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Meeting Report of Joint Society of Basic Urologic Research (SBUR) and European Society of Urological Research (ESUR) Symposium Fall 2017

Cindy K Miranti[1], Hari K Koul[2,3]

[1]Department Cellular and Molecular Medicine, University of Arizona College of Medicine, Phoenix, Arizona, USA; [2]Department of Biochemistry & Molecular Biology, [3]Feist-Weiller Cancer Center (FWCC), LSU Health Sciences Center, Shreveport, USA

The 2017 joint SBUR and ESUR symposium focused on genetic and epigenetic mechanisms that drive benign and malignant urologic disease. Highlighted disease areas included BPH, urinary disorders, and urologic cancers. The biological mechanisms explored within these disease states included classical genetic and chromatin-based epigenetic changes, alternative roles for chromatin modifying enzymes, alternate splicing, miRNAs, and exosomes. New therapeutic targeting approaches involving nanoparticles and regenerative pharmacology were also presented. Additional highlighted technologies included a new platform for quantitative digital pathology and the use of urine, exosomes, extracellular vesicles, and circulating tumor cells as biomarkers.

The meeting opened with AUA lecture focused on Epigenetics and Therapy of Urologic Cancers presented by *David Jarrard, MD from the University of Wisconsin Madison, WI*. An important question in prostate cancer biology is what underlies the remarkable incidence prostate cancer susceptibility with aging. The epigenome is susceptible to modulation by many factors associated with aging, including dietary and oxidative stress. David gave a scintillating talk discussing over two decades of his work that revealed that the peripheral zone of the prostate from men with prostate cancer, where prostate tends to arise, commonly contains biallelic Insulin-like growth factor (Igf2) expression a potent growth factor, in contrast to other organs where the gene is only expressed from one allele and thus demonstrates what is termed genomic imprinting. Importantly, he demonstrated that an age-related degradation of imprinting occurs in the murine and human prostate and underlies the development of prostate cancer. He showed that loss of CTCF binding protein during aging mechanistically causes the relaxation in Igf2 imprinting seen during aging and furthermore targets specific regions of the genome for hyper-methylation and inactivation. These considerations suggest that a degradation of the epigenome leads to a field of cancer susceptibility in the prostate serving as a marker for the disease, as well as a potential avenue for preventative therapy and treatment.

Current challenges in BPH research include identifying the mechanisms that lead to its development and finding better treatments. Dr. Aria Olumi, MD from Harvard Medical School reported on a recent study in which they found that 30% of human adult prostate glands do not express 5α reductase 2 (5-AR2). 5-AR2 is responsible for development and growth of the prostate, and the target enzyme used for standard anti-androgen treatment of BPH. This finding may explain treatment failure in some BPH patients. They discovered that 5-AR2 is silenced through epigenetic mechanisms involving inflammatory mediators such as TNFα and IL6 leading to induction of DNMT1 and methylation of the 5-AR2 promoter. Elevated TNFα correlated with age and increased 5-AR2 gene methylation. The absence of 5-AR2 was coupled with an “androgenic to estrogenic switch” and increased proliferation in the prostate gland, suggesting that alternate pathways, beside anti-androgenic targets, may be useful for managing BPH. Dr. Jose Teixeira, PhD at Michigan State University presented a new mouse model of BPH, which might explain how BPH could arise through deregulation of the underlying stroma.

Conditional deletion of the tumor suppressor genes PTEN and LKB1, specifically in the caudal stromal remnant of the Mullerian duct, resulted in prostatic growth of the stroma and subsequent hyperproliferation of the epithelium. Dr. Magdalena Grabowska, PhD from Case Western Reserve University identified loss of nuclear factor I/B (NFIB) as an inducer of BPH in NFIB null mice. Loss of NFIB resulted in increased numbers of keratin 5 (K5) positive basal cells as well as transient intermediate K5+/K18+ cells through an AR-independent mechanism.

Urinary disorders of the bladder and the challenge of treatment remains a major urologic problem. Dr. Laura Lamb, PhD from Beaumont Hospital presented new mouse models of radiation-induced cystitis and underactive bladder, highlighting how these models could be used to test new treatment strategies. Dr. Pradeep Tyagi, PhD at University of Pittsburgh discussed the importance of nerve growth factor (NGF) in driving the aberrant bladder sensations in overactive bladder (OAB), and how targeting NGF has led to the discovery of new epigenetic targets that drive OAB pathophysiology. Dr. Koudy Williams, PhD from Wake Forest University presented a new concept of Regenerative Pharmacology as a cheaper and more effective approach to stimulating tissue regeneration. His argument was nicely supported by data demonstrating that injection of the chemokine CXCL12, normally produced by cell at the sites of injury, was much more effective at restoring urinary sphincter structure and function than the injection of skeletal muscle precursor cells.

The highlight of this year's meeting was invited Leland W.K. Chung Keynote Lecture on Mitochondrial Reprogramming in Cancer by Dario Altieri, MD, President & CEO Wistar Institute Cancer Center, Philadelphia, PA. Given that therapeutic resistance in prostate cancer is driven by multiple mechanisms of drug resistance, and constitutive insensitivity to leading therapies for other tumors, leads to poor survival in CRPC, Dario's lecture emphasized on new therapeutic approach to CRPC. Dario presented ground baking work that his team has done with respect to experimental evidence that highlighted a novel role of mitochondria as pivotal disease drivers in these patients, fueling the machinery of tumor cell motility and invasion, while countering apoptosis of disseminated tumor cells. Dario presented mechanisms of mitochondrial reprogramming that affect a broad array of tumor responses in bioenergetics, redox balance and gene expression. Dario presented evidence for a small molecule targeting one of the mitochondrial adaptive pathways. The lecture concluded that many of these adaptive pathways are druggable, offering fresh therapeutic opportunities in the management of patients with advanced prostate cancer.

The genetic and epigenetic basis of urinary cancer development, its detection, and treatment were highlighted in several presentations. Dr. Margaret Knowles, PhD from Leeds Institute focused on defining the mutational landscape in bladder cancer, demonstrating that mutations in chromatin modifier genes are most highly associated with non-invasive bladder cancer. She identified inactivating mutations in CREB-BP/EP300 within the HAT domain that predicted for aggressive disease. Inactivation of CREB-BP/EP300 is predicted to silence genes by preventing H3K27ac. Inactivation of KDM6A, also common in bladder cancer, enhances EZH2 to promote H3K27 tri-methylation and further suppress transcription of the CREB-BP/EP300 targets. These chromatin modifiers are thought to disrupt bladder cell differentiation to promote tumorigenesis. She further demonstrated that EZH2 inhibitors can synergize with KDM6A mutants to suppress tumor growth. Thus, targeting these mutations could provide a localized therapeutic approach that may prevent the emergence of more aggressive muscle-invasive tumors. Dr. Dan Theodorescu, MD, PhD from the University of Colorado, Denver focused on an exciting approach to leverage cancer biology into cancer therapy. He specifically described innovative molecular tools and approaches that led to their discovery and validation of Ral GTPases as major driver genes in bladder cancer and the development of therapeutics targeted at proteins. He described a groundbreaking discovery approach that their group invented and used to identify RBC8, a small molecule Ral inhibitor. He presented data demonstrating that RBC8 inhibited *in vitro* Ral binding to its effector RalBP1, Ral mediated cell spreading in fibroblasts and growth of human cancer cells. RBC8 had favorable oral and intravenous pharmacokinetics and tumor drug uptake with no apparent toxicity. Moreover he presented

data demonstrating that higher potency RBC8 derivatives, BQU57 and BQU85 inhibited human cancer xenograft growth *in vivo* supporting further clinical development. Dr. Darryl Martin, PhD from Yale University School of Medicine discussed improved specificity in therapeutic targeting of bladder cancer by modifying the surface of nanoparticles to recognize specific tumor cells. Therapy was further enhanced by using the surface-specific targeting nanoparticles containing siRNAs that disrupt tumor-specific signaling pathways. Dr. Kerstin Junker, MD, PhD from Saarland University Medical Center described the importance of miRNA, particularly in extracellular vesicles, and their potential influence on intercellular communication between tumor cells and the tumor microenvironment. She highlighted the importance of using miRNA in extracellular vesicles for biomarker development.

Prostate cancer remains the most common cancer in men and a major focus of the SBUR community. Areas of emphasis this year were genetics, epigenetics, and the mechanisms behind the emergence of neuroendocrine prostate cancer. Dr. Christopher Barbieri, MD of Weill Cornell Medical College discussed a unique subset of prostate cancers that harbor mutations in the SPOP gene. The mutations are strictly missense and heterozygous, suggesting a dominant negative effect. SPOP is part of an E3 ligase complex that affects chromatin and DNA damage responses. Combined SPOP mutation and heterozygous loss of PTEN in mice accelerated prostate cancer development through enhanced mTor signaling. Additional proposed mechanisms include suppressed HR DNA repair, enhanced NHEJ to promote interchromsosomal rearrangements and alterations in chromatin structure that changes AR-mediated transcription. Dr. Michael Ittmann, MD, PhD at Baylor College of Medicine presented results from his studies on paired loss of the tumor suppressor RGS12 and enhanced MNX1 expression in African American prostate cancers. MNX1 is an HBox protein implicated in other cancers and may control fatty acid synthetic pathways. Another target of RGS12 is RalBP, whose loss enhances growth and metastasis. Thus, these mutations may explain some aspects of the more aggressive phenotypes seen in AA patients and they are developing novel ways to target this pathway. Dr Sarki A. Abdulkadir, MD, PhD at Northwestern University presented his studies on interactions between Myc and PIM kinase. PIM is elevated in HGPIN and phosphorylates Myc to stabilize its expression and enhance transcription. Targeting PIM kinase reduces Myc activity and sensitizes tumor cells to radiation. Thus, targeting PIM may be a better way to target Myc.

Non-classical epigenetic mechanisms of prostate cancer were nicely highlighted by several talks. Dr. Haojie Huang, PhD from Mayo Clinic presented his data on EZH2. Elevated EZH2, the methyltransferase responsible for histone H3K27 trimethylation and gene silencing via polycomb, is elevated in advance prostate cancer. However, it appears to operate by both polycomb- and methyltransferase-independent mechanisms. Using RNA-immunoprecipitation and RNA sequencing, he identified EHZ2-dependent binding to the 5'UTR of p53 mRNA that resulted in increased p53 expression. The methyltransferase activity of EZH2 was not required for the effects on p53. Dr. Scott Dehm, PhD from the University of Minnesota focused on defining the splicing mechanisms by which truncated, non-ligand binding variants of the androgen receptor (AR) arise. He identified a new mechanism whereby alternative polyadenylation can give rise to AR-V7 and AR-V9 variants and inhibiting alternative polyadenylation restores sensitivity of prostate cancer cells to AR-targeted therapies. Dr. Cindy Miranti, PhD from the University of Arizona identified CREB as crucial regulator of chromatin switching during terminal prostate luminal cell differentiation. Using an in vitro differentiation model of human prostate luminal cells, she demonstrated that loss of PTEN, through premature phosphorylation and activation of CREB by Akt, prevents induction of the chromatin binding protein ING4 required for terminal luminal cell differentiation. Thus, PTEN loss disrupts the terminal phase of the luminal cell differentiation program by preventing chromatin remodeling. Finally, Dr. Jin-Tang Dong, PhD of Emory University School of Medicine described how an acetylation/deacetylation switch, that modifies KLF5, both suppresses and induces prostate cancer growth. Acetylated KLF5 serves as a positive effector of TGF-β to suppress growth, while its deacetylation promotes growth.

The recent use of more highly active anti-androgen therapies for the treatment of castration

resistant prostate cancer (CRPC) has resulted in the more frequent emergence of neuroendocrine-like prostate cancers (NE-PCa) that no longer depend on AR. The mechanisms that drive NE-PCa and how to treat it is a major hurdle in the field. Dr. Amina Zoubeidi, PhD from the University of Vancouver discussed how the genetic/epigenetic plasticity of cancer stem cells and neuroendocrine cells drive NE-PCa development. She identified the transcription factor BRN2 as a major driver and a promising target for overcoming NE-PCa. Dr. Ralph Buttyan at the University of British Columbia presented evidence for "developmental reprogramming" of prostate cancer cells through a neural stem cell-like intermediate. This intermediate neural stem state is readily induced by anti-hormone or taxane treatments, is initially reversible, but eventually leads to irreversible CRPC. Thus, these mechanisms can help explain how NE-PCa can develop during therapy.

Several new technological approaches were highlighted for their application to urologic research. Dr. Beatrice Knudsen, MD, PhD at Cedars-Sinai Medical Center presented a new platform for digital image analysis and computational pathology that allows for more precise and quantitative diagnostic analysis of clinical samples. She demonstrated how multiplexing of 6-8 markers on a single sample could be integrated to provide more information and more accurate assessment of the disease. This could be a powerful tool for personalized and precision medicine and a quick way to assess multiple markers in a single patient's tissue sample. Teresa Liu, standing in for Dr. William Ricke, PhD at University of Wisconsin outlined new mass spectrometry methods that can be used on urine samples to identify specific urologic diseases or stratify patients for treatment. Finally, a panel presentation and discussion forum highlighted the pros and cons of identifying, characterizing, and using extracellular vesicles and circulating tumor cells (CTCs) as biomarkers. Discussion on extracellular vesicles was led by Dr. Larisa Nonn, PhD from University of Illinois at Chicago and Dr. Michael Freeman, PhD from Cedars-Sinai Medical Center. The discussion included highlights on the technical challenges of isolating and identifying different classes of vesicles and a detailed analysis of a new extracellular vesicle termed an 'oncosome' generated by budding of the nuclear membrane. The discussion on CTCs was led by Dr. Todd Morgan, MD from University of Michigan and Dr. Edwin Posadas, MD of Cedars-Sinai. They highlighted the challenges and biases of isolating CTCs and how CTCs are relatively representative of the tumors. But they also noted that application of the knowledge gained from CTC profiling remains a hurdle.

S1 Travel Awardee

TRPV1 Modulates Pelvic Pain in a Murine Model of Chronic Prostatitis

Kenny Roman, Anthony J Schaeffer, Praveen Thumbikat

Northwestern University, Chicago, IL, USA

Background: Patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) develop multiple symptoms that include chronic pain (from various pelvic regions) and the cause is poorly understood. Therefore, reliable treatment options are lacking. The goal of the proposed project is to elucidate the pathogenesis of CP/CPPS by identifying TRPV1 receptor as a mediator of chronic pelvic pain in an autoimmune mouse model of CP/CPPS, called experimental autoimmune prostatitis (EAP). Also, this project will determine the role of TRPV1 in peripheral inflammation by measuring the levels of activated mast cells and inflammation scoring of the prostate tissue. Methods: C57BL/6 (B6) and TRPV1 knockout (KO) male mice (2-3 months old), were used for this study. Mice were injected with prostate antigen (1 mg/ml) subcutaneous to elicit EAP. To determine whether mice exhibited prostate-specific pelvic pain we measured for suprapubic tactile allodynia on days 0, 7, 10, 14, and 20. The prostate, dorsal root ganglia (DRG), and spinal cord were excised from mice with EAP and respective control cohorts at day 20. The prostate, DRG, and spinal cord were processed for immunoblotting or immunohistochemistry. Results: Our behavioral measurements verified that EAP mice develop pelvic pain at day 7 thru day 20. However, TRPV1-KO mice do not develop increased tactile pelvic allodynia compared to control cohorts at day 20. Our immunoblots showed that p-ERK1/2 increased in the DRG and spinal cord (lumbosacral) of mice with EAP at day 20, compared to control. However, the DRG and spinal cord excised from TRPV1-KO with EAP at day 20 showed no changed in p-ERK1/2 expression compared to respective controls. In addition, the excised prostate lobes were stained with Toluidine blue and we observed increased mast cell activation in mice with EAP at day 20, compared to control. In contrast, mast cell activation was reduced in TRPV1-KO mice with EAP, compared to controls. Interestingly, inflammation scores showed that the prostates excised from TRPV1-KO mice with EAP have reduced inflammation compared to mice with EAP at day 20. Conclusions: Our project determined that 1) TRPV1-KO mice with EAP do not develop pelvic pain, 2) the lumbosacral DRG and spinal cord from TRPV1-KO mice with EAP do not have increased ERK1/2 phosphorylation, 3) TRPV1-KO mice with EAP showed diminished mast cell activation, and 4) overall prostate inflammation might be reduced in TRPV1-KO mice with EAP. We propose that targeted TRPV1 inhibition may alleviate chronic pain symptoms associated with CP/CPPS.

S2 Travel Awardee

Androgenic to Estrogenic Switch in Prostate Gland as a Result of Epigenetic Silencing of Steroid 5-α Reductase 2

Zongwei Wang[1], Libing Hu[1], Keyan Salari[1], Seth Bechis[1], Rongbin Ge[2], Shulin Wu[1], Shahin Tabetabaei[1], Chin-Lee Wu[3], Douglas Strand[4], Aria Olumi[1]

[1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [2]University of Massachusetts Medical Center, Worcester, MA, USA; [3]Massachusetts General Hospital, Harvard Medical School, Belmont, MA, USA; [4]UT Southwestern Medical Center, Dallas, TX, USA

Background: The steroid 5-α reductase type 2 (SRD5A2) is critical for prostatic development and growth. Strategies to block SRD5A2 using 5-alpha reductase inhibitors (5ARI) remain a mainstay in the treatment of benign prostatic hyperplasia (BPH). However, one-third of men are resistant to 5ARI therapies. We previously showed that expression of SRD5A2 is not static, since epigenetic modulations by DNA methyltransferase and pro-inflammatory cytokines somatically silence SRD5A2 during adulthood. Here we wished to identify whether absence of prostatic SRD5A2, when androgenic pathways are blocked, leads to modification of alternate hormonal pathways. Methods: Prostatic samples were obtained from patients with symptomatic BPH undergoing transurethral resection of prostate (TURP) surgery. Methylation of *SRD5A2* promoter was assessed using Methylated CpG Island Recovery Assay (MIRA). RNA was extracted for whole-transcriptome profiling analysis by Illumina Human BeadChip Arrays. Prostatic protein expression of SRD5A2, androgen receptor (AR), estrogen receptor (ER) subunits, and aromatase were determined in a panel of six BPH patients by Western blot, immunohistochemistry (IHC), and ELISA assays. Prostatic levels of testosterone (T), dihydrotestosterone (DHT), estradiol (E) were measured by HPLC-MS. In *in vitro* study, primary prostatic stroma cells and epithelial cell line, BPH-1, were cultured and treated with TNF-α and IL-6, and mRNA levels were determined by qPCR. Results: In prostate specimens that were methylated at the SRD5A2 promoter locus, estrogen response genes were identified as one of the most significantly upregulated gene family members as determined by gene expression analysis. The levels of T, E and aromatase were significantly upregulated, while DHT was significantly decreased. Absence of SRD5A2 significantly upregulated the phosphorylation of ERα (pERα), but did not significantly affect the levels of total ERα, total ERβ or pERβ. In primary prostatic stromal cells, administration of TNF-α, but not IL-6, suppressed the level of SRD5A2 and upregulated aromatase activity and ERα expression. However, treatment of prostatic epithelial cells with TNF-α or IL-6 did not change the androgenic or estrogenic signalling, suggesting that stromal cells regulate the androgenic to estrogenic switch when SRD5A2 is absent. Conclusions: Our study demonstrates for the first time that there is an androgenic to estrogenic switch when SRD5A2 is absent in the prostate gland. Somatic epigenetic silencing of SRD5A2 changes the prostatic hormonal milieu, and may modulate prostatic homeostasis and growth. Targeting the aromatase-estrogen-ER axis may serve as an effective treatment strategy in BPH patients who lack SRD5A2 expression.

