associated with prognosis. Select AR alterations are associated with abiraterone/enzalutamide resistance in PCa. The goal was to characterize AR amplifications (amps) and somatic point mutations (muts) detected in cfDNA from PCa patients and to relate those changes to non-AR alterations detected in the cfDNA landscape. **Methods:** cfDNA data was obtained from a heterogeneous group of unique PCa pts who underwent Guardant360 testing (Guardant Health, Redwood City, CA). This assay includes next generation sequencing for full exonic coverage of 73 genes and amplifications in 18 genes. The AR amps/muts detected in cfDNA testing were reported. **Results:** Over 6,800 genomic alterations including AR amps/muts were identified in 892 PCa patients. Pts were a median age of 70 (range = 41-93) at testing. 49% (n = 436) had AR amp only, 32% (n = 283) had nonsynonymous (NS)-muts only, 18% (n = 165) had both amp and NS-muts; < 1% (n = 8) had synonymous AR muts only. **Conclusion/Discussion:** This study has shown that the uptake of Dox by Dox-resistant 4T1-R is significantly less than Dox-sensitive 4T1-S which is partly attributed to the higher expression of drug-efflux pump P-gp on the surface of the resistant cells. The targeted knockdown of P-gp has been enhanced when the particles carrying P-gp siRNA was labeled with aptamer. Concurrently, the uptake of Dox into the Dox-resistant 4T1-R breast cancer cells has increased significantly when the P-gp was silenced by P-gp siRNA-encapsulated aptamer-labeled nanoparticles. **CONCLUSIONS:** This preliminary study concludes that downregulating P-gp expression by targeted delivery of P-gp siRNA using aptamer-labeled lipid based hybrid nanoparticles could effectively increase the intracellular trafficking of doxorubicin in Dox-resistant mouse breast cancer cells. **GRANT SUPPORT:** This work is funded in part by the Louisiana Cancer Research Consortium, NIMHD grant number TL4GM118968 and T34GM007716, NIGMS grant number UL1GM118967 and R25GM060926, CUR from Xavier University of Louisiana, LBRN Pilot and NSF.
32% (n = 287), PIK3CA 29% (n = 259), MET 25% (n = 224), CDK6 26% (n = 229), EGFR 24% (n = 219), FGFR1 21% (n = 189), and APC 12% (n = 112). DNA repair gene alterations were detected in combination with AR alterations, BRCA2 8% (n = 69), BRCA1 5% (n = 42), and ATM 3% (n = 28).

Conclusions: To our knowledge, this is the largest dataset ever reporting AR alterations in cfDNA. Both amps and muts, were frequently found in cfDNA from PCa pts. The common AR muts were L702H, T878A, H875Y and W742C/L, which are linked to resistance to abiraterone, enzalutamide or bicalutamide. Determining the association with other somatic cfDNA alterations and clinical outcomes may be critical for conceiving optimal treatment strategies.

Evaluating the Inhibitory Effects of LY2510924, A Cyclic Peptide CXCR4 Antagonist, in Human Colon Cancer Metastasis Using an Orthotopic Xenograft Model

Presenter David Levine BA, PI Li Li MD, PhD, University of Queensland/Ochsner Medical Center, Clinical and Translational Research

David Levine, Lihn Hellmers, Grace Maresh, Xin Zhang, Ravan Moret, Heather Green, David Margolin, Li Li

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Purpose: Previously, we have identified a class of colorectal cancer (CRC) tumor-initiating cells expressing cell surface markers CD133 and CXCR4 that play a role in therapy resistance and metastases. This study tested the inhibitory effects of LY2510924 (LY), a cyclic peptide CXCR4 antagonist, as monotherapy and with fluorouracil (5FU) on CRC tumor growth and distant organ metastasis in an orthotopic xenograft model.

Methods: Luciferase-tagged human CRC cells (HT-29, HCT-116, SW620) were injected intra-rectally in mice without or with lymph node stromal cells (HK). All treatment groups received HK cells. LY was administered for 14 consecutive days starting either one day before or five days after cancer cell injection. 5FU was administered once/week weeks 6-9, preceded one hour by leucovorin injection. Progress was monitored by bioluminescent imaging. At necropsy, tissues were collected for analysis.

Results: HK cells significantly enhanced tumor growth and metastases. The HT-29 experiment yielded no difference between treatment and untreated groups. The SW620 experiment treatment groups had significantly smaller tumors compared to untreated, but no reduction in metastasis. The HCT-116 model showed neither drug having effect as monotherapy. Both groups with combined therapy showed significant reduction in tumor size compared to untreated groups, and the LY Day 5-19+5FU group yielded reduction in metastases.

Conclusion: HK cells support tumorigenesis and metastasis. CRC cells responded to LY and 5FU differently. LY’s effect may depend on the extent of Co-TIC presence. Thus, individualized targeted therapy including inhibitors like LY should be explored for CRC patients.

Support: LY2510924 was provided by Eli Lilly.