S3 Travel Awardee

A Novel Approach to Treat Neuroendocrine and Metastatic Prostate Cancer (NE-PCa): Targeting S100A4 Protein by Small Molecule Inhibitor

Tabish Hussain[1], Arsheed A Ganaie[1], Teri Johnson[1], Reihana Maqbool[1], Badrinath R Konety[2], Mohammad Saleem[1]

[1]The Hormel Institute University of Minnesota, Austin, MN, USA; [2]University of Minnesota, Minneapolis, MN, USA

Background: NE-PCa an aggressive variant of prostate cancer (PCa) is non-responsive to androgen deprivation therapy (ADT) and shows poor prognosis in patients. We recently showed that S100A4 is an oncoprotein that drives the development of NE-PCa in transgenic GEM model. We showed that genetic targeting of S100A4 inhibits the development of NE-CaP. These data formed the basis of our hypothesis that S100A4 is a drug amenable protein, and could be exploited as a drug-target for treating NE-PCa in patients. We hypothesize that inhibition of S100A4 protein using small molecular inhibitors is an ideal approach to treat NE-PCa disease. Results: We show that S100A4 secreted by prostatic tumors confers metastatic and NE-PCa characteristics to indolent tumor cells. We show that serum-S100A4 levels highly elevated in PCa patients positively correlate to tumor stage. Next, we screened a library of 5000 molecules *in-silico* for S100A4 binding, and identified inhibitors (SMI1 and SMI2). Using isothermal titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR) assays, we show that SMI1 and SMI2 physically bind to recombinant S100A4 protein. Using Myosin-II protein disassembly and turbidity as indices of S100A4 activity, we showed that SMI1 and SMI2 inhibit S100A4 activity. We previously showed that soluble S100A4 induces the invasiveness via RAGE receptor on tumor cells. Using an ELISA assay we show that SMI2 inhibits the binding of soluble S100A4 protein to RAGE protein. We developed TdT/luc-expressing stable NE-CaP models from PC3M, PC3M-LN4 and TRAMPC2 and tested efficacy of inhibitors using these models *in-vitro* and *in-vivo*. SMI1 and SMI2 treatment decreased the activation of downstream targets (MMP9 and NFκB) of S100A4 in NE-PCa models. PCa cells are known to cross endothelial barrier of blood vessels and home at bones. Next using transendothelial and bone homing/alizarin assay, we tested the anti-metastatic efficacy of the inhibitors and show that SMI2 treatment inhibited the (i) potential of NE-PCa cells crossing the endothelial barrier and (ii) attachment of NE-PCa cells to bone marrow-derived hMSCs. Notably, SMI1 and SMI2 therapies inhibited the growth, prostatosphere formation, migration and invasion of NE-PCa models. Finally, SMI1 and SMI2 therapy caused a significant reduction in the growth/proliferation of NE-PCa tumors in syngeneic and athymic xenograft mouse models. Conclusion: S100A4 inhibition is a novel therapeutic approach NE-PCa treatment and S100A4 inhibitors are the potential drug candidates for treating NE-PCa in humans.

S4 Travel Awardee

Loss of Epigenetic Regulation Results in Congenital Malformations of the Urogenital System

Catherine Seager[1], Mary Taglienti[1], Feng Shen[2], Jordan Kreidberg[1]

[1]Boston Children's Hospital, Boston, MA, USA; [2]Washington University School of Medicine, St. Louis, MO, USA

Background: We are investigating the role of epigenetics in the development of the urogenital system with the goal of understanding how impaired epigenetic regulation may lead to congenital anomalies such as ureteropelvic junction obstruction and ureterovesical junction obstruction. Reciprocal epithelial mesenchymal inductive interactions are critical to regulating gene expression during development of the kidney and the lower urogenital tract. The mature pelvis and ureter consist of an inner epithelial compartment and an outer mesenchymal compartment. The ureteric bud gives rise to the epithelium of the collecting system while the smooth muscle and fibroblasts of the ureter are derived from a TBX-18-expressing mesenchyme. We are using gene targeting in mice to inactivate the *Eed* gene, an essential compoment of Polycomb Regulatory Complex 2, in either the epithelial or mesenchymal compartments during development of the urogenital system. Methods: *Eed* was inactivated in the epithelial and mesenchymal compartments of the collecting system and ureter using *HoxB7-Cre* and *TBX18-Cre* respectively. Kidneys and lower urogenital tract phenotypes were characterized through histological and gene expression analyses. Functional assessment of drainage was performed by ink injection study. Results: Conditional inactivation of *Eed* in the epithelium (*Eed$^{flox/flox}$; HoxB7-Cre*) resulted in animals with severe bilateral hydroureteronephrosis by postnatal day 21 (P21). Kidneys from *Eed$^{flox/flox}$; HoxB7-Cre* mice on P1 were decreased in size as compared to controls indicating that correct epigenetic regulation in the ureteric bud lineage is necessary for the maintenance of nephrogenesis. *Eed$^{flox/flox}$; HoxB7-Cre* mice showed decreased expression of uroplakin at the ureteropelvic junction (UPJ) while ink injection studies suggested impaired drainage at the level of the bladder. Conditional inactivation of *Eed* in the ureteral mesenchymal progenitors (*Eed$^{flox/flox}$; TBX18-Cre)* resulted in bilateral hydroureteronephrosis by P21. There was increased expression of smooth muscle actin (SMA) and SM22-alpha at the ureteropelvic junction and ink injection studies showed impaired drainage at the level of the UPJ. Vesicoureteral reflux was noted on injection of dye into the bladder. Conclusions: Inactivation of the *Eed* gene in epithelial and mesenchymal cell-type progenitors of the ureter and collecting system results in abnormalities of gene expression during ureteral and renal development. These studies are providing insights about how urogenital anomalies in humans may be caused by abnormal epigenetic regulation during fetal and early postnatal development.

S5 Travel Awardee

ONECUT2 is a Targetable Master Regulator of Lethal Prostate Cancer Variants

Mirja Rotinen[1], Sungyong You[1], Julie Yang[1], Simon Coetzee[1], Wen-Chin Huang[1], Fangjin Huang[1], Xinlei Pan[1], Alberto Yáñez[1], Dennis Hazelett[1], Chia-Yi Chu[1], Leland Chung[1], Stephen Freedland[1], Dolores Di Vizio[1], Isla Garraway[2], Ramachandran Murali[1], Beatrice Knudsen[1], Michael Freeman[1]

[1]Cedars-Sinai Medical Center, Los Angeles, CA, USA; [2]UCLA, Los Angeles, CA, USA

Background: Androgen deprivation therapy is associated with the emergence of aggressive variants of metastatic castration-resistant prostate cancer (mCRPC) that exhibit little or no dependence on the androgen receptor (AR). Here we identify the developmental transcription factor ONECUT2 as a negative regulator of the AR axis, that emerge in aggressive prostate cancer (PC) variants to control transcriptional networks linked to CRPC and neuroendocrine (NE) differentiation. We further show that ONECUT2 can be targeted with a small molecule that inhibits CRPC metastasis in mice. Methods: ONECUT2 was confirmed as a mCRPC-relevant protein and to be targetable by: computational modeling and bioinformatics, enforced expression, silencing, microarray, ChIP-Seq, immunohistochemistry, immunofluorescence, quantitative imaging, functional assays, in vivo experiments and surface plasmon resonance. Results: We have performed a master regulator analysis using transcriptome datasets from 260 samples of mCRPC and developed a model transcription factor network that associates OC2 with metastatic progression. Gene expression profiling of ONECUT2-engineered PC cell lines, has allowed us to generate a ONECUT2 activity signature that reveals high positive correlation with pro-neural and NE prostate cancer (NEPC) signatures, and a negative correlation with AR activation pathways. We find that ONECUT2 is a negative regulator of AR expression and a repressor of its transcriptional program through direct binding to AR target genes. We also find that ONECUT2 is significantly increased in human NEPC and that confers NE properties to CRPC through direct up-regulation of the NEPC driver PEG10 and direct down-regulation of the NEPC inhibitor FOXA1. Finally, we show that ONECUT2 is required for cell growth and survival and that it can be targeted with a small molecule that, by binding to its C-terminal DNA binding domain, inhibits CRPC growth and metastasis in mice. Conclusions: OC2 is a master regulator of aggressive mCRPC variants that drives AR-dependent adenocarcinoma toward NEPC differentiation by blocking AR/FOXA1-activity and inducing PEG10. OC2 can be targeted with a small molecule that inhibits growth and metastasis in mice. Thus, patients with OC2 active tumors could benefit from OC2 inhibitor therapy.

P1

Effects of Phthalates on Steroidogenesis in Prostate Cancer Cells

Clara Jeong, William Ricke

University of Wisconsin-Madison, Madison, WI, USA

Background: Prostate cancer (PRCA) represents 14% of all new cancer cases in the U.S. and is the second leading cause of cancer related death in males. The five-year relative survival rate was 30% for males with advanced metastatic PRCA. The standard treatment of PRCA is androgen deprivation therapy (ADT), which reduces levels of androgens required for prostate cell growth. Androgen deprivation therapy (ADT) is initially effective for PRCA treatment, but PRCA becomes resistant to ADT after 1-3 years and resume growth which is defined as castration resistant prostate cancer (CRPC). Intratumoral steroidogenesis is one possible mechanism that contribute to the CRPC progression. Phthalates are used in plastics and many products to soften and increase their flexibility. Phthalates exposure are associated with carcinogenesis risk in breast and liver. Phthalates were shown to inhibit human testes steroidogenesis and are capable of increasing steroid metabolizing enzymes. Whether phthalates exposure is associated to prostate cancer risk and whether phthalates can alter intratumoral steroidogenesis in prostate is unknown. The objective of this study was to determine if phthalates treatment alters the production of steroid hormones in prostate cancer cells. The central hypothesis was nono-ethylhexyl phthalate (MEHP; major metabolite of DEHP) treatment promotes testosterone production in prostate cancer cells and increase cell proliferation. Methods: Various human prostate cancer cell lines including LNCaP, C4-2B, and CWR22Rv1 cells were treated with 1 μM MEHP with or without the presence of 10 nM Testosterone. After 24 hour treatment, 2 mL of cell culture media were collected followed by MBTE liquid-liquid extraction. Levels of muti-steroid hormones were determined by QTRAP5500 LC-MS/MS analysis. Results: MEHP treatment resulted in an increase of testosterone levels compared to the control in both with or without the presence of 10 nM testosterone in C4-2B cells. 4-androstenedione levels increased by MEHP treatment compared to the DMSO control. Interestingly, MEHP treatment significantly decreased the estradiol levels compared to the DMSO control, while it showed an opposite effect in the presence of 10 nM testosterone. Conclusion: Phthalates alter the steroidogenesis in prostate cancer cells and the changes depend on the level of precursors in the system. RT-qPCR analysis is being conducted for the cell lysates to determine if the expression of steroid metabolizing enzymes are altered by MEHP treatment. Cell proliferation assays are being conducted to see if MEHP-mediated changes in steroid hormone levels could lead to higher proliferation.

P2

Genetic Regulation of Prostate Cancer by the Androgen Receptor and the Class E Basic Helix-Loop-Helix Transcription Repressor BHLHE40

Paramita M Ghosh, Zsofia Kiss

VA Northern California Health Care System, Mather, CA, USA

Background: Prostate cancer (CaP) is characterized by dependence upon androgen receptor (AR) signaling; hence androgen deprivation therapy (ADT) is the standard-of-care for advanced CaP. Androgen-sensitive CaP usually progresses to castration resistant prostate cancer (CRPC) following ADT; this results in aberrant AR expression and signaling. We previously demonstrated by genome-wide analysis of AR binding and target regulation that many direct AR targets were transcriptional regulators - subsequently we identified in an unbiased next-generation sequencing study that the class E basic helix-loop-helix protein 40 (BHLHE40/DEC1/STRA13/SHARP2), a known transcriptional regulator, is an AR target protein downregulated in CRPC. The overall goal of the current studies is to determine whether BHLHE40 coordinates with the AR to regulate CaP progression to CRPC. Methods: Western blot analysis and qPCR compared the levels of BHLHE40 in hormone dependent LNCaP cells versus in various CRPC cell lines. Transient overexpression of BHLHE40 was used to determine the possible function of BHLHE40 in these cell lines. We used immunofluorescence imaging and fractionation experiments to assess the localization of BHLHE40. Manipulation of the AR by transient overexpression, or knock down was used, coupled with chromatin immunoprecipitation (ChIP) experiments, to confirm AR binding to the promoter region of BHLHE40. Results: Comparison of BHLHE40 expression from publicly available (Oncomine) databases showed that BHLHE40 expression is downregulated in most CaP tumors from human patients, which was borne out by our own observations in CaP vs. non-tumor tissues from prostatectomy samples. BHLHE40 transcript and protein levels in hormone dependent LNCaP cells were much higher than in CRPC cell lines C4, C4-2, R1 and Rv1. Overexpression of BHLHE40 in LNCaP cells suppressed growth by only about 50% while it completely stopped cell growth in C4-2 cells. Immunofluorescence imaging coupled with fractionation experiment has further confirmed that BHLHE40 localizes to the nucleus. AR overexpression strongly increased BHLHE40 expression; however, knock-down of AR by siRNA did not significantly alter BHLHE40 levels, indicating that in the absence of AR, BHLHE40 expression is regulated by other transcription factors. Significantly, the AR bound to the specific androgen response element (ARE) on the BHLHE40 promoter in LNCaP cells. Furthermore, chromatin immunoprecipitation (ChIP) experiments showed that in LNCaP, but not in C4-2 cells, the AR stably bind to BHLHE40 promoter with high affinity. Conclusion: BHLHE40 is a strong growth repressor in CRPC cells, but not in hormone dependent CaP that is likely AR regulated in CRPC cells when activated by various co-regulators.

P3

Sensitizing Castration-resistant Prostate Cancer to Anti-androgens by Targeting Gastrin-releasing Peptide Receptor

Renjie Jin

Vanderbilt University, Nashville, TN, USA

Androgen receptor (AR) addiction is a driving force behind the development of castrate resistant prostate cancer (CRPC), and the initial therapeutic efficacy of second-generation high affinity anti-androgens validates AR as the tractable target for drug therapy in metastatic CRPC (mCRPC). Inevitably, mCRPC patients treated with these second generation anti-androgens, which target the ligand-binding domain (LBD) of AR Full Length (AR-FL), will develop secondary resistance. In many cases, failure to abiraterone acetate or enzalutamide appears to be mediated through the induction of AR splice variants (ARVs). ARVs are constitutively active and lack the LBD, thereby allowing CRPC to maintain AR activity despite therapies that target the AR-FL. Because the development of mCRPC is lethal, developing an approach to block ARVs expression is critical to resolve. Previously, we have demonstrated that long-term androgen deprivation therapy (ADT) increases the neuroendocrine (NE) hormone - Gastrin Releasing Peptide (GRP) and its receptor (GRP-R) expression in PCa cells. In addition, activation of GRP/GRP-R signaling increases NF-κB activity and ARVs expression, and contributes to progression to CRPC. In this study, we report that blocking of GRP/GRP-R signaling efficiently inhibits NF-κB activity and ARVs (AR-V7) expression in prostate cancer cells. In addition, blocking of GRP/GRP-R signaling by targeting GRP-R can sensitize CRPC cells to antiandrogen treatment. Further, combination of GRP-R antagonist (targeting ARVs) with antiandrogen (targeting AR-FL) is sufficient to inhibit tumor growth in CRPC mouse model.

P4

BCG Intravesical Therapy Modulates Immune Response Against Bladder Cancer by Suppressing Pd-L1 Expression

Junhyeok Heo[1], Kyung Seok Han[2], Kang Su Cho[2], Woong Kyu Han[1], Joon Chae Na[2], Sook Young Kim[2], Sung Joon Hong[1]

[1]Department of Urology and Urological Science Institute, Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Republic of Korea; [2]Department of Urological Science Institute, Yonsei University College of Medicine, Seoul, Republic of Korea

Background: Bacillus calmette-guérin (BCG) intravesical therapy has been a standard treatment to prevent recurrence and progression in non-muscle invasive bladder cancer. However, despite long clinical experience with BCG, the mechanism of its therapeutic effect is still under investigation. Recent innovations of cancer immunotherapy are based on suppressing the tumor induced immune suppressor such as PD-1 and its ligands PD-L1. Although BCG is the most effective immunotherapy in bladder cancer, the precise role and mechanism of immune checkpoints in the interaction between BCG and bladder cancer cells is unclear. Therefore, we investigated the role of PD-L1 in BCG intravesical therapy for bladder cancer. Methods: RT-qPCR analysis of PD-L1, PD-L2, PD-1, and CD28 were performed in tumor and paired normal tissues. Immunohistochemistry was performed to assess distribution of CD4, CD8, PD-1, and PD-L1 in tumor and paired normal FFPE sample. Immunoblotting was performed to assess PD-1, PD-L1 and PD-L2 basal expression of several human bladder cancer cell lines (253J, T24, UC3, KU1919) and normal human urothelial cell line (SVHUC1). Orthotopic bladder carcinoma bearing mice were developed to evaluate the effects of BCG. FACS was conducted for analysis of T cell sup-population and PD-L1 expression. Results: PD-L1 and PD-L2 are highly expressed in human bladder cancer cell line compared to human normal bladder cell line. Tumor and paired normal tissues of 30 patients showed that tumor is approximately 2.78, 3.64 times more express PD-L1 and PD-L2 than paired normal tissues, respectively. $CD4^+Foxp3^-$ and $CD8^+$ cells are more infiltrated in human and mouse bladder carcinoma. BCG intravesical therapy does not significantly influence on T cell sup-population but down-regulates expression of PD-L1 in mouse bladder cancer and tumor-infiltrating immune cells. PD-L1 mRNA was increased and correlated with IFN-γ and IL-2, however, expression of PD-L1 protein was decreased after BCG exposure in orthotopic bladder cancer. Conclusions: PD-L1 is highly expressed in bladder cancer and $CD4^+$ and $CD8^+$ cells are significantly infiltrated in bladder cancer. BCG intravesical therapy induces anti-tumor immune responses by post-transcriptionally down-regulating expression of PD-L1 in bladder cancer and tumor infiltrating immune cells.