Rab13 Delivered by Lymph Node Stromal Cell-derived Extracellular Vesicles Effects Colorectal Cancer Growth and Metastasis

Field: Clinical and Translational Research

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BACKGROUND: Ninety percent of Colorectal Cancer (CRC) deaths are caused by metastasis. This is closely connected to the interaction between CRC and the lymph node (LN) stromal microenvironment. We aimed to identify RNAs delivered to tumors by LN stromal extracellular vesicles (EV) and determine whether targeting them reduces primary tumor growth and metastases. METHODS and RESULTS: We identified RNAs from stromal cells and EVs by Next-Generation Sequencing, of which over 150 were enriched greater than 2-fold in EVs vs. cells, and 13 were common to EVs in both LN stromal and HK stromal cells. RT-PCR confirmed these 13 RNAs were more highly expressed in HK-EVs than HK cells. Among 6CRC cell lines analyzed by RT-PCR, HT29 cells had the lowest expression of one of these genes, RAB13, a Ras-related small GTPase with roles in membrane trafficking. The RAB13 gene was silenced in HK cells by siRNA transfection and confirmed by RT-PCR (93% silenced by 5 days). When RAB13 was silenced in HK cells, their supernatant (containing EVs) showed a decrease in its ability to induce cancer cell proliferation in vitro (p=0.0002). In our orthotopic mouse model of CRC, luciferase-tagged CRC cells mixed with RAB13-silenced HK cells did not promote tumor growth as well as non-silenced HKS (p=0.0018). At necropsy, the CRC tumor weights were significantly lower in the RAB13-silenced HK group than in the non-silenced HK group (p=0.0138). Furthermore, liver and lung metastases were significantly lower when RAB13 expression was silenced in HK cells (p=0.0444).

CONCLUSION: This study demonstrates the importance of the RAB13 gene in the stromal cell promotion of CRC tumor initiation and metastasis. We hope to use these findings to help develop new therapeutics based on the clarification of this cancer-related biological pathway.
Application Of Patient-Derived Xenografts As Translational Models To Screen For Novel Kinase Pathways In Triple Negative Breast Cancer

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Triple negative breast cancers (TNBCs) constitute approximately 12% of all breast cancers and are approximately twice more prevalent in African-American populations. Louisiana has a high proportion of African-American residents (32.5% in 2015), and among the highest incidences of TNBC in the country. Louisiana patients also have a high incidence of co-morbidities that affect breast cancer biology and outcomes, including type 2 diabetes and obesity. TNBCs have an aggressive clinical presentation due to high rates of metastasis, recurrence and chemoresistance, and targeted therapy remains elusive. Discovery of novel therapeutic targets, including a subset of previously uncharacterized kinases in TNBC could provide important insights into future targeted therapies. However, current models utilized in target discovery research are limited by the inability to accurately recapitulate the complex architecture and heterogenous genetic and molecular composition of breast cancer. Furthermore, immortalized cell lines have been selected in a two-dimensional environment and may have lost important epigenetic features of original tumors they derived from. Recently, our laboratory has successfully established ten TNBC patient-derived xenograft (PDX) models representing various health disparities, responsiveness to chemotherapies, and diverse TNBC molecular phenotypes and metastatic behaviors. Our primary objective was to dissect and evaluate the various individual components (tumor cell biology, stroma, immune, extracellular matrix) that can be targeted in TNBC. We utilize these models in vivo, ex vivo and in vitro to examine how unique kinases and small molecule inhibitors affect the distinct tumor characteristics. In addition to in vivo treatment studies, we generated cell lines and mammospheres (TU-BcX-2K1, TU-BcX-2O0, TU-BcX-49S, TU-BcX-4IC, TU-BcX-4EALNb, TU-BcX-4M4) and we utilize novel techniques such as tissue decellularization to examine extracellular matrix components. We also analyze mechanistically relevant transcript (qRT-PCR) and protein (Western Blot, immunohistochemistry) expression patterns that are unique to each PDX model to evaluate the effect of small molecule inhibitors on these transcripts and proteins. Our aim is to leverage novel patient-derived models from under-studied patients with a range of clinical presentations to guide the selection of therapeutically targetable pathways and molecules in specific molecular subtypes of TNBC.

Translational Pancreatic Cancer Research: Establishing an Unique Orthotopic Mouse Model to Test Chemotherapeutic Drug for Human Pancreatic Carcinoma

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Background: Pancreatic adenocarcinoma (PaCa) is the third leading cause of cancer death in the U.S. with a 5-year survival rate less than 3-6%. The risk of death significantly increases with regional and distant metastasis to the lymph node (LN). Common treatments for early stage PaCa include a combination of resection of primary tumor and chemotherapy, while for later stages it mostly relies on chemotherapy regimens. Here, we used an orthotrophic xenograft mouse model to test the efficacy of the chemotherapeutic drug Gemcitabine (GEM) on PaCa progression and metastasis in the presence of LN stromal cell (LNSC).

Methods: Luciferase-tagged PaCa cell lines, PANC-1 and HS766T cells were injected intra-pancreas of NOD/SCID mice with or without LNSCs. Both tumor growth and metastasis were measured weekly by bioluminescent imaging (BLI). At week 7, GEM (100 mg/kg) was given intraperitoneally twice a week to the mice. At the endpoint, primary tumors were weighed, mouse liver and lung metastasis were analyzed by BLI, H&E and immunohistochemistry (IHC) staining using biomarkers, cytokeratin-5, vimentin, and Ki67.

Results: In our orthotopic xenograft models, tumors consistently grew in pancreas and metastasis to liver. H&E and IHC staining showed that our orthotopic xenograft model recapitulated the histological structure and exhibit the characteristics of PaCa comparing to that of an original patient tumor specimen. The co-injection of LNSC promoted PaCa tumor growth and metastasis in a dose dependent manner, which was significantly reduced by GEM treatment (p<0.05).

Conclusion: Our orthotopic xenograft model with similar histological architecture and biomarker expression recapitulates the tumor microenvironment. The significant decrease of tumor size with 100 mg/kg GEM was similar to the results reported in clinical
Kidney injection. 6-8 week old male NOD/SCID mice were shaved on the left side and anesthetized using 2% isoflurane. A small incision to the flank of NOD/SCID mice for expansion. Excised tumors were digested to a single cell suspension using collagenase IV for intratumoral injections. A novel drug, which is specific for cc-RCC, was administered intravenously (IV) 4 hours prior to tumor removal upon necropsy. The collected tumors were fixed in 4% formaldehyde overnight followed by 30% sucrose over 4 hours and preserved in Cryo-Gel. Frozen sections were stained with H&E and fluorescent staining for analysis. H&E staining confirms not only the presence of tumor implanted in the kidney but also the original morphology of the patient tumor was maintained. Fluorescent staining confirmed the cc-RCC specific drug was delivered to the growing tumor.

**Background:** Angiogenesis is the process of developing new blood vessels from the preexisting vasculature. It is plays an important role in pathogenesis of many diseases hence several treatments targeting angiogenesis have been developed. Currently used anti-angiogenic agents are typically synthetic compounds that can be toxic for some patients and are expensive. Accumulating evidence shows that naturally occurring plant products can have similar efficacy as synthetic compounds with fewer negative side effects, less toxicity and at a lower cost. We hypothesize that these four natural extracts [Pomegranate Peel (PPE), Tongkat Ali (TA), Aloin, and Grape Seed Extract (GSE)] will impede neovessel growth in the human tissue-based *in vitro* model system.

**Methods:** Tissue from adult inferior vena cava (IVC, n=4) was assayed in our proprietary *in vitro* Human Vein Angiogenesis Model. Following IVC dissection, 2 mm discs were created and embedded in a three-dimensional fibrin-thrombin clot. Each vein disc was overlaid with nutrient culture media (M199 + 10% FBS) and treated with natural compounds in a dose-response manner. Treatment doses for each compound [PPE (0.1%, 0.01%, 0.001%, 0.0001%), TA (0.1%, 0.05%, 0.01%, 0.001%), Aloin (10⁻¹⁰ M, 10⁻⁸ M, 10⁻⁶ M, 60 µM, 30 µM, 10 µM) and GSE (200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml)] were selected based on literature. Neovessels were visually scored and evaluated for three angiogenic parameters: percent initiation (%I), angiogenic growth (AG), and combined angiogenic response (CR). Angiogenesis data was analyzed for significance using paired t-test.

**Results:** The PPR [0.01%] and TA [0.01%] doses achieved over 70% inhibition of %I whereas GSE [200 µg/ml, 100 µg/ml] doses accomplished higher than 30% inhibition of %I relative to the control. Both AG and CR were significantly inhibited by these particular doses of PPE [0.1%, 0.01%], TA [0.05%], and GSE [200 µg/ml, 100 µg/ml] compared to the control. All tested Aloin doses had no effect on all three angiogenic parameters.

**Conclusions:** Our results demonstrate a robust efficacy of PPE, TA and GSE in inhibiting angiogenesis in IVC *in vitro*. These natural compounds had comparable or improved anti-angiogenic properties compared to anti-angiogenic agents (i.e. avastin, valproic acid) we have previously tested in our model system. This study provides a compelling rationale that the novel and effective natural therapies can be used to prevent angiogenesis in cancer and other angiogenesis-driven diseases.
Background: 4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a poly-chlorinated pyridine cholesteryl carbonate with a MOA via bis-alkylation of DNA @ N7-guanine and N4-cytosine that has completed Phase I and II clinical study [AACR #1185, 2016] and shows long term responses and survival of patients with primary brain cancers and with melanoma, breast, and lung cancers with metastases to brain. In particular patients with NSCLC are discussed. The aims were to assess clinical response when DM-CHOC-PEN is administered i.V. at MTD and to monitor duration of responses and safety (IND 68,876). We report here the responses and toxicities seen in patients with NSCLC involving the CNS.

Patients & Methods: In Phase I, DM-CHOC-PEN was administered as a 3-hr IV infusion once every 21 days to patients with advanced cancer; cohorts received escalating doses from 39 - 111 mg/m². The Phase II dose schedule was 2-tiered: 85.8 mg/m² for patients with liver involvement and 98.7 mg/m² for patients with normal livers. Results: Fifty two (52) patients have been treated to date – 26 in Phase I (cancer patients with or without CNS involvement) and 26 in Phase II (with CNS involvement). The drug was well tolerated; the most common adverse effects were fatigue (17%), reversible liver dysfunction (9%) and nausea (11%). No neuro/psychological, hematological, cardiac or renal toxicities were observed. PK modeling revealed that AUCs were parallel for all dose levels (39-111 mg/m²). The Cmax for DM-CHOC-PEN and DM-PEN (4-demethylpenclomedine, a metabolite) were 3 and 24 hours, respectively. Both hematological, cardiac or renal toxicities were observed. PK modeling revealed that AUCs were parallel for all dose levels (39-111 mg/m²). The Cmax for DM-CHOC-PEN and DM-PEN (4-demethylpenclomedine, a metabolite) were 3 and 24 hours, respectively. Both

RCC patients. In previous experiments using orthotopic xenograft models, we determined LN stromal cells (HK) enhanced parameters of RCC tumor progression. RCC cell line ACHN is dependent on HK cells, while SN12K1 cells are relatively independent of HK cells in