P5

Progranulin/Epha2 Axis, A Novel Driver of Bladder Cancer

Shi-Qiong Xu[1], Simone Buraschi[1], Thomas Neill[1], Leonard G Gomella[1], Antonino Belfiore[2], Renato V Iozzo[1], Andrea C Morrione[1]

[1]Thomas Jefferson, Philadelphia, PA, USA; [2]Universita' Della Magna Graecia, Catanzaro, Italy

Background: Bladder cancer is a major health issue with 79,000 new cases in the USA and 14,500 estimated deaths in 2017. The growth factor progranulin is overexpressed in bladder cancer and detectable in urine and plasma. Progranulin levels are critical for motility, *in vivo* tumorigenesis and sensitivity to cisplatin treatment. Until recently, the progranulin receptor remained unidentified, precluding a full understanding of progranulin action in tumor biology. We recently made a major breakthrough by identifying the receptor tyrosine-kinase, EphA2, as the progranulin functional receptor. The canonical ligand for EphA2 is EphrinA1. However, *we propose a new paradigm where the primary ligand for EphA2 is progranulin in bladder cancer. We specifically hypothesize that progranulin-induced MAPK- and Akt-dependent EphA2 Ser-phosphorylation drives EphA2 signaling to enhance tumorigenicity and progranulin signaling influences the expression of metastatic genes as well as promotes pro-invasive EphA2/protein complexes to drive tumor growth and metastasis*. Methods: EphA2 expression in bladder tissues was analyzed by HIC. EphA2 phosphorylation and pro-migratory protein complexes were identified by pull-down assays and proteomics. EphA2 depletion was achieved by stably transfecting shRNA plasmids. Motility and anchorage-independent growth assays were performed. Sensitivity to cisplatin was assessed by cell survival curves. To identify potential pro-metastatic targets regulated by the progranulin/EphA2 axis, we used a Human Tumor Metastasis RT^2 Profiler PCR Array. Results: Progranulin mRNA levels were significantly higher than EphrinA1 in bladder cancer cells and publicly-available bladder cancer microarray studies. Progranulin was upregulated in bladder cancer compared to EphrinA1. EphA2 expression was only detectable in the urothelium of normal bladder tissues while it was upregulated in urothelial carcinoma tissues. In addition, EphA2 was highly expressed in urothelial carcinoma cell lines. Progranulin stimulated ERK and Akt-dependent EphA2 phosphorylation on Ser897. Significantly, EphA2 phosphorylation on Ser897 was abolished in UMUC-3 cells stably depleted of endogenous progranulin. EphA2-depleted UMUC-3 cells were inhibited in motility and anchorage-independent growth. Importantly, EphA2 depletion sensitized UMUC-3 cells to cisplatin. Finally, we identified a novel pro-migratory protein complex, which includes liprinα1, vinculin and ERC1 and potential pro-metastatic downstream targets, cadherin11 and heparanase. Conclusions: Our data will not only provide important information to further define the mechanisms regulating tumor formation in bladder cancer, but it will also yield valuable insight for translational research as characterization of the progranulin/EphA2 signaling axis could contribute to the identification of novel targets for therapeutic intervention along with more accurate diagnostic and prognostic markers for patients afflicted with this devastating disease.

P6

Combined Targeting of EZH2 and Androgen Receptor in Castration-resistant Prostate Cancer Cells

Eswar Shankar[1], Daniel Franco[2], Omair Iqbal[2], Stephen Moreton[3], Rajnee Kanwal[1], Sanjay Gupta[4]

[1]Department of Urology, Case Western Reserve University, The Urology Institute, University Hospitals Cleveland Medical Center, Cleveland, OH, USA; [2]Department of Urology, Case Western Reserve University, Cleveland, OH, USA; [3]Louis Stokes Veterans Administration Medical Center, Cleveland, OH, USA; [4]Department of Urology, Case Western Reserve University, The Urology Institute, University Hospitals Cleveland Medical Center, Louis Stokes Veterans Administration Medical Center, Cleveland, OH, USA

Background: Androgen receptor (AR) has emerged as an important therapeutic target in the treatment of metastatic prostate cancer as evidenced by androgen deprivation therapy (ADT). While almost all metastatic prostate cancers initially respond to ADT, in general the disease reemerges in the form of castration-resistant prostate cancer (CRPC). Enzalutamide, a second generation AR antagonist, exhibited survival advantage in CRPC patients, show relapse within a year, activating AR in these tumors. Recent genomic studies reveal AR-regulated genes contribute to CRPC emergence. Enhancer of zeste homolog 2 (EZH2), the enzymatic member of the polycomb repressor complex PRC2, catalyzing trimethylation of histone H3 at lysine 27, is upregulated in metastatic prostate cancer; its overabundance in localized cancers increases risk of recurrence after surgery. Apparently EZH2 and AR collaborate to activate target genes by cooperative recruitment. Association between EZH2 and AR might be a cause for developing chemoresistance, suggesting a novel combination therapy could be efficacious in CRPC treatment. We hypothesized that combined targeting of EZH2 and AR could significantly inhibit proliferation and metastasis of CRPC cells. Methods: Human prostate cancer C4-2B and 22Rv1, representative of CRPC tumors, were treated with EZH2 inhibitor GSK126 and AR antagonist Enzalutamide (ENZU) individually and in combination, followed by assessment of cell viability, cell cycle analysis, migration, invasion and expression of various target genes by Western blotting. ELISA was employed to assess the change in activity of histone methylation in these cells. Results: Treatment of C4-2B and 22Rv1 cells individually with GSK126 and ENZU (2.5-80 μM) for 24 h exhibited a partial suppressive effect in cell growth. Using GSK126 and ENZU combination at 1:1, 1:5, 1:10 and 1:20 micro-molar ratio exhibited increased cell growth inhibition, where 1:10 ratio showed maximum efficacy in inhibiting cell growth in both cell lines. This combination caused marked increase in G1-phase cell cycle arrest, inhibition of migration and invasion in both cell lines. Furthermore, combination treatment led to significant reduction in the protein expression of AR, AR-V7, EZH2, SUZ12, EED, p-Akt (Ser473) in both cell lines, compared to individual treatments. Combined treatment also caused significant decrease in the levels of H3K27 methylation and its activity. Conclusion: Combination treatment reduced AR and EZH2 expression supporting the hypothesis that this modality is more efficacious in inhibiting the growth of CRPC cells. This opens new possibilities of uncovering a novel path of treating CRPC by simultaneously targeting EZH2 and AR using a combinatorial approach.

P7 - Travel Awardee

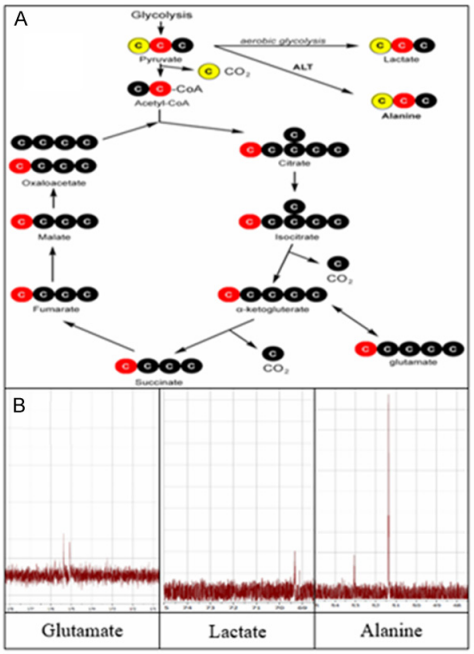
Using Metabolic Pathways to Improve Diagnosis and Risk-stratification of Prostate Cancer

Aymen Alqazzaz[1], Dexue Fu[1], Arman Karimi[2], Gustavo Ferreira[2], Mary C McKenna[2], Mohummad Minhaj Siddiqui[1]

[1]Division of Urology, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD, USA; [2]Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD, USA

Background: Prostate cancer has well-characterized changes that take place metabolically in the citric acid cycle (CAC) such as increased lactate formation, reversal of physiologic buildup of citrate and breakdown into downstream CAC products. Metabolic MR spectroscopy utilizing ^{13}C-labeled pyruvate now allows for clinical characterization of metabolic pathways in tissue. However, the traditionally utilized substrate of [1-^{13}C]pyruvate has limitations because as the pyruvate is broken down, the labeled carbon does not enter the CAC and instead is lost as CO_2 (Figure 1A, yellow carbon). We propose that [2-^{13}C]pyruvate, is better to characterize changes in the CAC (Figure 1A, red carbon). Specifically, ^{13}C-pyruvate can be converted to acetyl CoA and subsequently metabolized in the CAC, reduced to lactate (Warburg effect) or transaminated to alanine. By exposing prostate cancer cells to [2-^{13}C]pyruvate and determining the relative labeling of lactate, glutamate, and alanine, we can elucidate which metabolic pathways (CAC, Warburg, transamination) are utilized in prostate cancer metabolism. The aim of this study was to determine how the metabolism of [2-^{13}C]pyruvate via the CAC, reduction to lactate, and transamination differed in high and low malignancy prostate cancer cells.

Figure 1



Methods: Tumorigenic prostate cells LNCaP and PC3 were exposed a medium containing [2-^{13}C]pyruvate for 4 hours. PCA extraction was then performed to obtain the metabolites. ^{13}C labeled compounds formed intracellularly and those released into the medium were analyzed using NMR chemical shifts with focus on peaks associated with pyruvate, lactate, alanine, glutamate, and other compounds related to the CAC. Dioxane was used as an internal standard for NMR characterization. Results: The labeled carbon from the [2-^{13}C]pyruvate can be identified in the downstream metabolites of the citric acid cycle, after reduction to lactate, and/or transamination by determining labeled metabolites by ^{13}C-NMR analysis (Figure 1B). This allows for the ability to calculate the relative metabolism of pyruvate via the three pathways; transamination (74%), lactate formation (16%), and CAC (10%). Conclusions: [2-^{13}C]pyruvate can be successfully utilized as a tool to explore the alterations in pathways of metabolism, including the citric acid cycle, in prostate cancer cells.

P8

Androgen Regulates Arginine Metabolism in Prostate Cancer

Dexue Fu[1], Min Xu[1], Aymen Alqazzaz[1], Jee-Hoon Song[1], Hubert Huang[1], Lingbu Wang[1], Mohammad Afnan Khan[1], Krish Chandrasekaran[1], Arif Hussain[1], Ganesh Sriram[2], Mohummad Minhaj Siddiqui[1]

[1]University of Maryland School of Medicine, Baltimore, MD, USA; [2]University of Maryland College Park, Baltimore, MD, USA

Background: Arginine (Arg) metabolism has demonstrated recent promise in prostate cancer management as a potential therapeutic target for prostate cancer. Clinical trials are ongoing to target Arg metabolism in prostate cancer and have demonstrated promising early results. The aim of this study was to investigate if Arg metabolism was associated with prostate cancer progression from androgen dependent (AD) to androgen independent (AI) phenotype. Methods: Four sets of conditions were tested: LNCaP control, LNCaP in the presence of a potent androgen receptor agonist, dihydrotestosterone (DHT), LNCaP with MDV3100 (an androgen receptor inhibitor), and a subline of LNCaP (CSS90) which has become AI. Non-targeted metabolomics was performed using gas chromatography and mass spectroscopy (GC/MS). The Affymetrix HTA 2.0 gene chip was performed on RNA expression analysis. The Seahorse XF analyzer which allows for real-time measurement of cellular oxygen consumption. Western blot was used to show the effect of arginine starvation on protein expression, autophagy and metabolic signaling. Real-time PCR was performed on mRNA expression. MTT assay and ATP assay were used to measure the effect of starvation on the cancer cell growth. Flow cytometer was used to evaluate apoptosis. Results: LNCaP cells at a baseline are Arg-dependent. Arg starvation led to a 63% decrease in LNCaP cell viability ($p<0.0001$) in 24 hrs. By contrast, the AI CSS90 only demonstrated a 23% decrease in cell viability when depleted of Arg ($p<0.01$ with Arg control, $p<0.001$ between LNCaP and CSS90) demonstrating AD cells were much more dependent on exogenous Arg. Enhanced AR signaling with DHT further sensitized cells to arginine depletion with a 73% decrease, and low level AR inhibition decreased response to arginine depletion with only a 21% decrease in cell viability ($p<0.001$ for all changes). Modulation of androgen signaling with DHT and MDV3100 demonstrated similar increased reliance of exogenous Arg for AD cells with increased androgen signaling with a 2.2 fold increase in Arg dependent oxygen consumption with DHT and 95% drop with MDV ($p<0.0001$ for both). Interestingly Arg starvation induced apoptosis and cytotoxic-autophagy in 72 hr, whereas following androgen stimulation with DHT, apoptosis and autophagy were decreased. Conclusions: Arginine is potentially an important aspect of prostate cancer metabolism and is modulated by androgen signalling. Arg targeted therapies may best be suited towards earlier stage tumors and Arg metabolism characterization may have potential as a biomarker of progression to AI prostate cancer.

P9

ABCB1 Remains as a Dominant Factor Driving Acquired Cabazitaxel Resistance

Alan Lombard, Chengfei Liu, Cameron Armstrong, Vito Cucchiara, Xinwei Gu, Wei Lou, Christopher Evans, Allen Gao

University of California, Davis, Sacramento, CA, USA

Background: Castration-resistant prostate cancer (CRPC) remains an incurable disease. Docetaxel provides a survival benefit for CRPC patients but only ~50% of patients will respond to docetaxel and the rest eventually progress due to development of resistance. Cabazitaxel, a second generation taxane, is now approved for docetaxel resistant CRPC but again, resistance ultimately renders this treatment ineffective. Here we characterize two novel models of cabazitaxel resistant CRPC derived from our docetaxel resistant cells. These models mimic the clinical progression of cabazitaxel failure in the post-docetaxel setting. Methods: TaxR and DU145-DTXR docetaxel-resistant cell line derivatives were created by chronically exposing C4-2B and DU145 cells respectively, to increasing doses of docetaxel. CabR and CTXR are cabazitaxel-resistant cell lines derived from TaxR and DU145-DTXR cells respectively, created through chronic exposure to increasing doses of cabazitaxel. Cell growth assays utilizing a coulter counter were used to assess cellular viability. Colony formation assays supported cell growth assays. qPCR was used to assess ABCB1 expression. Western blots were used to assess AR expression. Exosomes were isolated using Exoquick-TC reagent. Results: Cell growth and colony formation assays demonstrate that CabR and CTXR cells exhibit robust resistance to cabazitaxel versus parental cells while maintaining similar resistance to docetaxel. Based on our previous findings that ABCB1 mediated partial cross-resistance between these taxanes, we hypothesized that further augmenting ABCB1 expression may be responsible for added cabazitaxel resistance in our new models. qPCR demonstrates that both CabR and CTXR cells further overexpress ABCB1 ~2.5 fold. Treatment of cabazitaxel resistant cell lines with elacridar completely re-sensitizes both to original parental C4-2B and DU145 cell levels. Inhibition of ABCB1 using the anti-androgens bicalutamide and enzalutamide similarly re-sensitizes CabR and CTXR cells to cabazitaxel treatment. Interestingly, AR positive CabR cells retain sensitivity to AR-directed therapies indicating a lack of cross-resistance between these drugs. Finally, we show that ABCB1 transcript level differences can be detected and distinguished in exosomes derived from our models of taxane resistance. Conclusions: Our data further support that ABCB1 is a dominant factor driving taxane resistance and that resistance may be a function of ABCB1 expression. We test putative strategies to sensitize resistant cells to treatment and demonstrate that AR-directed therapies are still viable for taxane resistant CRPC. Finally, we provide evidence that exosomal ABCB1 detection could be developed into a novel biomarker of taxane resistance. Future efforts will be directed toward clinical validation of these findings.

P10

Metabolomic Profiling of Prostate Cancer Upgrading During Active Surveillance

Bruce J Trock[1], Edward Karoly[2], John Luster[2], Mufaddal Mamawala[1], Sacha Wolf[1], Herbert Ballentine Carter[1]

[1]Johns Hopkins School of Medicine, Baltimore, MD, USA; [2]Metabolon, Inc., Durham, NC, USA

Withdrawn

P11

A Selective LSD1 Inhibitor Enhanced Anti-prostate Cancer Effect of Enzalutamide by Induction of the Nur77 Mediated Apoptotic Pathway in Prostate Cancer Cells

Noriko Yokoyama[1], Victor Pham[1], Yi-Chao Zheng[2], Hongmin Liu[2], Xiaolin Zi[1]

[1]University of California, Irvine, Orange, CA, USA; [2]Zhengzhou University, Zhengzhou, China

Background: Androgen receptor (AR) amplification is common in fatal castration resistant prostate cancer. Lysine (K)-specific demethylase 1A (LSD1) is also overexpressed in prostate cancer and its over expression predicts disease recurrence and poorer survival of prostate cancer patients. AR binds to LSD1 and changes its enzymatic specificity to H3K9me1/2, resulting in an enhanced AR transcriptional activity. We therefore have developed novel selective LSD1 inhibitors and examined whether LSD1 inhibitor can enhance anti-prostate cancer efficacy of a second-generation anti-androgen agent Enzalutamide. Methods: A novel series of 1, 2, 3-triazole-dithiocarbamate hybrid molecules were designed and synthesized via "click" chemistry. LSD1 and MAO-A enzyme inhibitory activities of these compounds were examined by LSD1 and MAO-A inhibitor screening assay kits. The combined effects of a lead LSD1 inhibitor LHM101 and enzalutamide on the growth and apoptosis of 22Rv1, C2-4B and C4-2B enzalutamide resistant cell lines were examined by MTT assay and FACS analysis. Gene silencing or knock-down experiments were performed by using siRNA. Real-time PCR and Western blotting analyses were used to evaluate the expression of AR target genes (e.g. PSA and TMPRSS2), orphan nuclear receptor TR3/Nur77, and intracrine androgen synthesis enzymes. Results: A lead LSD1 chemical inhibitor LHM101 with an IC_{50} value of 2.11 μM and selectivity over MAO-A up to 1250 fold has been screened out from a focused small molecule library. Biochemical analysis revealed that LHM101 was a reversible and flavin adenine dinucleotide competitive LSD1 inhibitor. Molecular modeling predicted that LHM101 reasonably docked into the FAD pocket of LSD1. LHM101 re-sensitized the cytotoxic effect of Enzalumide in the C4-2B Enzalutamide resistant cell line. LHM101, Enzalutamide and their combination all increased the expression of Nur77, leading to an enhanced apoptotic effect of Enzalutamide on prostate cancer cell lines. The growth inhibitory and apoptotic effects of LHM101 at least in part requires the existence of Nur77. Furthermore, LMH101 reduced the Enzlutamide induced expression of several intracrine androgen synthesis enzymes, including AKR1C3, HSD3B2 and HSD17B6. Conclusions: These results suggested that our newly developed LSD1 inhibitors deserve further investigation for a combination therapeutic approach with Enzalutamide in treatment of fatal prostate cancer.

P12 - Travel Awardee

Macrophage Plasticity in Bone Metastatic Prostate Cancer<!–EndFragment–>

Chenhao Lo, Etienne Baratchart, David Basanta, Conor Lynch

Moffitt Cancer Center, Tampa, FL, USA

Background: Metastatic prostate cancer (mPC) typically arises in bone. Understanding how mPC manipulates the bone microenvironment (BME) is crucial for therapy development. Macrophages (M0) that polarize to pro and anti-inflammatory phenotypes have well-described roles in cancer progression and, in bone remodeling/injury repair. However, temporal dynamics of M0 infiltration and polarization, and the resultant effect on bone mPC cells, bone building osteoblasts (OBL) and bone resorbing osteoclasts (OCL) remains unexplored to date. To address this gap in knowledge, we propose to use an integrated mathematical modeling approach since it allows for the dynamic analysis of multiple parallel cellular interactions. Methods: Initially, we parameterized ordinary differential equation (ODE)-based models with what is empirically known about M0 in normal bone repair. We supplemented this information by analyzing macrophage plasticity in vivo subsequent to intratibial injury. Bone marrows were isolated at time 0, 1, 2, 3, 7 and 14 days (n=5/group) and profiled by flow cytometry for pro- and anti-inflammatory monocyte macrophage content (CD11b, Ly-6G, Ly-6C, ARG-1 and iNOS). Contralateral tibias were analyzed for bone volume, osteoblast and osteoclast numbers. These data were then used for ODE simulations. Results: Inflammatory monocytes rapidly infiltrate the bone injury site and polarize to pro-inflammatory (Mi). Once the Mi clear the stromal debris (Days 0-2), mature macrophages polarize to anti-inflammatory status (Mα) to trigger wound healing (Days 1-3). OBL expand and mineralize the bone (Days 1 to 10) while OCL resorb bone (Days 3-14). Bone turnover and remodeling results in the release of growth factors like transforming growth factor β (TGFβ) that can suppress Mi and return the BME to homeostasis. These results were in agreement with ODE model outputs demonstrating a significant rapid Mi and Mα influxes that dissipate within 3 days, and give rise to OBL and OCL that eventually recede over a 2 week period as the bone undergoes remodeling and returns to homeostasis. Our preliminary studies with prostate cancer cell lines (TRAMPc1 and Myc-CAP) that are syngeneic with immunocompetent mice show a direct impact on the polarization of bone-derived M0. Conclusions: We have generated an ODE model that takes into account macrophage polarization over time during bone repair and important how pro- and anti-inflammatory macrophages interact with bone stromal cells. The mathematical model predictions are in agreement with our biological experiments in vivo and will allow us to interrogate how macrophages impact the behavior of the bone metastatic prostate cancer microenvironment.