Conclusion: DM-CHOC-PEN is safe at these dose levels and has produced objective responses with manageable toxicities in NSCLC involving the CNS. Complete data on patient responses and observed toxicities will be presented. We propose a 2-stage mechanism for drug entry into the CNS and into NSCLC cells

Acute myeloid leukemia (AML), a cancer of blood and bone marrow, is characterized by an excess number of immature myeloid blasts, marked by a high degree of heterogeneity in gene mutations and chromosomal abnormalities. Identification of these aberrations plays an integral role in terms of diagnostics, prognostics, and treatment. Traditionally, these aberrations have been detected with classical cytogenetics and improved upon with fluorescence in situ hybridization (FISH). However, classical G-banding and FISH have obvious technical limitations in resolution and sensitivity. New technologies, such as array comparative genomic hybridization (aCGH) + Single Nucleotide Polymorphism (SNP) microarray have improved the detection of subtle and cryptic genomic variations in myeloid leukemias. Here we report the karyotype, FISH, and microarray findings of a patient with AML, in order to elucidate the significance of the integrated analytic strategy. Bone marrow aspirate from a 39-year old female was received in our laboratory for cytogenetic and FISH analysis for AML. Chromosome analysis revealed a complex karyotype with several structural and numerical abnormalities involving chromosomes 1, 5, 17, 20, and 21, as well as a marker chromosome. FISH further characterized the deletions in 1q21.3 (CKS1B), 5q31.2 (EGR1), and 17p13.1 (TP53), as well as 21q22.1 (RUNX1) amplification. Microarray studies were then performed on fixed pellet, to further characterize the abnormalities, if possible. These results confirmed the aberrations seen in chromosomes 5, 17, and 20, and helped the characterization of the structural rearrangements involving chromosomes 1 and 20, 5 and 17, a marker chromosome, as well as 21q amplification. Specifically, the RUNX1 gene was revealed to be deleted during the 21q amplification. These results suggest a reciprocal complementary strategy of integrating G-banding, FISH, and microarray analyses in cancer cytogenetics application. In this case report, the extensive genome-wide analyses of AML patient with complex karyotypes enabled us to depict the chromosomal aberrations more precisely. Also, these distinct aberrations can potentially be used as biomarkers for risk stratification, detection of minimal residual disease, and the advancement of new therapeutic interventions in the future.

Renal Cell Carcinoma Progression Modulated By Lymph Node Stromal Cell Via Altering Malignant Cell Protein Expression

Introduction: Renal cell carcinoma (RCC) is the most common solid tumor of the adult kidney, with a cancer specific mortality of 30–40%. Lymph node (LN) involvement is a strong negative factor for RCC prognosis. Metastases are incurable and affect up to 25% of RCC patients. In previous experiments using orthotopic xenograft models, we determined LN stromal cells (HK) enhanced parameters of RCC tumor progression. RCC cell line ACHN is dependent on HK cells, while SN12K1 cells are relatively independent of HK cells in

David Nguyen - Tulane

Quoc-Han Nguyen - Ochsner

An AML Case Study: Elucidating The Significance Of The Integrated Application Of Karyotyping, Fish, And Microarray In Cancer Cytogenetics

David Nguyen, Yuwen Li, Hans C. Andersson, Tian-Jian Chen, Theresa C. Brown

Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA, USA

Acute myeloid leukemia (AML), a cancer of blood and bone marrow, is characterized by an excess number of immature myeloid blasts, marked by a high degree of heterogeneity in gene mutations and chromosomal abnormalities. Identification of these aberrations plays an integral role in terms of diagnostics, prognostics, and treatment. Traditionally, these aberrations have been detected with classical cytogenetics and improved upon with fluorescence in situ hybridization (FISH). However, classical G-banding and FISH have obvious technical limitations in resolution and sensitivity. New technologies, such as array comparative genomic hybridization (aCGH) + Single Nucleotide Polymorphism (SNP) microarray have improved the detection of subtle and cryptic genomic variations in myeloid leukemias. Here we report the karyotype, FISH, and microarray findings of a patient with AML, in order to elucidate the significance of the integrated analytic strategy. Bone marrow aspirate from a 39-year old female was received in our laboratory for cytogenetic and FISH analysis for AML. Chromosome analysis revealed a complex karyotype with several structural and numerical abnormalities involving chromosomes 1, 5, 17, 20, and 21, as well as a marker chromosome. FISH further characterized the deletions in 1q21.3 (CKS1B), 5q31.2 (EGR1), and 17p13.1 (TP53), as well as 21q22.1 (RUNX1) amplification. Microarray studies were then performed on fixed pellet, to further characterize the abnormalities, if possible. These results confirmed the aberrations seen in chromosomes 5, 17, and 20, and helped the characterization of the structural rearrangements involving chromosomes 1 and 20, 5 and 17, a marker chromosome, as well as 21q amplification. Specifically, the RUNX1 gene was revealed to be deleted during the 21q amplification. These results suggest a reciprocal complementary strategy of integrating G-banding, FISH, and microarray analyses in cancer cytogenetics application. In this case report, the extensive genome-wide analyses of AML patient with complex karyotypes enabled us to depict the chromosomal aberrations more precisely. Also, these distinct aberrations can potentially be used as biomarkers for risk stratification, detection of minimal residual disease, and the advancement of new therapeutic interventions in the future.
tumor formation and distant organ metastasis. In this study we attempted to identify mechanisms by which LNSC promote RCC progression via the expression of various proteins on cancer cells.

**Methods:** RCC cells lines (ACHN and SN12K1) were cultured with or without HK cell supernatent and cell lysates were subjected to proteomic analysis. Significant protein markers were selected using information from a publicly-available database [http://www.proteinatlas.org/](http://www.proteinatlas.org/). Ten differentially expressed proteins that were significant in RCC or other cancers were confirmed by Western Blot and quantitative analysis.

**Results:** 1540 proteins were detected in cancer cells by proteomic analysis. 128 proteins were present in both cell lines with or without HK cell supernatent treatment. 10 protein markers, CTSD, SAMHD1, FAM114A1, RFC5, NAPA, SRP68, SNX6, AIMP1, PSMD6, and YWHAE, were selected for further investigation based on their published significance in RCC or other cancer survival data. Preliminary Western Blots showed both cell lines express all ten proteins. In addition, quantitative analysis validated the proteomic findings showing differential expression of proteins between the two RCC cell lines caused by HK cell supernatent.

**Conclusions:** The presence of conditioned media from HK stromal cells altered the expression levels of proteins in RCC. Further studies into the pathways affected by these proteins and, subsequently, blocking those pathways may lead to novel treatments for RCC patients.

**Presenter:** Quoc Moret, Ravan, BS, Laboratory of Translational Cancer Research, Ochsner Health System, rmoret@ochsner.org

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**The Involvement Of Human Papillomavirus In The Pathogenesis Of Non-Small Cell Lung Cancer**

Fayez Kheir¹, Michael J. Strong², Avi Patel (Presenter)³, Melody Baddoo¹, Erik K Flemington¹, Michael Hagensee³, Kris Reiss³, Li Li³, *Zhen Lin*

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**LCRC Scientific Program Assignment:** Clinical and Translational Research

**Background:** Lung cancer (LC) is the leading killer among cancers in the United States with an estimated 154,050 deaths expected to occur in 2018. It is also the second most diagnosed cancer in the U.S., and is expected to be responsible for approximately 234,030 new cases in 2018. Based on the treatment and prognosis, LCs can be classified as either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). NSCLCs account for about 85-90% of LC cases and have two major types: adenocarcinomas (LUAD) and squamous cell carcinoma (LUSC). To date, the etiology of LC is still largely unclear. Even though smoking is a definitive causative factor for LCs, most smokers don't develop LCs. Furthermore, at least 15% of male LC patients and 53% female LC patients in the U.S. are lifelong non-smokers, suggesting that factors other than smoking may be responsible for lung carcinogenesis. It is important to understand other etiological factors of LC, which may provide a better foundation for disease prevention and treatment. We and others hypothesized that human papillomavirus (HPV) may play a causative role in LCs due to the close proximity of the oropharynx to the lungs, where HPV is known to cause a substantial portion of cancers.

**METHODS:** With our unique sequencing based informatics approaches, we have investigated the involvement of HPV in LCs using more than 1,000 RNA/DNA-seq data sets from multiple national large cancer cohorts including samples obtained from our local collaborators at Tulane, LSUHSC, and Ochsner Cancer Center.

**RESULTS AND CONCLUSIONS:** Our data indicate that HPV is likely to be a primary viral driver of NSCLC. This is especially likely in the immunocompromised populations such as HIV+ patients and transplant patients. Our findings also shed light on the underlying mechanism of HPV-mediated malignancies, which may be particularly significant for future disease management.

**Conclusions:**

**Presenter:** Rachel Sabol - Tulane

**Mechanochemical Therapy Resensitizes Tamoxifen Resistant Breast Cancer in vitro**

**Field of Research:** Clinical and Translational Research

**Presenter:** Rachel A. Sabol, Tulane University
Tamoxifen (tam) is the most commonly used anti-cancer therapeutic agent in estrogen receptor positive (ER+) breast cancer (BC) which accounts for ~70% of BC cases. Tam treatment decreases a woman’s risk of recurrence by 50%; however, BC that is initially responsive to tam often develops resistance. In this study we evaluate a potential strategy to overcome resistance by using tam in combination with high intensity focused ultrasound (HIFU). HIFU is a clinically used non-invasive tumor ablative therapy that uses acoustic energy deposition. Previous studies have demonstrated that HIFU in combination with cancer therapeutics can have synergistic effects. In this study we found that treatment of MCF7 cells with HIFU and tam has additive anti-proliferative effects and mediates increased cell death. Additionally, we used tam resistant (TR) MCF7 cells that had been exposed to low dose tam over time until they acquired resistance. When MCF7 TR are treated with tam there is no change in viability; however, treatment with HIFU in combination with tam decreased viability of both MCF7 and MCF7 TR to 19% and the viability of the cell lines was indistinguishable. We next evaluated the effect on MCF7 Y537s mutant ESR1, where ER is mutated to be constitutively active. Treatment of MCF7 Y537S had no significant decrease in viability of combination therapy compared to viability after HIFU alone. Analysis of ERalpha gene expression showed that HIFU treatment increased ERalpha expression in MCF7 TR cells, thus resensitizing these cells to tam and allowing these therapies to work synergistically. Our team developed a system to evaluate the potential of this combination of therapies in a patient-derived xenografts (PDX) model. PDX have emerged as a novel translational tool for cancer research with the potential to more accurately recapitulate the molecular and behavioral aspects of cancer. The WHIM20 PDX is a tamoxifen resistant tumor where the patient developed the Y537s mutation in ESR1. Ex vivo experiments on PDX tumor pieces demonstrated that combination therapy of HIFU and tam work synergistically to increase cell death of these tumors. Further, cryo SEM demonstrates ablation of cells when these therapies are used together. These studies present a novel translational strategy to overcome tamoxifen resistance in ER+BC.