P13 - Travel Awardee

P53 and Rb are Required in Ell2 Suppression of Prostate Cancer Cell Proliferation and Migration

Mingming Zhong[1], Laura E Pascal[1], Xiaonan Qiu[2], Yibin Zhou[3], Yao Wang[4], Zhou Wang[1]

[1]University of Pittsburgh, Pittsburgh, PA, USA; [2]Tsinghua University, Beijing, China; [3]The Second Affiliated Hospital of Soochow University, Soochow, China; [4]China-Japan Union Hospital of Jilin University, Changchun, China

Background: Eleven-nineteen lysine-rich leukemia 2 (ELL2) is an RNA Pol II elongation factor and is an androgen response gene. ELL2 expression is downregulated in high-Gleason score prostate cancer specimens. Retinoblastoma (RB) and p53 and are tumor suppressors that play important roles in prostate cancer. We recently reported ELL2 interaction with RB in the suppression of prostate cancer. ELL, a homolog of ELL2, was reported to interact with p53. This study was designed to test the roles of p53 and RB in ELL2 suppression of prostate cancer. Methods: Histological defects in prostate-specific ELL2 knockout mice on a C57BL/6J background were examined at age 18-20 mos. Co-immunoprecipitation was used to determine ELL2 association with p53, as well as RB. ELL2, p53 and RB were knocked down either individually or in combination using siRNA in C4-2 and LNCaP cells are confirmed by western blotting. The effects of knockdown in cellular proliferation and migration were determined by BrdU, colony formation, wound healing assay and transwell. Results: ELL2$^{-/-}$ male mice exhibited increased proliferation in the prostates and developed mouse prostatic intraepithelial neoplasia (mPIN). ELL2 co-precipitated with both p53 and RB. Knockdown of ELL2 alone in prostate cancer cell lines C4-2 and LNCaP induced an increase in cellular proliferation and migration. ELL2 knockdown enhanced cell proliferation and migration when either p53 or ELL2 was knocked down. However, ELL2 knockdown had no obvious effect on cell proliferation and migration when both p53 and ELL2 were knocked down. Conclusions: These results suggest that ELL2 is growth suppressive in the prostate and p53 and RB are required for ELL2 suppression of cell proliferation and migration in prostate cancer cells. These findings provide a foundation for investigating the mechanism of ELL2 suppression of prostate cancer through p53 and RB.

P14

Novel Small Molecule Inhibition of the Function and Level of Androgen Receptor in Castration-resistant Prostate Cancer

Zhenyu Yang[1], James Johnson[2], Laura Pascal[1], Dan Wang[1], Jianhua Zhou[1], Joel Nelson[1], Peter Wipf[3], Zhou Wang[1]

[1]Department of Urology, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Chemistry, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA

Background: Prostate cancer patients eventually develop castration-resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT), and CRPC is currently incurable. The androgen receptor (AR) plays a critical role in CRPC as well as in the development of resistance to enzalutamide and abiraterone. Both enzalutamide and abiraterone act either directly or indirectly through the AR ligand-binding domain (LBD). We previously identified a novel small molecule, 2-((isoxazol-4-ylmethyl)thio)-1-(4-phenylpiperazin-1-yl)ethanone (IMTPPE), that inhibits AR transcriptional activity and protein levels in several prostate cancer cell lines, and also inhibits CRPC xenograft tumor growth. JJ-450 is a cyclopropane analog of IMTPPE with desirable potency and drug-like properties. In this study, we characterize the effect of JJ-450 on the transcriptional activity and protein levels of AR, the proliferation of a series of prostate cancer cell lines, and the growth of an enzalutamide-resistant prostate tumor model. Methods: JJ-450 was synthesized as previously described. The effects of JJ-450 on cell proliferation and the expression of PSA and AR proteins were tested using clonogenic assay and Western blot, respectively, in C4-2 and 22Rv1 prostate cancer cell lines. The inhibition of AR-target gene mRNA expression was determined by real-time PCR. A luciferase assay was used to test JJ-450 inhibition of the transcriptional activity of AR, or AR constructs that lack LBD. Animal studies used a 22Rv1 xenograft tumor model. Results: Western blot analysis revealed that 2 μM JJ-450 inhibited PSA protein expression, and at 10 μM or higher, JJ-450 also inhibited AR and AR splice variants expression levels. Real-time PCR analysis indicated JJ-450 mediated inhibition of the R1881-induced mRNA expression of AR-target genes in several different prostate cancer cell lines. JJ-450 inhibited the PSA-luciferase activity in a dose-dependent fashion in C4-2 cells cultured in complete RPMI medium. JJ-450 also inhibited PSA-luciferase activity induced by GFP-NAR (AR lacks LBD). In a clonogenic assay, IMTPPE only inhibited the proliferation of AR-positive prostate cancer cells, but not AR-negative cells. Furthermore, JJ-450 inhibited the growth of a 22Rv1 xenograft tumor, a model for a prostate cancer resistant to enzalutamide. Conclusion: JJ-450 is a new lead compound for developing clinical candidates for the treatment of CRPC, including those resistant to enzalutamide and abiraterone.

P15 - Travel Awardee

Regulation of Androgen Receptor of Dhx15 in Prostate Cancer

Yadong Xu[1], Laura E Pascal[1], Fangming Deng[2], Zhou Wang[1]

[1]UPMC Shadyside, Pittsburgh, PA, USA; [2]Department of Pathology, NYU School of Medicine, Newyork, NY, USA

Background: The development to castration resistant prostate cancer (CRPC) after androgen deprivation therapy (ADT) remains one of the most difficult challenges in prostate cancer treatment. Androgen receptor (AR) plays a key role in the prostate carcinogenesis and development to castration resistance and is the most important therapeutic target of prostate cancer. Therefore, understanding the mechanism regulating AR may lead to new therapies for prostate cancer patients. Through a yeast mutagenesis screen, PRP43 was found to play an important role in the AR nucleocytoplasmic translocation, which is a crucial step in AR function. DHX15 is the mammalian ortholog of prp43 and a member of the DEAH-box (DHX) RNA helicase family. DHX15 was reported to contribute to the tumorigenesis including glioma, gastric cancer. However, the mechanism of DHX15 in tumorigenesis remains elusive. Previous study showed that DHX15 can bind to and stabilize Siah2, an AR E3 ligase which targets the repressed AR chromatin complexes for degradation, resulting in activation of AR-target genes involved in cell proliferation, cell motility and lipid metabolism. These findings lead to the hypothesis that DHX15, Siah2 and AR form a complex that plays an important role in the development of CRPC. This study further demonstrated the interaction among these three proteins and suggested a potential role of DHX15 in CRPC. Methods: C4-2 prostate cancer cell line was used as a model for CRPC. Cell proliferation was demonstrated with BrdU assay and colony formation assay. AR transcriptional activity was determined by western blot and PSA promoter-driven luciferase assay. siRNA was used to knockdown DHX15. The interactions among DHX15, Siah2 and AR were detected using co-immunoprecipitation assay. DHX15 expression level in human CRPC specimens was detected by tissue microarray (TMA) immunostaining analysis. Results: Knockdown DHX15 in prostate cancer cells repressed the cell proliferation and the AR transcriptional activity. DHX15, AR and Siah2 form a complex. Both N terminal and C terminal in DHX15 can interact with AR. The expression level of DHX15 was upregulated in human prostate cancer, particularly in CRPC specimens. Conclusions: DHX15 contributes to prostate cancer progression to castration resistance. DHX15/Siah2/AR axis may represent an excellent target for CRPC treatment.

P16

Radiation Induced Endoglin Mediates Prostate Cancer Radio-resistance Via Metabolic Reprogramming

Anisha Madhav, Frank Duong, Neil A Bhowmick

Cedars-Sinai Medical Center, Los Angeles, CA, USA

Background: Radiation therapy is the primary intervention for nearly half of the patients with localized advanced prostate cancer. The development of radiation resistant disease affects nearly 30-50% of patients undergoing radiotherapy. Based on initial observations that irradiation upregulated endoglin and antagonizing endoglin-dependent BMP signaling with a partially humanized monoclonal antibody, TRC105, resulted in a significant reduction in clonogenicity, compared to irradiation alone ($p<0.001$), we examined both tumor autonomous- and tumor microenvironment-dependent radiation-resistance mechanisms. Methods: We used noggin and novel endoglin neutralizing antibodies to specifically block BMP signaling in the context of irradiation in culture conditions and in mice. Three dimensional co-cultures of prostatic fibroblasts and epithelia provided data paralleling that found in orthotopically grafted tissue recombinants in mice for measures of epithelial expansion. Metabolic changes were determined by oxygen consumption response assays on a Seahorse instrument. Static metabolite analysis of irradiated stromal fibroblasts was performed by mass spectrometry. Results: We found that TRC105 delayed DNA damage repair compared to irradiation alone, however was not sufficient to account for the dramatic radiation sensitization achieved. Cancer cells undergo an energy deficiency following irradiation, due to the demands of DNA and organelle repair. We revealed that the ATP stores of irradiated cancer cells did not recover if endoglin/BMP signaling was antagonized, due to inhibition of mitochondrial gene transcription by PGC-1α. We found that radiation-induced endoglin and BMP signaling was sufficient and necessary for mitochondrial biogenesis of cancer epithelia. However, the radiation sensitization conferred by antagonizing cancer epithelial endoglin was lost in co-culture experiments with prostatic stromal fibroblasts. But, blocking fibroblast' endoglin signaling alone sensitized prostate cancer epithelia to radiation treatment. We found irradiation resulted in endoglin-dependent metabolic reprogramming of wild type fibroblasts to resemble a CAF metabolic phenotype, with elevated fatty acid oxidation. The resultant ketone bodies, β-hydroxybutyrate, was able to protect prostate cancer epithelia from radiation induced DNA damage and restore energy deficits. Fibroblast-derived ß-hydroxybutyrate contributed to DNA damage repair of the adjacent epithelia. Ultimately, stereotactic irradiation treatment of orthotopically grafted prostatic tissue recombinants were found to cooperatively reduce tumor volume in combination with TRC105, compared to irradiation alone. Conclusions: Together, our data suggested that stromal and epithelial endoglin have distinct roles in prostate cancer radiation resistance, yet both must be targeted to effectively for radiation sensitization. A novel synthetic lethality strategy exploiting a radiation-induced reverse Warburg effect dependent on endoglin signaling has the promise of improving outcomes in prostate cancer.

P17

Androgen Regulation of Fibroblast Growth Factor-5 in Prostate Cancer

Dalton McLean

University of Wisconsin, Madison, WI, USA

Background: The large family of Fibroblast Growth Factors (FGFs) have been implicated in a number of cancers including renal cell carcinoma, breast cancer, and glioblastoma. They have a wide range of implications in cell proliferation, survival, migration, and differentiation, making their signaling pathways attractive candidates for hijacking by cancer cells. Using a unique cell line model (BCaP) that portrays progression of prostate cancer from benign through metastasis, we have identified FGF5 as a potential mediator of prostate cancer progression. Methods: Utilizing the BCaP cell line model of prostate cancer progression, we assessed FGF5 expression and function *in vitro* and *in vivo*. We were also able to analyze both an outcomes and progression tissue microarray containing approximately 400 prostate cancer patient cores to assess FGF5 expression in a clinical setting. Results: There was a significant increase in both RNA and protein expression of FGF5 across progression of the BCaP line, with the highest levels being observed in the aggressive prostate cancer cells. Recombinant FGF5 significantly increased proliferation in Androgen Receptor (AR) positive cell lines. Conversely, in AR negative cell lines, proliferation was inhibited by FGF5. In addition, some AR-negative cell lines would express AR following FGF5 overexpression. AR also played a role in regulating FGF5 as addition of dihydrotestosterone to AR-positive cells results in a decrease in FGF5 protein and RNA. Finally, using the patient tissue microarrays, we found that FGF5 positive cells were significantly increased in cancer and metastatic cores compared to benign. It was also observed that higher PSA values correlated with lower FGF protein expression, corroborating *in vitro* work. Conclusion: These results suggest that FGF5 is increased in prostate cancer progression in cell lines as well as patient samples. Data also suggests that AR plays a role in the regulation of FGF5 and vice versa. In the developing prostate, AR-negative basal cells give rise to luminal AR-positive cells, which could be resulting from increased expression of FGF5. The mechanism of FGF5 and AR regulation will be further evaluated.

P18

Megacystis-microcolon-intestinal Hypoperistalsis and Prune Belly Syndrome: Overlapping Genetic Variants Cause Overlapping Phenotypes

Nida Iqbal, Thomas Jascur, Shaohua Zhang, Linda Baker

UT Southwestern Medical Center, Dallas, TX, USA

Background: MMIHS (OMIM# 155310) is a rare congenital disorder with significant phenotypic overlap and oftentimes misdiagnosis with the related disease, PBS (OMIM# 100100). We interrogated our cohort of MMIHS patients for mutations in *ACTG2, MYH11, MYLK* and *LMOD1* and searched for novel causal variants in unidentified cases. Because of the significant phenotypic overlap and reports highlighting incidence of both diseases in the same family, we screened a subset of PBS patients for variants in MMIHS causal genes. Methods: All studies were performed under an IRB approved protocol. 100-bp paired-end WES on lymphocyte DNA was performed in some cases. Sanger Sequencing was used for candidate variants, WES validation, and inheritance testing. Results: See Table 1. Conclusions: MMIHS and PBS are phenotypically related smooth muscle myopathies that may be caused by mutations in the same genes. We identified causal variants in *ACTG2* in 62.5% of our MMIHS patient cohort and have suggested candidate genes in the remaining 2 of 3 cases. We found 6 of 79 PBS patients tested to have variants in MMIHS causal genes. Our data suggest that PBS is a heterogeneous and complex disorder that in part, can be explained by mutations in MMIHS causal genes.

Table 1. Summary of autosomal dominant variants in PBS and MMIHS patients

Patient ID	Gene	Amino Acid	Novelty	Inheritance
MMIHS 1	Unidentified	N/A	N/A	N/A
MMIHS 2	*ACTG2*	p.R178H	Reported	*de novo*
MMIHS 3	*ACTG2*	p.R257C	Reported	*de novo*
MMIHS 4/PBS	*ACTG2*	p.R178C	Reported	*de novo*
MMIHS 5	*ACTG2*	p.M45T	Reported	*de novo*
MMIHS 6	*ACTG2*	p.R178H	Reported	*de novo*
MMIHS 7	Candidate gene *MYL9*	p.E112-S115del	Not reported	Paternal
MMIHS 8	Candidate gene *FN1*	p.L1364P; p.N55D	Not reported	Unknown
PBS 1	*ACTG2*	p.E58V	Not reported	*de novo*
PBS 2	*ACTG2*	p.L347P	Not reported	*de novo*
PBS 3	*MYH11*	p.R108W	Not reported	Unknown
PBS 4	*LMOD1*	p.S516F	Not reported	Unknown
PBS 5	*LMOD1*	p.N64S	Not reported	Unknown
PBS 6	*MYLK*	p.E98A	Not reported	Unknown

P19

Tumor Communication to Bone microenvironment When SCF is Deleted

Brittni M Foster, Lihong Shi, Bethany Kerr, Phyillis Elliot, Mary E Mobley, Koran Harris, Christina M Snyder

Wake Forest University School of Medicine, Winston Salem, NC, USA

Background: The tyrosine kinase CD117 or C-kit correlates with tumor progression with the highest expression being found in the bone. 80% of prostate cancers metastasize to the bone and once the primary tumor cells have spread to the skeleton, the survival rate of patient's drops from 90% to less than 30%. Stem cell factor (SCF) is CD117's sole ligand and it is well known for playing an important role in stimulating mature hematopoietic stem cells. In this study, by knocking out SCF in three different tissues in our murine model we examined gene expression and cell function in response to CD117 activation in prostate cancer. Methods: RM1 cells were sorted into CD117 positive and negative populations. These tumor cells were injected into our murine SCF knockout models. The three groups contain platelet-specific SCF KO, osteoblast-specific SCF KO, and prostate-specific SCF KO. Tumors were harvested at 12 days along with bone and blood samples. Gene expression was looked at to compare the different populations of CD117 tumor cells in the different KO models. TRAP staining was performed on the tibia samples collected from the mice to look at the changes in the bone microenvironment and how signaling affects the growth or destruction of bone. These positive and negative cell populations gene expressions were also compared in vitro using a 2D monolayer and 3D sphere models. Results: Here we look at the changes in gene expression and the bone microenvironment in our KO murine models in the presence or absence of CD117 on RM1 cells and its sole ligand, SCF expression in tissues. We see a significant increase of gene expression in our knockout models injected with CD117+ tumor cells. Conclusion: Knocking out SCF in tissues associated with metastasis alters CD117+ cell function.

P20 - Travel Awardee

Notch3 Promotes Prostate Cancer-Induced Bone Metastasis in a MMP3-dependent manner

Sourik S Ganguly[1], Xiaohong Li[1], Cindy K Miranti[2]

[1]Van Andel Research Institute, Grand Rapids, MI, USA; [2]The University of Arizona, Tucson, AZ, USA

Background: Notch signaling is known to be dysregulated in PCa, but its role in PCa-induced bone metastasis is not known. Currently approved therapies are focused on the targeting of events occurring inside the tumor and do not fully consider the contributions of the host microenvironment. Thus, better understanding of the host/tumor interactions that trigger and drive metastatic processes could provide additional avenues for therapeutic intervention. Methods: PCa cells, in which Notch3 expression was manipulated, were injected into the tibiae of SCID mice. Development of bone lesions was monitored by x-ray and measured using Metamorph software. The tibiae were harvested at end time points for histological analyses, qRT-PCR, or western blots. qRT-PCR and western blotting were performed on the homogenized tibiae to evaluate the molecular signaling. Cultured bone marrow from naïve mice was used for *in vitro* differentiation of osteoblasts or osteoclasts in the presence or absence of conditioned medium from cancer cells in which Notch3 expression was manipulated. The proliferations of osteoblasts or osteoclasts were read out by Crystal violet staining or MTT assay and the differentiations were read out by ALP or TRAP staining, respectively. Results: PCa cell lines that promote mixed osteoblastic bone lesions (C42B and 22RV1) express more Notch3 relative to cell lines, which promote osteolytic bone lesions (PC3). Overexpression of the activated form of Notch3 (NICD3) in PC3 cells decreased osteolytic lesions and decreased the number of osteoclasts in the tumor-bone microenvironment. Conversely, inhibition of Notch3 in PC3, 22rv1 and C42B cells with shRNA, promoted prostate cancer-induced osteolytic lesions when injected in the tibiae. Conditioned medium from PC3-NICD3 cells generated ALP-positive osteoblasts, and increased osteoblast proliferation in vitro. Conditioned medium from PC3-NICD3 cells also decreased osteoclasts and inhibited osteoclastgenesis. PC3-NICD3 cells injected into tibiae expressed more human-specific MMP3 than tibiae injected with control cells. Conversely, PCa cells expressing Notch 3sh RNA expressed decreased human specific MMP3. PCa cells expressing Notch3sh promoted increased osteoclasts number both *in vivo* and *in vitro*. Our results also indicate that NICD3 inhibits lytic lesion development and *in vitro* osteoclast differentiation in a cancer cell secreted MMP3-dependent manner. Conclusions: Notch signaling in PCa tumors favors osteoblastic metastasis by stimulating the production of MMP3 in the tumor microenvironment to inhibit osteoclast function and number while inducing osteoblast proliferation. Our results suggest that Notch signaling from cancer cells promotes osteoblastic metastasis and thus may be a therapeutic target for such metastatic lesions.

P21 - Travel Awardee

Androgen Receptor Variants Mediate DNA Repair Following Radiation in Prostate Cancer

Yi Yin[1], Rui Li[1], Felix Y Feng[2], Johann S de Bono[3], Scott M Dehm[4], Ram S Mani[5], Ganesh V Raj[6]

[1]Department of Urology, The University of Texas Southwestern Medical Center, Dallas, TX, USA; [2]Departments of Radiation Oncology, Urology, and Medicine, University of California at San Francisco, San Francisco, CA, USA; [3]Drug Development Unit and Prostate Cancer Targeted Therapy Group, The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, London, United Kingdom; [4]Masonic Cancer Center and Departments of Laboratory Medicine and Pathology and Urology, University of Minnesota, Minneapolis, MN, USA; [5]Department of Pathology and Urology, The University of Texas Southwestern Medical Center, Dallas, TX, USA; [6]Department of Urology and Phamacology, The University of Texas Southwestern Medical Center, Dallas, TX, USA

Recently, the combination of androgen deprivation therapy (ADT) and radiation therapy (RT) has been shown to block the androgen receptor (AR)-driven DNA damage response (DDR) and enhance RT-mediated cell kill of prostate cancer (PCa). Since ADT may induce expression of AR splice variants (ARVs) we hypothesized that ARVs can drive DDR and mediate resistance to combined ADT and RT. Herein, we demonstrate that ARVs can increase the clonogenic survival of PCa cells following RT in an ADT-independent manner. RT induces the interaction between ARVs and a DDR driver, the DNA-dependent protein kinase catalytic subunit (DNA-PKc). Pharmacological inhibition of DNA-PKc blocks its interaction with ARVs and results in persistence of DNA damage, increased tumor cell kill and improved PCa cell survival following RT. These results indicate that combinatorial targeting of DNA-PKc with ADT and RT may be an effective strategy for overcoming radioresistance when treating clinically localized PCa.

P22

The Impact of Altered Steroidogenesis on Estrogen Receptor Activation in Benign Prostatic Hyperplasia

Teresa T Liu, Clara H Jeong, Emily A Ricke, William A Ricke

University of Wisconsin-Madison, Madison, WI, USA

Background: Benign prostatic hyperplasia (BPH) impacts 50% of men in their 50 s and 90% of men in their 80 s. Many men with BPH develop lower urinary tract symptoms (LUTS) that significantly decreases their quality of life. While BPH development and progression is complex and multi-faceted (inflammation, proliferation, collagen), it has also been associated with altered steroid homeostasis. With advancing age and increased BPH/LUTS, there is a coincident decrease in the testosterone (T) to estrogen (E) ratio in circulation. This suggests an integral role for an appropriate balance of sex steroid hormones for the maintenance of the normal prostate. ERα and ERβ, the two estrogen receptors, serve opposing roles in the prostate (pro-proliferative vs. pro-apoptotic) suggesting the ERα:ERβ ratio also critical in the progression of disease. We hypothesize that the alteration in steroid hormone enzymes directly impact the expression and activation of estrogen receptors leading to disease progression. Methods: Using immunohistochemistry (IHC), we will measure protein levels of steroid enzymes responsible for the metabolism of androgens to estrogens in human normal and disease prostate. Using multiplex IHC techniques, we will correlate the expression of these enzymes with the activation of ERα and ERβ. We will examine the expression of ER target genes to approximate the degree of ER activation. Using liquid chromatography-mass spectrometry (LC-MS), we will also quantify the circulating steroid hormone levels in our established steroid hormone induced mouse model. Results: Meta-analysis of publicly available datasets show a significant positive correlation between ERα expression and AKR1C1 expression and a negative correlation between ERβ and AKR1C1 (p=0.09). AKR1C1 is responsible for the metabolism of DHT to 3β-diol, an ERβ ligand. Additionally, preliminary IHC shows an increase in AKR1C1 in normal prostate tissue, predominantly in the cytoplasm of the epithelial cells. Using a hormone-induced mouse model of BPH, we have successfully measured eleven serum steroid hormones including T, dihydrotestosterone, 17β-estradiol, and 3α-/3β-diol by LC-MS. Conclusions: The correlation of AKR1C1 with ERα and ERβ suggests that there is a mechanism by which the prostate maintains a critical ERα:ERβ ratio. Using IHC to examine the expression of steroid enzymes in conjunction with the estrogen receptors will give us clearer insight into the contribution of hormone homeostasis has on progression. Additionally, with the sensitivity of our hormone panel, we can evaluate the circulating hormone levels and correlate it to: enzyme, receptor, and target gene expression leading to better disease stratification and personalized treatment.

P23 - Travel Awardee

Effect of CXCR6/CXCL16 Axis on Efficacy of Docetaxel in Prostate Cancer

Neeraj Kapur[1], Hina Mir[1], Guru Sonpavde[2], Shailesh Singh[1]

[1]Morehouse School of Medicine, Atlanta, GA, USA; [2]Dana Farber Cancer Institute, Boston, MA, USA

Background: Chemotherapeutic interventions in prostate cancer (PCa) often become ineffective due to emergence of chemo-resistance. The molecular mechanism underlying chemo-resistance is not well defined yet. Here, we demonstrate the involvement of CXCR6/CXCL16 axis on the efficacy of docetaxel (DTX), which is often offered to treat PCa. Methods: Effect of CXCR6-CXCL16 axis blockade on PCa cell (LNCaP, PC3, and DU145) viability in response to DTX was determined using MTT assay. Flow cytometry and RT-qPCR was used to determine changes in CXCR6 and CXCL16 expression in response to DTX-treatment in PCa cells. ELISA was used to detect soluble CXCL16 in conditioned media from DTX treated and untreated cells. Efficacy of DTX on PCa cells with or without CXCR6 stimulations and/or CXCR6 blockade was determined by flow cytometry based apoptosis assay. Further, phospho-specific antibody microarray was performed to determine CXCL16-induced pro-survival molecules in PCa cells, which were confirmed by western-blot analysis. CXCR6-mediated change in expression of epithelial to mesenchymal transition (EMT) markers was investigated using RT-qPCR and western-blot. Results: Poor efficacy of DTX was observed after CXCL16 treatment. Efficacy of the drug was significantly improved after CXCR6 blockade. DTX treatment accentuated CXCR6/CXCL16 expression in PCa cells and also induced ADAM-10 mediated shedding of CXCL16 from PCa cells. Further, soluble CXCL16 compromised the therapeutic response to DTX by inducing pro-survival signaling molecules such as GSK-3β, NFκB, ERK/1/2, and survivin. PCa cells also showed significantly decreased expression of epithelial marker (E-cadherin) and increased expression of mesenchymal markers (β-catenin, α-SMA, vimentin, snail) following CXCL16 treatment reinforcing the role of CXCR6-CXCL16 axis in metastatic progression of the disease. Conclusion: Thus, our study shows that PCa cells instigate chemotherapeutic resistance against DTX by increasing pro-survival CXCR6-CXCL16 signaling. Hence, combining CXCR6 blockade with DTX based chemotherapy would be a better treatment option for advanced and drug resistant PCa.

P24

Dual Action Potent Anticancer Small Molecule Immunotherapeutic Antagonizing Immunosupression in the Tumor Microenvironment

Erin M Kischuk, Joydeb Majumder, Jonathan Fine, Travis C Lantz, Timothy L Ratliff, Gaurav Chopra

Purdue University, West Lafayette, IN, USA

Withdrawn

P25 - Travel Awardee

Anti-cancer Activity of Novel Chalcones Against Highly-metastatic Prostate Cancer Cells

Metsiel Donate[1], Maria Sanchez, Master Degree[1], Joseph Mooney[2], Magaly Martinez Ferrer[1], David Sanabria[3]

[1]CCC UPR, San Juan, Puerto Rico; [2]Inte American University of Puerto Rico, San Juan, Puerto Rico; [3]Inter American University of Puerto Rico, San Juan, Puerto Rico

Background: Prostate cancer (PCa) is the third leading cause of cancer related death in United States. Docetaxel (Dxtl) is one of the current chemotherapeutic drugs that is used for treating PCa in US. However, due to its potential toxicity treatment with Dxtl has been relegated to metastatic castration-resistant prostate cancer patients who have progressed after androgen deprivation therapy and presented symptoms arising from the progression of the disease. Thus, there is a critical need to develop new modalities of more effective and less toxic chemotherapies. Recent studies have shown the effectiveness of natural-ocurring chalcones (e.g. Curcumin, Curc) as anticancer agents in PCa resistant to Dxtl. Moreover, it was evidenced that these compounds induced apoptosis and suppress the activity of NF-kβ, protein associated with the mechanism of resistance in PCa cells. The objective of this study is to investigate the effect of C5-Curc and IJMG-205 (synthetic analogs of Curc) in prostate cancer. Methods: The total synthesis of both C5-Curc and IJMG-205 were successfully performed in three synthetic steps. The synthesis of these compounds started with the protection of a phenolic benzaldehyde with tetrahydropyranyl protecting group in acidic condition. Subsequent Aldolic reaction of the tetrahydropyranyl protected phenolic benzaldehyde with either acetone or Boc-piperidone, and removal of the tetrahydropyranyl protecting group under mild acidic conditions afforded the desired chalcones IJMG-205 and C5-Curc in 44 and 75% yield, respectively. Chemical characterization of both C5-Curc and IJMG-205 was performed by using 1H-NMR, 13C-NMR, and FT-IR. Once these compounds were prepared, PC3 (androgen independent) and 22RV1 (androgen dependent) cell lines were treated with C5-Curc and IJMG-205. Curcumin and Docetaxel were used as control. The MTS assays were performed according to manufacturing recommendations. Results: The IC_{50} values for all compounds are summarized in the following table:
Treatment (concentrations):
IC_{50}
PC-3
22RV1
C5-Curc: (5-30 µg/mL)
24.83 µg/mL
22.70 µg/mL
IJM-205: (5-30 µg/mL)
22.89 µg/mL
17.10 µg/mL
Curc: (0.001-30 µg/mL)
13.79 µg/mL
14.26 µg/mL
Dxtl: (0.002-0.01 µg/mL)
0.009 µg/mL
0.006 µg/mL
Conclusion: Our results showed a decrease in cell viability of Curc-derived therapies C5-Curc and IJMG-205 in both cell lines when compared with their control and reduced toxicity when compared with Curc and Dxtl in normal prostate cells. Our findings clearly demonstrate that chalcones may be potential drugs in the treatment of PCa.

P26

The Co-alteration Landscape of ARID1A Mutated High Grade Bladder Cancer

Eugene J Pietzak, Victor McPherson, Sumit Isharwal, Shawn Dason, Francois Audnet, Ahmed Zehir, Niki Schultz, Michael Berger, Jonathan Rosenberg, Dean Bajorin, Hikmat Al-Ahmadie, Gopa Iyer, Bernard Bochner, David Solit

Memorial Sloan Kettering Cancer Center, New York, NY, USA

Background: Mutations in the SWI/SNF chromatin-remodeling gene ARID1A commonly occur in bladder cancer. Preliminary data on ARID1A mutations in bladder cancer suggest they are associated with higher grade and stage disease, and may be associated with treatment resistance. ARID1A mutations and other SWI/SNF alterations have been found to result in synthetic lethal vulnerabilities to inhibitors of EZH2 methyltransferase, PIK3CA, PARP, and SRC in several other tumor types, but these effects may be tissue specific or dependent on co-alteration patterns, thus we sought to investigate the co-alteration landscape of ARID1A mutated bladder cancer identify potential therapeutic vulnerabilities. Methods: Targeted exome sequencing was performed on high-grade bladders with a >300 cancer-associated gene panel. ARID1A mutated tumors were compared to wild-type tumors to identify patterns of co-occurrence and mutual exclusivity. Fisher exact test was used for comparison and Benjamini-Hochberg false discovery procedure was used to correct for multiple comparisons. Results: We identified 533 high-grade bladder tumors (390 MIBC/143 NMIBC) with SWI/SNF complex alterations occurring in 42%. Truncating mutations in ARID1A were the most common alteration found in 21%. ARID1A missense mutations occurred in 8%. ARIDB and ARID2 were both mutated in 10% of tumors, but were predominantly missense mutations of unknown significance. SMARCA4 and SMARCB1 were mutated in <3%. There was a tendency for co-occurrence between ARID1A mutations and mutations in PIK3CA, TP53, and CDKN1A but this did not reach statistical significance. When comparing ARID1A mutated tumors to wild-type for other associations on the gene panel, no mutually exclusive genetic associations were seen, but five genes were significantly co-occurring (RAD50, PIK3C3, ZFHX3, TERT promoter, and BRCA2; all FDR $q<0.05$). We then investigated these genes in the TCGA dataset (n=413) to find only RAD50 ($p=0.06$) and BRCA2 ($p=0.006$) were also enriched in ARID1A mutated tumors on Fisher exact test. Further analyzing our 533 cohort, we identified other genes in the non-homologous end joining pathway (BRCA1, PALB2) and other DNA damage repair genes (ERCC2, FANCC, ATR, CHEK2) to be more commonly altered in ARID1A mutated bladder cancer on Fisher exact test, but these did not reach significance after correcting for multiple comparisons. Conclusions: Investigating the co-alteration landscape of ARID1A mutated bladder cancer reveals several potential therapeutic targets. The high co-occurrence of DNA damage repair gene alterations suggests that ARID1A mutated bladders tumors are genomically unstable and further investigating their susceptibility to PARP inhibitors and ATR-checkpoint kinase inhibitors, among other agents, is warranted.

P27

Sox2 Expression Marks Androgen-independent Progenitor Cells in the Adult Murine Prostate

Erin M McAuley, Daniel Moline, Sophia Lamperis, Gladdell Paner, Russell Szmulewitz, Donald Vander Griend

University of Chicago, Chicago, IL, USA

Withdrawn

P28

Role of Eukaryotic Translation Initiation Factor 4 Gamma 1 (eif4g1) in Prostate Cancer

Praveen K Jaiswal, Sweaty Koul, Hari K Koul

Louisiana Health Science Center, Shreveport, LA, USA

Background: cap-dependent translation is essential to maintain high protein synthesis in rapidly dividing cancer cells. Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) participates in cap-dependent protein translation by serving as a scaffold and interacting with several other initiation factors in EIF4F complex, that recruits ribosomes to the capped end of mRNA to initiate cap-dependent translation. In the present study, we analyzed gene expression data in clinical data sets, and prostate tumor tissue in TRAMP model and commonly used Prostate Cancer (PCa) cell lines. We also evaluated the function of EIF4G1 in commonly used PCa cell lines. Methods: mRNA expression was analyzed from TCGA and Trento/Cornell/Broad clinical data sets. PCa cells LNCaP, C4-2b, 22Rv1, DU145, PC3 and normal prostate cell line RWPE-1 and TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) tissues were used in present study. Protein levels were measured by immunoblotting. siRNA was used to knock down EIF4G1. RT-PCR was used to quantitate mRNA expression level. Cell viability and proliferation was measured by MTT and Crystal violet assay. Cell cycle distribution was analyzed on BD LSR II FACS machine. Results: We observed that protein levels of EIF4G1 are higher in PCa cell lines as well as prostate tumor tissues from TRAMP mice as compared to normal prostate cells and prostate tissue from wild-type mice. Similarly, EIF4G1 was significantly ($p<0.0001$) overexpressed in tumor specimens as compared to normal tissue. We also observed a graded increase in EIF4G1 expression with increasing tumor grade (Gleason Score) from TCGA dataset. Analysis of data from Trento/Cornell/Broad clinical data set revealed overexpression of EIF4G1 in 43% tumor specimens. Knock down EIF4G1 expression in LNCaP and C4-2b cells impaired colony formation and cell proliferation. Furthermore, silencing of EIF4G1 resulted in altered expression of cell-cycle associated genes (p21 and p27) and G1 cell cycle arrest. Conclusions: Together, our results indicate that EIF4G1 may function as an oncoprotein in PCa, and as such may represent a novel and promising therapeutic target in Prostate cancer.

P29

Mesenchymal Compartmentalization of Mir-1 and Mir-143 in Prostate Tissue and Loss of Expression in Tumor-associated Stroma

Binod Kumar, Avi Rosenberg, Su Mi Choi, Karen Fox-Talbot, Angelo De Marzo, Larisa Nonn, W Nathaniel Brennen, Luigi Marchionni, Marc K Halushka, Shawn E Lupold

Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Mesenchymal-specific genes can be misinterpreted as potential tumor suppressors when relatively lower transcript levels are detected in carcinomas, as was recently discovered for miR-143/145. In light of this, we examined the epithelial and mesenchymal nature of several putative tumor suppressive and oncogenic miRNAs in human prostate cancer (PCa). Methods: We applied Expression Microdissection (xMD) and Laser Capture Microdissection (LCM) to isolate stromal and epithelial tissue from radical prostatectomy specimens. Individual miRNAs were quantified by droplet-digital RT-PCR. Bioinformatic analyses determined the correlation of miRNAs with stromal marker expression and clinical outcomes. Human prostate stromal cultures were developed and transfected with miRNA mimics/inhibitors or treated with receptor tyrosine kinase (RTK) inhibitors or ligands. Results: The expression of miR-1 and miR-143 was predominantly mesenchymal. Conversely, miR-141 was almost exclusively epithelial. The levels of miR-1 and miR-143 were significantly lower in tumor-associated prostate stroma than in normal prostate stroma. Also, stromal marker expression directly correlated with miR-1 and miR-143 in the TCGA-PRAD, while miR-141 was inversely correlated. Reduced miR-1 and elevated miR-21 were associated with biochemical recurrence. In stromal cultures, miR-143 was stimulated by RTK inhibitors and suppressed by their ligands. Conclusions: We demonstrate that miR-1 and miR-143 are predominantly stromal miRNAs, thus challenging their roles as prostate cancer tumor suppressors. We further reveal that the levels of miR-1 and miR-143 are diminished in tumor associated stroma, implicating a new role for these miRNAs in the tumor microenvironment. We further show that reduced miR-1 and elevated miR-21 are associated with biochemical recurrence, and that miR-21 levels are associated on top and beyond Gleason grade and stage in multivariate analysis. This data suggests that miRNAs in tumor associated stroma may provide prognostic information for PCa and miRNA-targeted therapy must consider cell-type specificity of miRNA expression.