Changes in Chronic Inflammatory Cells by Bariatric Weight Loss Surgery

Sanchez-Pino, Maria Dulfary PhD1; Kim, Yonghyan MS2; Liu, Jiao MD2; Wyczewchowska, Dorota PhD1; William Richardson MD, FACS, FASMB5; James Wooldridge MD, FACS2; Randall Mynatt PhD1; Timothy Foster PhD1; and Augusto Ochoa MD1.

Obesity is characterized by chronic low grade systemic inflammation, where myeloid cells play a critical role. Myeloid-derived suppressor cells (MDSC; Monocytic [M-MDSC] and Granulocytic [PMN-MDSC] subsets) have been identified in cancer and other chronic inflammatory diseases. A few studies have shown a significant accumulation of MDSC in blood and different tissues in diet-induced obese mice and we also observed a significant accumulation of MDSC in mice made obese by a high fat-high sucrose diet compared with lean animals. The mechanisms linking obesity and MDSC activation or the effects of weight loss are unknown.

Methods: We have compared the frequency of MDSC in blood and omental adipose tissue in obese patients (BMI≥40) with non-obese controls (BMI≤25), and evaluated the effect of bariatric surgery over time (3, 6 and 12 months) and in vitro models to determine the obesity condition driving MDSC development. Results: Our preliminary data shows that PMN-MDSC are significantly increased in patients prior to surgery, but drastically decrease 3 months after bariatric surgery, associated with improvement of obesity-related inflammation and metabolic dysfunction. Furthermore, our in vitro studies demonstrated that coculture with adipocytes and the uptake of lipoproteins transforms normal myeloid cells into highly immunosuppressive MDSC by inducing the expression of Arginase I.

Conclusion: Increased caloric intake and obesity microenvironment stimulate the induction of MDSC, which promote a chronic inflammatory environment, while weight loss intervention reduces MDSC, eliminating the inflammation and controlling the metabolic dysfunction condition. Thus, understanding the cellular and molecular mechanisms by which obesity leads to the induction of MDSC is essential to understand the role of those MDSC in the obesity-related pathologies.

Supported by grants from NIH (5R01DK100756-01, R01AI112402)

Nanoencapsulation of α-Mangostin Inhibits Pancreatic Carcinogenesis By Targeting Cancer Stem Cells In KC And KPC Mice

Raj Kumar Verma1, Wei Yu1, Anju Shrivastava2, Rakesh K. Srivastava3, and Sharmila Shankar1,4

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Activation of sonic hedgehog (Shh) in cancer stem cell (CSC) has been demonstrated with aggressiveness of pancreatic cancer. In order to enhance the biological activity of α-mangostin, we formulated mangostin-encapsulated PLGA nanoparticles (Mang-NPs) and examined the molecular mechanisms by which they inhibit human and KC mice (Pdx126; LSL-KrasG12D) pancreatic CSC characteristics in vitro, and pancreatic carcinogenesis in KPC (Pdx126; LSL-KrasG12D; LSL-Trap531210) mice. Mang-NPs inhibited human and KrasG12D mice pancreatic CSC characteristics in vitro. Mang-NPs also inhibited EMT by up-regulating E-cadherin and inhibiting N-cadherin and transcription factors Slug, and pluripotency maintaining factors Nanog, c-Myc, and Oct4. Furthermore, Mang-NPs inhibited the components of Shh pathway and Gli targets. In vivo, Mang-NPs inhibited the progression of pancreatic intranoeplasia to pancreatic ductal adenocarcinoma and liver metastasis in KPC mice. The inhibitory effects of Mang-NPs on carcinogenesis in KPC mice were associated with downregulation of pluripotency maintaining factors (c-Myc, Nanog and Oct4), stem cell markers (CD24 and CD133),
Smoking History and PDL-1 Blockade: Predicting Response to Nivolumab Treatment in Metastatic Melanoma and Renal Cell Carcinoma
Caitlin Sullivan, MD, MS, Jonathan Lu, MD, Diana Vesselinovitch, MS, Neharika Khurana, MS, Marc Matrana MD, MS, FACP

Aims: Identifying patients who will benefit from immune-checkpoint inhibitor therapy is a challenge as proven predictive indicators remain to be elucidated. High tumor mutational burden (TMB) represents a possible biomarker for response to PDL1 blockade such as in nivolumab or pembrolizumab. Genomic analyses have shown that patients with heavy smoking history are more likely to have high TMB. However smoking status alone has not been examined independently in relation to treatment response. We sought to determine whether a relationship existed between smoking history and response to treatment in metastatic renal cell carcinoma (mRCC) and metastatic melanoma (mMelanoma).

Methods: We conducted a retrospective analysis of Ochsner Health System patients with mRCC receiving a minimum of two cycles of nivolumab or pembrolizumab between 12/2015 and 01/2018. Pre- and post-treatment target lesions were analyzed using RECIST criteria to calculate best response to treatment. Patient demographic information was gathered including age, sex, smoking history, and performance status pre and post treatment.

Results: Heavy smokers (>10 packyears) had a significantly higher response to immunotherapy than light (< 10 years) and never smokers in both mRCC (69% vs 32%, p = 0.0284) and mMelanoma (100% vs 65%). PFS, OS and Kaplan-Meier analysis to be presented at meeting.

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Heavy Smokers</th>
<th>Light/Never Smokers</th>
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<tbody>
<tr>
<td>Number of Patients</td>
<td>20</td>
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</tr>
<tr>
<td>Male (%)</td>
<td>16 (80)</td>
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<td>Median Age (Range)</td>
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<td>67 (37-91)</td>
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<td>Race: Black (%)</td>
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<tr>
<td>White (%)</td>
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<td>29 (80)</td>
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<td>Other (%)</td>
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<td>1 (3)</td>
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<tr>
<td>Disease Control Rate (%)</td>
<td>15 (75)</td>
<td>17 (47)</td>
</tr>
</tbody>
</table>

Initial Whole Blood-Based Gene Expression Profile Assays In mCRPC Pts.
Koji Tsumagari1,2, Patrick Miller3, Marcus Moses3, Elisa Ledet3, Oliver Sartor2,3
1radiation Oncology 2Tulane Medical School, 3Tulane Cancer Center

Background: Although second generation androgen receptor (AR) targeting therapy, abiraterone, improve therapeutic effect for patients of mCRPC, acquired resistance occurs. Biomarkers are clearly needed to predict the efficacy of AR targeting drugs for mCRPC patients and much work is occurring on this important issue. Circulating tumor cells are attractive biomaterials because of non-invasive collecting methods. In this study, we assessed whole blood RNA as a non-invasive methodology to access biomarkers of potential interest.

Methods: We used whole bloods (~5mL) preserved in PAXgene tube from 10 patients with mCRPC with acquired resistance follow Abiraterone and 11 controls without prostate cancer. Total RNA was extracted followed by qRT-PCR for assessment of 10 transcripts including ARV7, HOXB13, GHLR2, KLK3, KLK2, FOXA1, SchLAP1, KIF2C, MIA, and NCAM1. All amplicons were normalized to expression of β-actin.

Results: ARV7 (2/10), GRHL2 (2/10), HOXB13 (4/10), KLK3 (7/10), and KLK2 (4/10) amplicons were detected only in the mCRPC prostate pts. FOXA1 (7/10) and SchLAP1 (3/10) amplicons were detected in mCRPC pts at higher concentrations in mCRPC pts as compared to controls (p<0.001 and p=0.02, respectively). In contrast, KIF2C (5/11), MIA (11/11), and NCAM1 (11/11) amplicons were present in pts but in lower concentrations in mCRPC as compared to controls (p=0.03, p<0.001, and p<0.001, respectively).

Conclusions: We identified 5 transcripts that can be determined from whole blood RNA assays using abiraterone resistant mCRPC pts, additional transcripts were expressed at higher or lower concentrations as compared to controls. Although this is a small cohort and more data are clearly needed, these findings highlight the potential role for whole blood RNA in assess abiraterone resistant mCRPC pts.

Metabolism, Pharmacokinetics, and Bioavailability of ZB716, a Steroidal Selective Estrogen Receptor Downregulator (SERD)
Changde Zhang1, Shanchun Guo1, Lin Yang1, Jiawang Li2, Shilong Zheng2, Qiu Zhong1,2, Qiang Zhang1,2 and Guangdi Wang1,2
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2018 LCRC Annual Retreat Booklet
**Field of Research:** Clinical and Translational Research

**Background:** ZB716 is a selective estrogen receptor downregulator (SERD) with excellent oral bioavailability and superior efficacy. ZB716 is a potential second line drug in fighting tamoxifen resistant breast cancer though oral administration.

**Specific Aims:** In this study, we investigate the in vitro and in vivo metabolism, pharmacokinetics, and the excretion of ZB716.

**Research Methodology:** ZB716 was incubated with liver microsomes or liver cytosol to study its in vitro cytochrome P450 oxidation, sulfation, and glucuronidation biotransformation metabolism. Female Sprague-Dawley rats were fed with ZB716 through oral administration. Their blood, urine, and feces were collected and were investigated with a UPLC-Q-Exactive mass spectrometer to study the in vivo metabolism, pharmacokinetics, and the excretion of ZB716.

**Results:** The metabolic profile of ZB716 showed fulvestrant and ZB716-sulfone as the two major oxidative metabolites. ZB716 also underwent some degree of sulfation and glucuronidation in vitro. The major oxidative metabolites of ZB716 were found in rat plasma, feces, and urine samples. No sulfation and glucuronidation metabolites from ZB716 were found in plasma. Limited amounts of sulfate conjugates and glucuronides of ZB716 were detected in feces. The glucuronidation on 3-OH position of fulvestrant was the main metabolite found in urine, suggesting that this specific site of phase 2 metabolism is blocked in ZB716 and formation of glucuronide 3-fulvestrant must be preceded by metabolic transformation of ZB716 to fulvestrant. The pharmacokinetic study of ZB716 showed a half-life (t1/2) at 17.03 hour, the area under curve value (AUC) of 1451.82 ng/ml*h, and the maximum plasma concentration (Cmax) at 158.12 ng/ml reached at 2 h after a single dose of 10 mg/kg by oral gavage.