P30

Chromosomal Inequality Causes the Sex Disparities in Bladder Cancer Through a KDM6A-dependent Epigenetic Mechanism

Satoshi Kaneko, Xue Li

Boston Children's Hospital, Boston, MA, USA

Background: The sex chromosome complement differs between sexes but whether such fundamental difference contributes to the gender disparities in cancer remains to be determined. Methods: We used BBN-induced mouse BCa model, which is coupled with the "four core genotype (FCG)" mouse model consisting wild type XY male (XYM), XX female (XXF), the complete sex reversed XY female (XYF) and XX male (XXM), and the urothelium-specific Kdm6a condition knockout mice. In addition, the Cancer Genome Atlas (TCGA) BCa data sets were also used to determine the effects of human *KDM6A* in BCa progression and prognosis. Results: Regardless of sex hormones, an extra copy of X chromosome leads to a better protection against BCa via the KDM6A dependent epigenetic mechanism. *KDM6A* is expressed in significantly higher levels in the XX than XY urothelial cells. Both wild type and the catalytically-dead *KDM6A* inhibit BCa cell proliferation and anchorage-independent growth. Conversely, conditional knockout of mouse *Kdm6a* significantly increases BCa risk among females. *Cdkn1a* and *Perp,* the key *Tp53* gene targets that inhibit cell cycle and promote apoptosis, respectively, are significantly down regulated in *Kdm6a* mutants. Expression of *Cdkn1a* but not *Perp* depends on *Kdm6a* demethylase activity. Furthermore, low levels of *KDM6A* are tightly linked to the advanced stages of human BCa; and KDM6A mutations and reduced expression predict poor outcomes of the disease-free survival among female BCa patients. Conclusions: The chromosomal inequality is an intrinsic cause of the gender disparities in BCa due in part to the *KDMA6A* demethylase activity-dependent and -independent mechanisms. These findings provide compelling evidence explaining why men are much more likely than women to have cancer, and further suggest that the preventative strategies of targeting the epigenome may reduce cancer risk in males.

P31

Genomic Alterations in the cfDNA of Castrate Resistant Prostate Cancer Patients

Elisa M Ledet, Joshua Schiff, Bryce Christensen, Marcus Moses, Lynne Chapman, Ashkan Shahbandi, Peter Steinwald, Patrick Cotogno, Jodi Layton, Brian Lewis, Oliver Sartor

Tulane University, New Orleans, LA, USA

Background: Cell free DNA (cfDNA) present in plasma of cancer pts can reflect tumoral alterations. Genomic alterations in cfDNA may be associated with prognosis and selected AR alterations are associated with abiraterone/enzalutamide resistance in CRPC. The goal of this evaluation was to characterize AR amplifications (Amps) and somatic point mutations (Muts) detected in CRPC cfDNA and to relate those changes to non-AR alterations detected in the cfDNA landscape. Methods: A heterogenous group of 121 metastatic CRPC patients (pts) with evidence of clinical progression from Tulane Cancer Center underwent cfDNA analysis using Guardant 360 test (Guardant Health, Redwood City, CA). 50 of these patients were tested multiple times during treatment. This evaluation included full exonic coverage of 70 genes and amplifications in 18 genes. Mutations reported herein include known pathogenic mutations as well as variants of unknown significance. Results: 52.9% (n=64) of the mCRPC pts evaluated had an AR alteration. Of the pts with AR alterations, 43.8% (n=28) had AR Amps, 37.5% (n=24) had AR Muts, and 18.8% (n=12) had both. In addition to amplifications, 19 different AR Muts were detected. AR Muts included: T878A (n=15), H875Y (n=8), W742C (n=11), AR L702H (n=7), and others. AR alterations comprised on average about 7% of tumoral cfDNA. To better understand the relationship between AR alterations and other commonly detected cfDNA aberrations, associations between BRAF (27.3%), TP53 (44.6%), and MYC (19.0%) and AR were assessed. Among these genes, TP53 alterations were all Muts. MYC (n=21) and BRAF (n=27) alterations were predominantly Amps though Muts were also detected in MYC (n=3) and BRAF (n=9). TP53 Muts were not significantly associated with AR alterations. BRAF and MYC alterations significantly associated with AR alterations (p= 0.0012 and p= 0.0223). Pts were re-tested upon disease progression; among these patients, 52.2% (n=24) had an increase in overall mut burden. Conclusions: AR alterations in cfDNA impact both disease progression and response to therapy. Co-segregation of AR, BRAF, and MYC alterations may have significant prognostic and therapeutic implications. Further research and larger sample size are needed to further elucidate associations between disease progression, treatment response, and the development of somatic alterations over time in CRPC.

P32 - Travel Awardee

Mesenchymal Stem Cells Promote Osteogenesis and the Evolution of Apoptosis Resistant Bone Metastatic Prostate Cancer

Jeremy J McGuire[1], Leah Cook[2], Jeremy Frieling[2], Conor Lynch[2]

[1]Tumor Biology/Cancer Biology PhD Program, Moffitt Cancer Center/University of South Florida, Thonotosassa, FL, USA; [2]Moffitt Cancer Center/University of South Florida, Tampa, FL, USA

Introduction: Bone metastatic prostate cancer is incurable and hallmarked by areas of extensive osteoblastic bone formation. Bone is a natural reservoir for mesenchymal stem cells (MSCs) that give rise to multiple cell types including osteoblasts. Our *in vitro* data show that prostate cancer cells recruit MSCs and that human and murine bone metastatic prostate cancer specimens have significant MSC numbers compared tumor naïve controls. We therefore hypothesized that MSCs could contribute to the prostate cancer growth and associated bone formation. Methodology: Isolated murine MSCs were characterized by flow (CD29+/SCA1+/CD45-) and differentiation assays. *In vivo*, mice (n=8/group) were intratibially inoculated with MSCs (2×10^4), PAIII prostate cancer cells (2×10^4), or a 1:1 ratio (MSCs:PAIII). Bone disease was measured via X-ray and μCT and tumor growth by cleaved caspase3 (CC3) and phospho-histone H3 (pHH3) immunofluorescence. Cytokine arrays were used to identify MSC derived apoptotic factors while receptor and signaling pathways were assessed by immunoblot. IL-28 receptor was ablated via shRNA. STI-201 was used to determine STAT3 dependency. Results: *In-vivo*, MSCs suppressed PAIII growth until day 11 ($p<0.05$), but subsequently, PAIII growth increased significantly compared to controls. We also noted increased bone formation in MSC injected groups. *In vitro* co-culture and conditioned media studies demonstrated MSCs promoted PAIII apoptosis within 5-hours. Education of PAIII with successive exposures to MSC CM *in vitro* yielded PAIIIs (F2 PAIII) that were resistant to MSC CM, etoposide and docetaxel induced apoptosis. These observations were confirmed with DU145 and Myc-CaP cell lines. Subsequent *in vivo* studies (n=10/group) demonstrated that F2-PAIII grew significantly faster in combination with MSCs compared to controls. Cytokine array analysis revealed MSC derived IL-28 as a potential apoptosis mediator. IL-28 depletion from MSC CM or IL-28 receptor shRNA knockdown decreased MSC induced apoptosis. We observed no difference in IL-28 receptor expression between MSC naive and educated prostate cancer cells but a difference was detected in pSTAT3 and pSTAT1 signaling in MSC educated cancer cells that was reflected in their sensitivity to the STAT3 inhibitor, STI-201. Conclusions: Bone marrow MSCs initially repress prostate cancer growth through IL-28 exposure that ultimately promotes the evolution of apoptotic resistant cancer cells that are resistant to chemotherapy. However, MSC educated prostate cancer cells are sensitive to STAT3 inhibition. Bjartell et al (PMID: 27344294) have shown that 95% of human bone metastatic prostate cancers are positive for pSTAT3 providing rationale for the exploration of STAT3 inhibitors to treat this incurable disease.

P33

Activated Gli is the Primary Driver of Prostate Cancer Cell Growth

Na Li, Sarah Truong, Mannan Nouri, Jackson Moore, Ralph Buttyan

The Vancouver Prostate Centre, Vancouver, BC, Canada

Background: Androgens regulate the growth of prostate cancer (PCa) cells. These steroids act through the androgen receptor (AR) protein, so classically, AR is felt to be the primary driver of PCa cell growth. Recently, we showed that transcriptionally-active ARs (liganded full-length and C-terminal truncates) bind to Gli transcription factors at their C-terminal protein processing domain and that this binding blocks the proteolytic degradation process that converts active Gli proteins into Gli repressors. As a consequence, AR binding to Gli2/Gli3 maintains them in a high molecular weight active form. Our studies of this non-canonical Gli activation process now indicate that Gli, instead of AR is the primary driver of androgen-dependent (AD) and -independent (AI) PCa cell growth and show that we can uncouple AR's transcriptional activities from its effects on PCa cell growth. Methods: We tested small molecule Gli inhibitors (GANT61 and HPI-1), exogenously-delivered Gli2-derived decoy peptides (that compete with AR and displace endogenous Gli from the AR complex) and knockdown (KD) by Gli3/Gl2 siRNA for their effects, in vitro, on growth of various AD and AI PCa cells. Relative growth was measured by the Cyquant assay (indexes DNA content) or on the Incucyte cell quantification system that visually determines relative cell numbers over time. Expressions of AR and Gli proteins were assessed on Western blots and expressions of mRNA encoding known AR target genes or genes associated with cell growth were determined by real-time qPCR. Results: Gli inhibitors strongly suppressed growth of AD and AI cells at concentrations commensurate with their inhibitory effects on Gli-mediated transcription without any effect on AR or Gli3 expression. These inhibitors, however, increased the expression of known AR target genes (KLK3 and KLK2) while suppressing expressions of growth-related genes. Gli2-derived decoy peptides also suppressed growth of AD/AI cells; did not affect AR protein levels and also increased expressions of AR target genes. Finally, Gli3 knockdowns strongly suppressed growth in AD/AI $Gli3^{Hi}$ cells but not in $Gli2^{Hi}$ cells. Here, Gli3 knockdown reduced AR protein levels through a protein destabilization process and both standard AR targets and growth-inducing genes were repressed. Conclusions: AR-dependent activation of Gli in PCa cells is linked to cell growth and blockade of Gli activity without suppression of AR activity is sufficient to suppress the growth of PCa cells. These studies suggest that Gli, not AR, is the primary driver of PCa cell growth.

P34

Fibroblast Heterogeneity and Inflammatory Cell Recruitment in Prostate Cancer

Nadia M Attalah[1], Renee E Vickman[2], Omar E Franco[2], Timothy L Ratliff[1], Simon W Hayward[2]

[1]Purdue University Center for Cancer Research, West Lafayette, IN, USA; [2]Northshore University Health System, Evanston, IL, USA

Background: Carcinoma associated fibroblasts (CAF) are a heterogeneous group of cells in the tumor microenvironment that can promote tumorigenesis in the prostate, presumably via juxtacrine and paracrine interactions. The full extent of heterogeneity in CAF is not presently described and its consequences are not understood. A detailed description of the prostate tumor microenvironment could be a critical component in defining indolent versus aggressive disease and should also suggest novel therapeutic and disease stabilizing pathways. Methods: In vivo testing of tissue recombinants generated with CAF or normal fibroblasts was performed and the resultant tumors analyzed using FACS to assess the effects of the CAF on inflammatory cell recruitment. Fluidgm's C1 integrated fluidics system was used to generate single cell cDNA libraries and RNAseq performed to establish human prostate CAF phenotypes on an individual cell basis. Bioinformatic analysis was performed to derive principal component analysis and cell clustering. Further analysis of the gene expression profiles was performed to determine how the various clusters of cells could potentially communicate between themselves and with other cells, notably with the immune/inflammatory network. Results: In vivo analysis demonstrated that CAF were able to drive tumorigenesis in a reporter epithelium (per many previous studies). FACS demonstrated that compared to normal human prostate fibroblasts, the presence of CAF resulted in increased recruitment of myeloid cells including macrophages and granulocytes. The scRNAseq analysis using unsupervised clustering approaches, revealed five subpopulations or clusters of CAF based upon gene expression profiles. Further analysis of these suggested interactions with immune/inflammatory cells, for example, one cluster expresses high levels of CCL2, which is involved in macrophage recruitment. Another large cluster expresses CCL11, known to be involved in chemoattraction of eosinophils. Eosinophils, in turn, express IL-4 which plays a role in driving expression of the type II macrophage (M2) phenotype. Conclusions: CAF fall into a relatively small number of distinct sub-populations. This makes analysis of the specific roles of these groups feasible and open to biological and mathematical modeling. The clusters express extracellular mitotic and chemotactic signaling molecules that may be involved in the recruitment of inflammatory cells as well as in direct growth regulation of the tumor by promoting formation of an M2-dominant microenvironment.

P35

PEDF Acts as a Bridging Protein in a Novel Lipid-centrosomal Signaling Axis in Prostate Cancer-associated Fibroblasts

Susan E Crawford, Francesca Nardi, Philip Fitchev, Omar E Franco, Lijun Huang, Jelena Ivanisevic, Adrian Scheibler, Simon W Hayward

Northshore University Research Institute, Evanston, IL, USA

Background: Cancer cells in the tumor microenvironment (TME) make metabolic adaptations to ensure a continuous source of energy and amplify cell cycle regulators such as centrosomes to sustain their high proliferative capacity. Lipid homeostasis can become dysregulated in obesity when non-adipocytes acquire ectopic lipid or when there is a signaling blockade of triacylglycerol (TAG) lipolytic proteins such as pigment epithelium-derived factor (PEDF). Centrosomes accelerate growth of cells by altering microtubule assembly and cell cycle checkpoints. It is not known whether cancer associated fibroblasts (CAFs) acquire defects in lipid signaling and/or centrosomes. We postulated that CAFs increase intracellular lipid content by altering TAG lipolytic proteins to fuel proliferation and that this metabolic switch directly promotes centrosomal amplification. Methods: CAFs derived from prostate cancer tumor specimens were analyzed for TAG regulatory proteins, PEDF, ATGL, CGI-58, and G0/G1 switch gene 2 (GOS2) by Western blot and immunofluorescence (IF). These data were compared to normal fibroblasts (NFs). Centrosomes were analyzed by staining for pericentriolar matrix protein, pericentrin, and quantified by IF. CAFs were treated with recombinant PEDF protein to test the function of protein on centrosomal number and lipid content. Lipid droplets (LDs) containing neutral lipid were assessed using Oil-Red-O and Bodipy stains. Results: NFs expressed high levels of PEDF protein and contained endogenous neutral lipid stored in LDs that encircled the perinuclear region. As expected, they had one or two centrosomes close to the nucleus, denoted by positive pericentrin staining. In striking contrast to NFs, CAFs expressed little to no PEDF protein and demonstrated significantly more LDs diffusely dispersed throughout the cytoplasm (NF 66.79 ± 6.77 vs. CAF 188.5 ± 17.82; $p<0.001$). Additionally, centrosomal amplification was evident in both the perinuclear and peripheral regions of the cells (NF 1.17 ± 0.064 vs. CAF 69.12 ± 10.57; $p<0.01$). Restoration of PEDF in CAFs normalized the centrosome number and lipid content and PEDF co-localized with centrosomal protein, pericentrin. Conclusions: These data suggest the existence of a novel lipid-centrosome signaling axis in CAFs. A deficiency in PEDF appears to be one mechanism for the pro-lipogenic and centrosomal phenotype in CAFs. The dual functions of PEDF bridge lipolysis and centrosomal amplification, possibly by acting as a metabolic-sensing centrosomal matrix protein. Restoration of PEDF in CAFs could represent a new targeted approach to normalize lipid homeostasis and centrosomal amplification in the TME and suppress tumor growth.

P36

Nuclear Factor I/B Interacts with Androgen Receptor to Regulate Response to Anti-androgens

Wisam N Awadallah[1], Robert J Matusik[2], Magdalena M Grabowska[1]

[1]Case Western Reserve University, Cleveland, OH, USA; [2]Vanderbilt University Medical Center, Nashville, TN, USA

Background: Androgen receptor (AR) is the therapeutic target in prostate cancer. Current strategies target the ligand binding domain of AR, either through direct competition with androgens or through inhibition of androgen synthesis. Unfortunately, these targeting strategies are less effective in prostate cancer patients whose tumors express AR splice variants (AR-Vs), which lack the ligand binding domain and are therefore constitutively active. Our studies have focused on identifying AR cofactors that can regulate both full length AR (AR-FL) and AR-Vs in order to identify novel therapeutic targets. Previous studies determined that nuclear factor I/B (NFIB) represses AR-regulated gene expression and is frequently associated with genomic AR and FOXA1 binding sites in an androgen dependent prostate cancer cell line (LNCaP). Our current studies focused on determining the role of NFIB in the AR/FOXA1 complex in castrate resistant prostate cancer, where AR-Vs are present. Methods: We used co-immunoprecipitation studies in JEG-3 cells, which do not express AR-FL, AR-V7, FOXA1, or NFIB to determine whether FOXA1 was required for NFIB interaction with AR-FL or AR-V7. To determine the role of NFIB in castrate resistant prostate cancer, we generated NFIB overexpressing castrate resistant (C4-2B) prostate cancer cell lines and evaluated changes in cell proliferation, prostate specific antigen enhancer (PSAE)-reporter activity, and gene expression in response to dihydrotestosterone (DHT) and the anti-androgens enzalutamide and bicalutamide. Results: Our studies demonstrated that NFIB can interact with both AR-FL and AR-V7, one of the more prominent AR-Vs, independently of FOXA1. The overexpression of NFIB resulted in increased sensitivity to the anti-androgens bicalutamide and enzalutamide in proliferation assays, but did not impact response to DHT at physiologic levels. In reporter assays, NFIB overexpression did not modulate response to DHT, but NFIB overexpression did decrease reporter gene construct expression in response to enzalutamide. Total *AR* gene expression was decreased in response to enzalutamide treatment in C4-2B-NFIB cells versus C4-2B-Vector. However, AR-target gene expression was unaffected (*NKX3-1, FKBP5)* or even increased (*TMPRSS2, KLK3)* in response to NFIB overexpression and enzalutamide treatment. Conclusions: Our studies demonstrate that NFIB overexpression sensitizes C4-2B cells, which are castrate resistant, to the anti-androgens bicalutamide and enzalutamide, likely by decreasing total *AR* gene expression. Subsequent studies are evaluating how NFIB overexpression drives increased expression of *KLK3* and *TMPRSS2* in the presence of enzalutamide.

P37

Thrombospondin-1 Regulates Lipolytic Activity in Prostate Cancer Cells

Susan Wcislak[1], Ayesha Chawla[1], Nizar Khamjan[1], Beth A Plunkett[2], Jennifer A Doll[1]

[1]University of Wisconsin Milwaukee, Milwaukee, WI, USA; [2]Northshore University Health System, Evanston, IL, USA

Background: Thrombospondin-1 (TSP-1) is a multifunctional secreted glycoprotein. In prostate cancer (PCa) tissues, TSP-1 expression is decreased. In mice, TSP-1 knockout induces prostatic hyperplasia, characterized by increased proliferation and microvessel density. Our lab has previously observed that feeding TSP-1 KO mice a high fat diet induced progression of the hyperplasia to cancer. One characteristic of this phenotype was increased lipid accumulation. To investigate this, we tested the activity of TSP-1 on regulation of lipid metabolism in PCa cells in vitro. Methods: Normal prostate epithelial cells (RWPE-1) and PCa cells (LNCaP, PC-3 and DU145) were plated at 20,000 cells/cm^2, allowed to attach for 24 h, serum-starved for 4 h, and then treated with oleic acid (1 mM), ± TSP-1 (1-20 nM), in serum-free media for 48 h. Serum-free conditioned media and cell lysates were collected. Lipid content was assessed using Oil R O staining of cells grown on chamber slides. The free glycerol assay (Sigma) was used to quantify lipolytic activity. Pigment-epithelium derived factor (PEDF), a known lipolytic activator, was quantified by ELISA. Values were normalized to cell numbers or protein content. Dose response was assessed by ANOVA and pair-wise comparisons by Student t-test with a *P*-value of ≤0.05 considered significant. Results: In RWPE-1 cells, TSP-1 treatment showed a decreased trend, but was not significant. In LNCaP cells, surprisingly, TSP-1 treatment increased cell numbers. In PC-3 cells, while TSP-1 slightly stimulated proliferation at 5 nM, at 10 nM, proliferation was inhibited. Lastly, in DU145 cells, TSP-1 inhibited proliferation. With lipolytic activity, TSP-1 treatment increased lipolytic activity of RWPE-1, LNCaP and PC-3 cells, while in DU145 cells, TSP-1 treatment suppressed lipolytic activity ($P<0.04$). By oil red O staining, TSP-1 appeared to change the distribution of lipids within PC-3 and DU145 cells; however, no quantitative measure of this has currently been conducted. Overall, in RWPE-1, LNCaP and PC-3 cells, TSP-1 treatment also increased PEDF expression in both the conditioned media (secreted PEDF) and in the cell lysate (cellular PEDF). In DU145 cells, however, while secreted PEDF levels were increased, cellular levels of PEDF decreased. Conclusions: These data are consistent with a direct functional role for TSP-1 in regulating lipid metabolism in prostate cells. The data also suggest that up-regulation of PEDF expression may be one mechanism through which TSP-1 increases lipolytic activity. Thus, loss of TSP-1 in the tumor microenvironment may impact lipid metabolism pathways, facilitating pro-tumorigenic lipid metabolism.

P38

Novel Race-specific Genetic and Epigenetic Determinants of Enzalutamide-resistance in Prostate Cancer

Arsheed A Ganaie[1], Tabish Hussain[1], Marina Ferrari[1], Matteo Astone[2], Luke Hoeppner[1], Todd Schuster[1], Paari Murugan[3], Badrinath R Konety[3], Mohammad Saleem[2]

[1]Hormel Institute, University of Minnesota, Austin, MN, USA; [2]Hormel Institute, University of Minnesota, Austin, MN, USA; [3]University of Minnesota, Minneapolis, MN, USA

Background: Enzalutamide has recently been introduced in clinics to treat castration-resistant prostate cancer (CRPC) condition, however has failed to significantly improve the overall survival in patients. Similar to bicalutamide, Enzalutamide therapy is reported not to be significantly beneficial in blocking emergence of refractory CRPC in high-risk patients such as in African-American (AA) men who exhibit (i) aggressive prostate cancer (PCa), (ii) worse survival than Caucasians (CA), and (iii) poor response to ADT. Intrinsic genetic differences play an important role in race-related PCa disparities, and there is no concrete data available explaining Enzalutamide-therapy failure in AA PCa patients. Methods: In this study, we developed genetically distinct Enzalutamide-resistance cell models representing PCa in CA and AA men, and studied their characteristics in terms of proliferation, prostatosphere formation, *in vivo* metastasis in a transgenic zebra-fish model and responsiveness to Enzalutamide therapy in athymic mouse model. Results: We show that Enzalutamide-therapy increases the enrichment of cancer stem (S/P) and epithelial-mesenchymal-transition (EMT) cell populations. We provide evidence that short-term and long-term (4-months) of Enzalutamide-therapy results in the induction of AR expression in resistant-Caucasian (LNCaP95-$_{Enz}$) model, whereas no effect on AR-pathway is observed in resistant AA models (RC-77T/E-$_{Enz}$ and E006AA-ht-$_{Enz}$). Because AKT pathway has been shown to drive CRPC in ADT-resistant cases, we tested efficacy of AKT-inhibitor therapy in Enzalutamide-resistant AA and CA models. Whereas Enzalutamide-resistant CA models were responsive to AKT-inhibitor therapy, AA models exhibited non-responsiveness. To understand the exact molecular mechanism of drug-resistance induced by Enzalutamide, we hypothesized that this phenomenon could be associated to the aberration of molecules (at genetic and epigenetic levels). We performed RNA-seq analysis to study global gene expression, mircoRNA and LncRNA expression profile in Enzalutamide-responsive and Enzalutamide-resistant AA PCa models. We show that non-AR mediated genetic and epigenetic mechanisms play significant role in drug-resistance in AA PCa. We established an AA-specific molecular signature of 2 genes; 3 micro-RNA's and 1 LncRNA. The data was validated in cell models and tumor specimens (primary and METS) of CA and AA patients. We identified BAMBi as a lead biomarker that differentiates responsive-PCa e from Enzalutamide-resistant PCa in AA men, and conducted functional studies to validate its significance as a therapeutic targe. Conclusions: To summarize, our study provides detailed information about the changes in genetic and epigenetic landscape which occur during the PCa progression and form the basis of novel race-specific biomarkers of Enzalutamide-resistance and therapeutic targets in AA PCa patients.

P39

Concurrent Deletion of Eaf2 and Ell2 Induces Murine Prostate Intraepithelial Neoplasia

Laura E Pascal, Mingming Zhong, Uma Chandran, Khalid Masoodi, Yachen Zang, Lora H Rigatti, Zhou Wang

University of Pittsburgh, Pittsburgh, PA, USA

Introduction and Objectives: Elongation factor, RNA polymerase II, 2 (ELL2) is an RNA Pol II elongation factor with functional properties similar to ELL that can interact with the prostate tumor suppressor EAF2. Loss of either ELL2 or EAF2 could enhance proliferation and migration in prostate cancer cell lines, and *EAF2* and *ELL2* gene expression are down-regulated in high Gleason score prostate cancer specimens. Here, prostate-specific deletion of Ell2 and conventional deletion of Eaf2 in a mouse model revealed a potential role for interaction between EAF2 and ELL2 in prostate tumor suppression. Methods: Transcriptomes from prostates of *Ell2* knockout and *Eaf2* knockout mice on a C57BL/6J background were compared to identify genes potentially regulated by both EAF2 and ELL2. Mice with combined deletion of *Eaf2* and *Ell2* were generated and examined for histological defects. Results: Combined deletion of *Eaf2* and *Ell2* induced high grade prostatic intraepithelial neoplasia. Microarray analysis revealed several differentially expressed genes that were similarly altered in *Ell2* knockout and *Eaf2* knockout mice. These genes were associated with proliferation, cellular motility and epithelial and neural differentiation. Conclusions: These results suggest that EAF2 and ELL2 may play interacting roles in the maintenance of prostate homeostasis. This work was funded in part by NIH grants R01CA186780, P50 CA180995, T32 DK007774, and 1R50 CA211242 (LEP) and scholarships from the Tippins Foundation (LEP). The microarray work was performed in the Genomics Research Core at the University of Pittsburgh. This project used the UPCI Animal Facility and was supported in part by award P30CA047904.

P40

PDEF Inhibits Prostate Cancer Metastasis by Promoting Luminal Differentiation

Fengtian Wang[1], Sweaty Koul[2], Prakash Srinivasan Timiri Shanmugam[2], Qin Dong[2], Hari K Koul[2]

[1]Louisiana State University Health Sciences Center-Shreveport, Shreveport, LA, USA; [2]Louisiana State University Health Sciences Center-Shreveport, Shreveport, LA, USA

Background: The emergence of metastatic castration resistant prostate cancer (PCa) is accompanied by the loss of prostate luminal cell identity. Transcription factors by association with enhancer and super-enhancer elements are key drivers of cell identity. Our previous studies have shown that Sam Pointed Domain Ets Transcription Factor a.k.a. Prostate Derived Ets Factor (SPDEF/PDEF), inhibits tumor metastasis *in vivo* and there is a graded loss of PDEF protein expression with increasing Gleason Score in PCa patient samples. PDEF has been reported to be one of the super enhancer associated transcription factors in the luminal PCa cell line LNCaP and it is highly expressed in prostate luminal cells. We propose that PDEF functions as a putative tumor metastasis suppressor and inhibits tumor progression by inducing luminal differentiation. The present study was designed to investigate the role of PDEF in metastatic PCa progression. Methods: PC3 cells were stably transfected with PDEF/control pBABE retroviral vectors. Global gene expression changes were probed using Affymetrix microarray. Microarray were analyzed with gene set enrichment analysis. qRT-PCR was performed to confirm gene expression and immunohistochemistry, immunofluorescence and immunoblot were performed to visualize protein expression. ChIP-seq data were extracted from SRP002475 and aligned with Bowtie. Peaks were identified by MACS2. Clinical data were extracted from GSE16560, GSE21034 and TCGA database. Statistical analysis was performed with GraphPad. Results: Data analysis from multiple clinical prostate cancer cohorts suggests the loss of PDEF is associated with tumor metastasis, tumor progression, and poor survival. Analysis of our microarray studies revealed that PDEF expression resulted in the negative enrichment of metastasis related gene sets and the positive enrichment of gene sets involved in luminal differentiation. Especially, PDEF restores canonical AR signaling in PC3 cells. Confirmed with qPCR, IB and IF, PDEF also inhibits the expression of neuroendocrine related genes, stemness related genes while promoting the expression of prostate luminal differentiation related genes. However, No PDEF binding peaks were associated with the regulatory elements of EMT transcription factors in ChIP-seq data. However, our analysis revealed a novel binding site of PDEF at the putative promoter region of prostate luminal cell marker cytokeratin 18. Furthermore, PDEF promotes the expression of cytokeratin 18 *in vitro* and *in vivo*. Conclusions: PDEF expression leads to luminal differentiation phenotype in advanced prostate cancer cells. PDEF inhibits cell migration and metastasis in part by down-regulating EMT related transcription factors and directly promoting the expression of cytokeratin 18.

P41

Members of the Transcription Factor Activator Protein 2 Family are Markers of the Basal Molecular Subtype of Human Bladder Cancer and Repressed by Peroxisome Proliferator Activated Receptor Gamma

Hironobu Yamashita[1], Zongyu Zheng[1], Lauren Shuman[1], Truc Tran[1], Osei-Amponsa Vasty[1], Joshua Warrick[1], Jay Raman[2], Trevor Williams[3], David Degraff[1]

[1]The Departments of Pathology and Surgery, Division of Urology, Penn State Hershey, Hershey, PA, USA; [2]The Division of Urology, Penn State Hershey, Hershey, PA, USA; [3]The University of Colorado Medical Center, Aurora, CO, USA

Background: Recent molecular analysis identified luminal and basal gene expression subtypes of human bladder cancer with prognostic significance. We were the first to report that loss of the luminal transcription factor, FOXA1 is associated with poor clinical outocme in bladder cancer, and that FOXA1 cooperartes with GATA3, and PPARG to reprogram human basal bladder cancer cells into cells expressing a luminal gene expression pattern. Interestingly, RNA-seq analysis showed that expression of the transcription factors TFAP2A and TFAP2C, which is reportedly involved in squamous differentiation, are repressed following PPARG activation. Therefore, in this study, we investigated the role of TFAP2A/TFAP2C in basal human bladder cancers. Methods: We performed computational data analysis using TCGA data set to determine if TFAP2A and/or TFAP2C expression is correlated with gene expression subtype. Therefore, we performed immunohistochemistry (IHC) of a tissue microarray consisting of over 200 clinical bladder cancer samples to determine the extent to which expression of TFAP2A and TFAP2C correlated with SqD, and clinical attributes of aggressive disease. Western blotting analysis was performed to investigate the expression of TFAP2A and TFAP2C in human bladder cancer cell lines. Furthermore, we performed tissue recombination xenografting using TFAP2C-overexpressing human bladder cancer cell to assess *in vivo* tumorigenicity. Results: Computational analysis shows increased expression of both TFAP2A and TFAP2C are significantly associated with a basal gene expression subtype in human bladder cancer ($p<0.01$). In addition, immunohistochemistry (IHC) of human bladder cancer tissue revealed TFAP2A expression is significantly associated with SqD and lymph node metastasis, while TFAP2C expression was significantly associated with SqD and distant recurrence. Western blotting analysis of 10 commonly used human bladder cancer cell lines showed that TFAP2A and TFAP2C is highly expressed in cells identified previously identified by us as robust models of basal bladder cancer. Activation of the driver of luminal gene expression and PPARG by rosiglitazone induced the significant reduction in TFAP2A expression in SW780, UMUC1 and 5637 cell lines. Moreover, reductions in TFAP2A expression following rosiglitazone treatment was prevented following treatment with a PPARG antagonist. Finally, tissue recombination xenografting using T24 and UMUC3 cells stably overexpressing TFAP2C enhanced tumor formation *in vivo*. Conclusions: TFAP2A and TFAP2C expression is significantly associated with the presence of a basal molecular subtype, SqD, and prognostic indicators of poor clinical outcome such as lymph node metastases and distant recurrence. In addition, TFAP2A is a PPARG repressed gene and overexpression of TFAP2C increases *in vivo* tumorigenicity.

P42

A Better Alternative for Prostate Cancer Diagnosis by Using Organic Compounds in Urine

Qin Gao[1], Xiaogang Su[1], Heinric Williams[2], Thomas Prince[2], Brielle R Schreiter[2], Wen-Yee Lee[1]

[1]University of Texas at El Paso, El Paso, TX, USA; [2]Urologic Oncology Geisinger Medical Center, Danville, PA, USA

Background: Prostate cancer (PCa) is the 3rd most common cause of male cancer mortality in the US. The limitation of prostate specific antigen (PSA) in PCa screening has resulted in an intense search for more reliable biomarkers. Studies showed that dogs could differentiate PCa patients from negative control by sniffing their urine. As the odor profiles are generated by volatile organic compounds (VOCs), the aim of this study was to identify PCa-specific VOCs in urine for PCa diagnosis. Methods: The study included 74 men who presented for transrectal ultrasound guided prostate biopsy for an elevated serum PSA (>2.5 ng/mL) or abnormal digital rectal exam. Of the 74 men, 33 were diagnosed with PCa, and their PSA levels ranged from 2.66 to 1987. VOCs in urine were analyzed by Gas Chromatography/Mass Spectrometry, and were identified based on their occurrence and relative quantity in the urine. Potential PCa-specific VOCs were screened by Wilcoxon rank-sum tests. Regularized logistic regression was applied to develop VOC based models for PCa diagnosis. Results: We identified 37 VOCs associated with PCa positive urine samples and 45 VOCs corresponding to PCa negative ones (Wilcoxon rank-sum test, $p<0.05$). After further selection by l1 regularization, 15 VOCs were included in the final logistic model. By cross-validating the predicted probabilities for the model, the area under the receiver operating characteristic curve (AUC) was 0.943, indicating a highly discriminative power of urinary VOCs in PCa. When based on PSA alone (available in 54 men), the AUC was only 0.553, and the sensitivity and the specificity were 0.47 and 0.71 respectively (cutoff point at 0.5), showing a poor discrimination performance. In comparison, we used the same sample pool to validate the aforementioned 15-VOC logistic model. Its AUC is 0.967 with the sensitivity and the specificity at 0.85 and 0.86, respectively. Among those selected 15 VOCs, several are involved in the fatty acid, serine, glycine and sarcosine metabolic pathways, all of which have been shown to be relevant in PCa pathogenesis. Conclusions: This study demonstrated the feasibility of using VOCs in PCa diagnosis. The VOCs found in this study will be further evaluated for their specific roles in PCa biology.

P43

Endodermal *Dnmt1* Maintains an Endoderm-Mesoderm Junction in the Developing Urogenital Tract

Diya Binoy Joseph, Anoop Chandrashekar, Lisa L Abler, Chad M Vezina

University of Wisconsin-Madison, Madison, WI, USA

Background: The urogenital sinus-nephric duct junction is an endoderm-mesoderm junction that separates the endodermal urethra and bladder from the mesodermal nephric ducts. The two epithelial compartments flanking this junction maintain their distinct molecular identities and morphology despite their close apposition. The mechanisms by which this germ layer boundary is maintained and how it enforces lineage restriction are unknown. Here, we tested the hypothesis that DNA methylation through DNA methyltransferase-1 (*Dnmt1*) maintains the urogenital tract endoderm-mesoderm junction and restricts the lineages of cells flanking this junction. Although dynamic changes in *Dnmt1* expression have been previously identified, the role of *Dnmt1* in regulating urogenital tract development has been largely unexplored. Methods: The Cre-lox approach was used to specifically ablate *Dnmt1* in the endoderm layer from which the bladder, prostate and urethra derive. Cre-inducible lineage reporters were used to assess the fate of endodermal cells that have lost DNMT1 expression. *In situ* hybridization and fluorescent immunohistochemistry were used to investigate the molecular changes that occurred with *Dnmt1* ablation. Results: Conditional ablation of endodermal *Dnmt1* in mouse embryos resulted in DNA damage-induced, P53-mediated apoptosis and inappropriate differentiation of urethra and bladder epithelium. Prostate bud count and thickness of bladder epithelium was significantly reduced in conditional *Dnmt1* mutants compared to controls. We present evidence that *Dnmt1* ablation causes structural and molecular changes in the endodermal compartment that may be permissive for endoderm-mesoderm junction breach. Endodermal *Dnmt1* ablation resulted in the uni-directional movement of mesodermal PAX2/PAX8 positive cells from the nephric duct across the endoderm-mesoderm junction and into the urethra and bladder. Displaced mesodermal cells gradually acquired the endoderm marker FOXA1 and bladder markers Keratin 5, P63 and Uroplakin during movement into the bladder. Conclusions: Here, we provide evidence that *Dnmt1* expression is required for endodermal tissue health and maintenance of the endoderm-mesoderm junction. Breakdown of the endoderm-mesoderm junction allows movement of mesodermal cells from the nephric duct into the urethra and bladder epithelium. We present the unexpected finding that displaced mesodermal cells in the urogenital tract can be reprogrammed to acquire characteristics of endodermal bladder tissue. The replacement of injured bladder epithelium with reprogrammed mesodermal cells has important implications for bladder regenerative therapies. *Dnmt1* conditional mutant mice provide a new *in vivo* model for examining mechanisms of autologous bladder epithelial replacement.