**Conclusions:** This study elucidated important metabolic, pharmacokinetic, and excretion characteristics of ZB716.

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**Abstract KIF3a-AR-V7 Interaction in Castration Resistance Prostate Cancer Progression**

**Background:** Prostate cancer (PCa) is the leading cause of cancer-related deaths in men because a majority of PCa patients develop castration resistant prostate cancer (CRPC) after first line therapy. New clinical insights into prostate cancer (PCa) reveal that activation of androgen receptor splice variants (AR-Vs) induces castration resistant prostate cancer tumor growth and subsequently yields resistance to chemotherapy. Understanding of the mechanism of AR-Vs' in CRPC is lacking. Our previous studies have shown the kinesin motor protein, Kinesin Family Member 3A (KIF3a), promotes proliferation and invasion in PCs. It is unclear, though, if KIF3a interacts with AR-Vs' and what the biological impacts of this interaction would mean in the context of CRPC tumor growth and activation.

**Hypothesis/Results:** Our studies have demonstrated that KIF3a interacts with AR-V7 and that KIF3a is required for AR-V7 activation in CRPC. We hypothesize that overexpression of KIF3a induces AR-V7 dimerization and activation to promote AR-V7 nuclear translocation and CRPC tumor growth, and that inhibition of the KIF3a-AR-V7 interaction by a small peptide within the Stalk domain of KIF3a will inhibit CRPC progression.

**Specific Aims:** 1) Determine the roles and mechanisms of KIF3a in AR-V7 dimerization and nuclear translocation. 2) Determine the small motif in the Stalk domain of KIF3a that is necessary for interaction of KIF3a and AR-V7 and inhibition of AR-V7 activity. 3) Design and deliver a small peptide from the motif to inhibit the KIF3a-AR-V7 interaction for CRPC suppression.

**Methodology:** We will evaluate the ability of the KIF3a Stalk domain to regulate AR-V7 homo- or hetero-dimerization with the androgen receptor (AR) using bimolecular fluorescence complementation (BiFC) and protein immunoprecipitation assays in HEK293T cells expressing AR-V7 and wild-type (WT) KIF3a. KIF3a mutant constructs will be generated to determine which motif within the Stalk domain facilitates AR-V7 interaction. The biological effects of the KIF3a-Stalk domain motif will be studied by performing assays to evaluate AR-V7 transcriptional activity, CRPC cell proliferation, and CRPC xenograft growth. A fused small peptide TAT-protein transduction domain (TAT-PTD) will be generated based on the KIF3a Stalk domain motif to determine if the peptide blocks the interaction between KIF3a-AR-V7 and subsequently inhibits CRPC tumor growth in our xenograft mouse models.

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**Recruitment and Retention of Bone Marrow Derived Epithelial Cells by Lymph Node Stromal Cells Supports Tumor Progression in Human Colorectal Cancer**

Xin Zhang<sup>1</sup>, Neeha Mathew<sup>1</sup>, Linh Hellmers<sup>1</sup>, Grace Maresh<sup>1</sup>, Ravan Moret<sup>1</sup>, Heather Green<sup>2</sup>, Simone Niclou<sup>3</sup>, David Margolin<sup>2</sup>, Li Li<sup>1</sup>

<sup>1</sup>Institution of Translational Research, <sup>2</sup>Department of Colon and Rectal Surgery, Ochsner Medical Center, New Orleans, Louisiana; <sup>3</sup>Luxembourg Institute of Health, Luxembourg

**Objectives:** Colorectal cancer (CRC) is the third leading cause of cancer death in US. Although mortality has decreased due to improved screening methods, patient prognosis is still severely affected by the formation of extranodal metastasis. A better understanding of the mechanism of tumorigenesis is crucial for uncovering novel therapeutic interventions. Here, using an MHC-matched GFP<sup>+</sup> bone marrow (BM) transplantation model, we investigated the mechanism of how the infiltrating BM-derived cells mediated CRC progression enhanced by lymph node stromal cells (LNSC).

**Methods:** BM cells from GFP<sup>+</sup> donor mice were transplanted into lethally irradiated NOD/SCID recipient mice. After 4 weeks, the recipient mice were inoculated with human CRC cell line HT-29 and HT-116 cells in the presence or absence of LNSC in our established orthotopic xenograft model. A small molecule inhibitor targeting CXCR4 (AMD3100) was tested. At the endpoint, the primary tumors were weighted, tumor cells were stained with a panel of antibodies against infiltrating cells surface marker, and analyzed by flow cytometry. The BM-derived infiltrating cells were also evaluated by immunohistochemistry staining.

**Results:** In our orthotopic xenograft model, the addition of LNSC significantly enhanced the CRC tumor weight (<p>0.01), which was reduced by AMD3100 (<p>0.01). BM-derived GFP<sup>+</sup> cells were found in the tumor cell digests and tumor tissue, indicating that BM cells infiltrate to CRC tumor sites. The frequency of infiltrating GFP<sup>+</sup> epithelial cell was increased by the co-inoculation of LNSC with...
tumor cells, which was significantly reduced by AMD3100 treatment ($p<0.05$).

**Conclusions:** Host BM-derived cells participate in CRC tumor progression. LNSC recruits more BM-derived endothelial cells to the tumor site and accelerates angiogenesis and tumor growth, which is partially mediated by interaction of CXCR4 on cancer cells and SDF-1 on LNSC. Targeting the crosstalk between tumor cells and LNSC may have therapeutic potential on angiogenesis and tumor progression in CRC.
Section 3

Core Facilities - Poster Presentations