P44

Addition of Stroma Enhances Branching Morphogenesis and AMACR Expression in a 3D Organoid Co-Culture Model of Prostate Cancer

Zachary Richards[1], Morgan Zenner[1], Joseph Marsili[2], Jacob Manlucu[2], Tara McCray[1], Klara Valyi-Nagy[3], Larisa Nonn[4]

[1]University of Illinois at Chicago, Chicago, IL, USA; [2]Northeastern Illinois University, Chicago, IL, USA; [3]University of Illinois Biorepository, Chicago, IL, USA; [4]University of Illinois Cancer Center, Chicago, IL, USA

Organotypic *in vitro* culture has emerged as a useful technology to model mammalian disease in many tissue types including the prostate. Primary prostate epithelial progenitors fully differentiate into a bi-layered organoid with basal and luminal compartments in 3D culture. Extensive studies in mice have utilized organoids to demonstrate lineage specificity and identify the cell-of-origin in prostate cancer (PCa). Limited studies using human derived organoids have recapitulated common genetic alterations in PCa and provide a means to study therapeutic efficacy *in vitro*. Although 3D culture has proven useful in many avenues of prostate research, nearly all conclusions are limited as they fail to incorporate prostate stroma. The prostate stroma is essential for glandular development, differentiation, and morphogenesis. Additionally, the stroma has direct influence on PCa progression and demonstrated to modulate the tumor microenvironment, therefore, incorporation of stroma into 3D organotypic modelling presents a current limitation of *in vitro* PCa studies. To overcome this limitation we sought to develop and characterize a 3D prostate organoid co-culture model to include stroma and better recapitulate the *in vivo* environment. To accomplish this, epithelial and stromal primary prostate cells from benign and cancer regions of radical prostatectomy (RP) tissues were grown in Matrigel in co-culture and mono-culture. Addition of stromal cells to organoid culture significantly increased branching morphogenesis and organoid take-rate after 7 days compared to organoids in mono-culture. Despite this effect, addition of stroma had no observable influence on organoid size, which was solely dependent on epithelial seeding density. Organoids were significantly smaller when seeded at 5×10^3 cells compared to those seeded at 100 cells per 100 uL in a 96-well plate with or without stromal cells. To assess the validity of the co-culture model as a means to study PCa, organoids derived from PCa cells (tumoroids) were co-cultured with stroma and expression of the PCa marker AMACR was quantified by RT-qPCR. Tumoroids co-cultured with stroma retained high levels of AMACR, which was negligible in 2D and mono-culture. Low level of AMACR expression was present in benign organoids in co-culture, but expression was significantly higher in tumoroids. In summary, the addition of stromal cells to organoid/tumoroid culture increased cell proliferation and significantly promoted expression of the PCa marker AMACR in tumoroids only. The addition of stroma to 3D culture may better recapitulate the in vivo tumor microenvironment, facilitate cell-to-cell contact, and enable necessary epithelial-stroma cross talk, thus providing a better in vitro model of PCa.

P45

Challenging the Free Hormone Hypothesis for Vitamin D in the Prostate Has Implications for the Prostate Cancer Disparity in African American Men

Zachary Richards[1], Jason Garcia[1], Ryan Deaton[2], Andres Acosta[1], Klara Valyi-Nagy[3], Virgilia Macias[1], Peter H Gann[4], Larisa Nonn[4]

[1]University of Illinois at Chicago, Chicago, IL, USA; [2]University of Illinois at Chicago, Research Histology & Tissue Imaging, Chicago, IL, USA; [3]University of Illinois at Chicago, UI Biorepository, Chicago, IL, USA; [4]University of Illinois at Chicago, UI Cancer Center, Chicago, IL, USA

African American (AA) men are disproportionately at risk for both prostate cancer (PCa) and vitamin D (vitD) deficiency compared to European American (EA) men. Based on the numerous chemopreventative properties of vitD and its status with PCa aggressiveness and mortality, vitD deficiency has been hypothesized as a biological contributor to the PCa disparity in AA men. We recently reported that despite having deficient levels of vitD in the serum, AAs have higher levels of vitD in their prostate tissue compared to EAs. This suggests a mechanism of active vitD transport across the membrane and challenges the long-standing Free Hormone Hypothesis, which asserts the activity of a hormone is due to the bioavailable fraction and its passive diffusion across the membrane. The majority of VitD circulates bound to the vitamin D binding protein (DBP) and is sequestered in the serum rendering it unavailable for passive diffusion. A mechanism of active DBP-vitD endocytosis has been well characterized in the kidney by the extracellular receptor Megalin. We hypothesized that Megalin is functional in prostate epithelium and facilitates adequate prostatic vitD levels in AAs with vitD deficiency. Here, we report Megalin protein expression in prostate tissue, LNCaP, and primary human prostate cells. Megalin function was further assessed in primary human derived organoids treated with 25D in the presence or absence of DBP. Surprisingly, vitD import and metabolism occurred faster in +DBP conditions and suggests that DBP-vitD active transport is more efficient than passive diffusion of vitD alone. Addition of a Megalin antagonist diminished activation of the vitamin D receptor (VDR) in a dose responsive manner. Additionally, Megalin protein levels in PCa were significantly lower compared to benign ($p<0.05$) on a tissue microarray quantified by immunofluorescent staining. In summary, our data support a model of Megalin-mediated active transport for vitD in the prostate and demonstrate ancestry-related and PCa differences in Megalin regulation. These findings challenge the free hormone hypothesis and postulate that vitD deficiency is more complex than previously thought.

P46

Characterization of Androgen Response in Organoids Derived from a Novel Metastatic Castration Recurrent PDX

Diamonds A Banks[1], Shruti Shah[2], Barbara Foster[2]

[1]Howard University, Washington, DC, USA; [2]Roswell Park Cancer Institute, Buffalo, NY, USA

Background: As more information about the connection between genetics and prognosis in prostate cancer (PCa) is discovered, doctors will be able to provide more patient specific treatments. However, the time and funds required to do these tests are burdensome to both scientist and patient. An efficient set of tools that scientists could use are 3-dimensional (3D) models called organoids and spheroids. Prostate organoids are tiny, primitive versions of the prostate that are grown in vitro with growth factors that promote prostate specific differentiation (i.e. formation of the lumen). Prostate derived spheroids maintain the stem cell niche. Cells with stem cell properties are thought to be the source of treatment recurrent disease. In this study, we use organoids and spheroids to characterize androgen responsiveness of a new lethal phenotype patient derived xenograft (PDX), LP-0801, which was derived from metastatic castration recurrent (mCRPC) tissue sample. Purpose: The purpose of this study is to characterize LP-0801 PDX in terms of androgen responsive growth rates and expression of differentiation markers using organoids and spheroids. Methods: LP-0801 was established by subcutaneously implanting tumor tissue into the flank of severe combined immunodeficiency (SCID) mice. Tumor tissue was obtained post mortem from a man with the lethal phenotype of prostate cancer that had failed androgen deprivation therapy. Three PDX LP-801 tumors were collected from 20-week old castrated mice. Prostate tissues from castrated and non-castrated mice were used as controls. Tumor samples were digested and used to establish organoid and spheroid cultures. To determine the effect of androgens, organoids were cultured in media with or without dihydrotestosterone (DHT). Androgen responsiveness was compared to established androgen independent and androgen responsive cell lines, respectively DU145 and LNCaP. To determine androgen response of the organoids and spheroids, five microscope images were taken to measure the average area. Within those five frames, we counted the organoids and spheroids within the depth. Histology of the organoids and spheroids were evaluated on hematoxylin and eosin stained slides of the organoid cultures. Results: LP-0801 were grow in 3D cultures culture conditions with and without androgens under conditions that promoted differentiated (organoids) or maintenance of the stem cell niche (spheroids). Androgens did not significantly affect LP-0801 growth, approximately 0.02% organoid growth from 1.0×10^4 cells. Both cell lines grew least 10x more organoids than LP-0801. Conclusion: LP-0801 organoids and spheroids grew and mirrored the androgen independent in vivo phenotype.

P47

Inactivation of *Foxa1* and *Pten* Results in Development of Carcinoma in Situ and Basal Subtype of Muscle Invasive Bladder Cancer

Vasty O Amponsa[1], Jenna M Buckwalter[1], Vonn Walter[1], Joshua Warrick[1], Xue-Ru Wu[2], Jay D Raman[1], David J DeGraff[1]

[1]Penn State Hershey College of Medicine, Hershey, PA, USA; [2]New York University, New York, NY, USA

Background: Bladder cancer (BLCa) is the second most common urologic malignancy with an estimated 79,000 new cases diagnosed in 2016. BLCa is often morphologically heterogeneous, characterized by conventional urothelial cell carcinoma (UCC) with mixed squamous cell differentiation (SqD) and other variant morphologies. Loss of the transcription factor forkhead box A1 (FOXA1) expression is associated with poor clinical outcome in human BLCa, as well as the development of UCC with SqD. A recent "pan-cancer" genomics study by The Cancer Genome Atlas (TCGA) group identified a subset of disease with a "squamous-like" signature and loss of function mutations in the tumor suppressor phosphatase and tensin homologue on chromosome ten (PTEN). The aim of this study is to investigate the potential for a synergistic relationship between *FOXA1* and *PTEN* in the development of BLCa with squamous characteristics. Methods: In order to determine if genetic alterations in *FOXA1* and *PTEN* co-occurred in BLCa, we analyzed the TCGA bladder dataset. We additionally utilized a Cre recombinase system under the control of a bladder-specific uroplakin II promoter (UpII-Cre) to conditionally knock-out (KO) *Foxa1* and/or *Pten* in the urothelium of mice. One group of mice was aged for 6 and 12 months. Mouse bladders were dissected and stained by histology (H&E) and immunohistochemistry (IHC) for markers of commonly altered pathways in this disease. Results: At 6 months of age, both single KO mice (*UpII-Cre/Foxa1*$^{-/-}$ or *UpII-Cre/Pten*$^{-/-}$) and double KO (*UpII-Cre/Foxa1*$^{-/-}$/*Pten*$^{-/-}$) mice developed urothelial hyperplasia relative to genetic controls (no Cre). Double KO (*UpII-Cre/Foxa1*$^{-/-}$/*Pten*$^{-/-}$) mice developed carcinoma *in situ* (CIS) with squamous characteristics and invasive BLCa. In addition, these tumors exhibited increased expression of Ki67 and high molecular weight cytokeratins (Krt5/6 and Krt14), and reduced expression of the urothelial differentiation factors Pparγ and Gata3. These observations confirm the presence of SqD, and are also indicative of a basal gene expression subtype following bladder-specific *Foxa1* and *Pten* KO. Conclusions: Genomic evidence from the TCGA study indicate somatic copy number loss of at least one allele of *FOXA1* and *PTEN* within the same tumor is common in advanced BC. We show combined loss of both alleles of *Foxa1* and *Pten* results in CIS exhibiting a component of SqD and invasive BLCa. These results in combination with observations observed in human BLCa tumors suggest loss of *FOXA1* and *PTEN* synergize to promote tumor progression and/or SqD and that alterations in these genes may have prognostic significance.

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Pro-fibrotic Signaling in Prostate Stroma Increases Prostate Collagen Fiber Density and Disrupts Normal Urinary Function in Male Mice

Kyle A Wegner[1], Jinjin Guo[2], Jill A McMahon[2], Nobuyo Maeda[3], Peiqing Wang[1], Dale E Bjorling[1], Paul C Marker[1], Andrew P McMahon[2], Chad M Vezina[1]

[1]University of Wisconsin-Madison, Madison, WI, USA; [2]University of Southern California, Los Angeles, CA, USA; [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Background: Prostatic collagen content associates with lower urinary tract symptoms in aging men, but whether it causes these symptoms or is a non-pathological sequaele of other aging-related factors has not determined. This study uses a genetic approach to isolate the variable of prostate fibrosis and determine whether it is by itself is sufficient to change urinary function in mice. Methods: A mouse harboring a conditional knock-in *cre* transgene driven by the steroid 5 alpha-reductase type 2 promoter (*Srd5a2*^{creErt2}) was synthesized by the GenitoUrinary Development Molecular Anatomy Project (GUDMAP) to enable genetic manipulation of prostate stromal gene expression and was bred to a tdtomato^{fl} reporter mouse to map *cre* expression. The *Srd5a2*^{creErt2} mouse was also bred to mouse harboring a profibrotic, conditional connective tissue growth factor (CTGF) allele that is unstable and rapidly degraded in the absence of Cre (*Ctgf-lo*) or stabilized and overexpressed in the presence of Cre (*Ctgf-HI*). Four daily 200 mg/kg tamoxifen doses were given to six week old male mice to activate Cre Changes in bladder pressures associated with filling and emptying were assessed with cystometry eight weeks after tamoxifen injection. Prostatic collagen content was assessed by staining tissue sections with picrosirius red, imaging under fluorescent light, and analyzing images with CT-FIRE software. Results: *Srd5a2*^{creErt2} was activated in a tamoxifen-dependent fashion in all prostate lobes and to a lesser degree in the seminal vesicle and testis. Ventral prostate lobes had the highest level of *cre*-dependent reporter expression. Expression was abundant in the thin fibromuscular tunica surrounding prostate ducts but was also observed in cells positioned between the basement membrane and peridcutal smooth muscle layer. The collagen density in *Ctgf-HI* mouse anterior prostate was greater than in *Ctgf-lo* mice, and this was accompanied significantly lower baseline bladder pressure and pressure required to trigger a urination event. Conclusions: The Srd5a2^{creErt2} mouse strain is an effective resource for manipulating gene expression in mouse prostate stroma. CMG anlaysis of Ctgf-HI mice revealed decreased baseline and threshold pressures which may indicate increased bladder capacity due to fibrosis-related obstruction. These data paired with the observed increase in collagen fiber density in the prostates of Ctgf-HI mice is the first evidence that prostatic fibrosis is sufficient to disrupt urinary function in mice. Supported by NIH Grants U54 DK099328, U01DK110807, and T32 ES007015.

P49

Delayed Treatment of Tcf4 Inhibitor, Pkf118-310, Attenuated Enzalutamide Resistant Prostate Cancer Cell Growth in Xenograft Mice

Geun Taek Lee, Isaac Y Kim

Section of Urologic Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Background: In treating patients with castration resistant prostate cancer (CRPC), enzalutamide, the second generation AR antagonist, has been considered a cornerstone of care. However, clinical benefits are limited to a median time of 4.8 months because resistance to enzalutamide inevitably emerges. In our preliminary RNA sequence study, it was found that the expression levels of neuroendocrine differentiation (NED) markers such as chromogranin A (ChgA) and parathyroid hormone related peptide (PTHrP) were highly elevated in an enzalutamide-resistant human prostate cancer (CaP) cell line when compared to the parental cell line. After analyzing the promoters of the NED related genes, we found that transcription factor-4 (TCF4), a transcription factor that has not been linked to CaP previously, mediated NED in response to enzalutamide treatment and was elevated in the enzalutamide-resistant CaP cell line. Inducible TCF4 overexpression also caused enzalutamide-resistance in mouse xenograft model. Methods: Xenograft mouse model using enzalutamide resistant LNCaP cell line (LNCaP-EnzR) was used for studying TCF4 function in enzalutamide resistant prostate cancer. TCF4 inhibitor, PKF118-310 which is inhibitor of interaction between TCF4 and β-catenin, was delayed treated for four weeks after tumor size reached up to 200 mm^3. Results: Delayed treatment of PKF118-310 with enzalutamide decreased enzalutamide resistance in LNCaP-EnzR. In addition, there is not much effect on the PKC118-310 injection only group. It means PKF118-310 treatment inhibited enzalutamide resistant prostate cancer growth by increasing enzalutamide sensitivity. Conclusions: TCF4 in one of key molecules for increasing enzalutamide resistant prostate cancer and its inhibitor PKF118-310 has good therapeutic potential for treatment of enzalutamide resistant prostate cancer.

P50

Pathogenesis of Inflammation in Human BPH

Gervaise Henry, Alicia Malewska, Ryan Mauck, Ryan Hutchinson, Jeff Gahan, Franto Francis, Jose Torrealba, Claus Roehrborn, Douglas W Strand

UT Southwestern Medical Center, Dallas, TX, USA

Background: The design of drugs that target the actual pathogenic pathways in BPH has been hampered by a precise understanding of the molecular and cellular changes associated with disease progression. The presence of chronic inflammation is predictive of larger prostate volume, symptom worsening, indication of surgery, acute urinary retention, and 5ARI treatment failure. Targeting inflammation is a promising therapeutic strategy for many chronic diseases, but a comprehensive profile of the immune/inflammatory milieu in human BPH phenotypic subtypes is lacking. Methods: Ten young organ donor prostate specimens and 60 glandular or stromal BPH specimens were prepared fresh from surgery into 1) digested single cells; 2) flash frozen pieces; and 3) paraffin-embedded blocks. Flow cytometry was used to isolate basal epithelia (CD45-/CD31-/CD326+/CD271+/CD26-), luminal epithelia (CD45-/CD31-/CD326+/CD271-/CD26+) and stroma (CD45-/CD31-/CD326-) for RNA sequencing. A multiplex bead-based assay was used to quantify pro-inflammatory cytokines/chemokines in flash frozen specimens. Multicolor flow cytometry antibody panels were designed to quantify T cell and macrophage subsets. Results: The normal prostate contains a small number of resident T cells, predominantly CD8+ cytotoxic cells. The total number of leukocytes increases dramatically in BPH vs. normal specimens, predominantly due to increased macrophages. Bioinformatics analysis of cell type-specific RNA sequencing reveals a strong upregulation of specific pro-immune/inflammatory signals in stromal/glandular BPH vs. normal specimens. Multiplexed pro-inflammatory cytokine/chemokine analysis in whole tissue reveals increased stromal cell IL-33 in stromal BPH, which was accompanied by an increase in Th2 CD4+ T cells as shown by FACS. In glandular BPH, MCP-1 was significantly increased in luminal epithelia along with an increase in M1 macrophages. Conclusions: A thorough understanding of the pro-immune/inflammatory prostate signals responsible for the leukocytic subtypes present is necessary for refining potential therapeutic targets. The differential recruitment of leukocytes in specific phenotypic subsets by stromal and epithelial signals suggests a distinct pathogenesis in stromal vs. glandular BPH. An understanding of the pathologic effects of Th2 on stromal hyperplasia and M1 macrophages on glandular hyperplasia is needed.

P51

CREB1 and ATF1 Differentially Regulate Terminal Prostate Luminal Cell Differentiation by Controlling the Timing of ING4 Expression, while CREB1 Prevents ING4 Expression Upon PTEN Loss in Prostate Cancer

McLane Watson[1], Penny Berger[1], Sander B Frank[2], Mary Winn[1], Cindy Miranti[2]

[1]Van Andel Research Institute, Grand Rapids, MI, USA; [2]University of Arizona, Tucson, AZ, USA

Background: We previously demonstrated that transient ING4 expression is required for luminal cell differentiation. Additionally, ING4 is downregulated in ~60% of primary prostate tumors and its loss is correlated with loss of PTEN. We further demonstrated in a primary prostate cancer model overexpressing ERG, MYC, and shPTEN (EMP) that loss of PTEN was responsible for ING4 downregulation, though the mechanism remained elusive. Methods: Utilizing RNA-seq and our *in vitro* differentiation model, we identified transcriptional nodes required for luminal differentiation. Of the ~600 differentially regulated genes during differentiation, the largest transcription factor signature (29% of genes) was CREB/ATF. A subset of these targets (Blimp1, Claudin1, Plk2, Chek1) were further validated by qRT-PCR and immunoblotting. Results: CREB/ATF bind constitutively to exposed CRE elements and can be activated by multiple kinases, including AKT. Both CREB1 and ATF1 are inducibly phosphorylated midway through luminal differentiation, with ATF1 preceding CREB1. Knockdown of CREB1 with shRNA increased ING4, accelerated differentiation, and induced premature luminal cell death. Conversely, knockdown of ATF1 blocked ING4 induction and prevented supra-basal layer formation. CREB1/ATF1 ChIP was enriched at the ING4 promoter at mid-differentiation, when ING4 expression peaks. Additionally, CREB1/ATF1 was constitutively bound to the promoter of JFK, an E3-ligase that targets ING4 and whose mRNA increases during differentiation. Thus, we propose that ATF1 is required to induce ING4 transcription while CREB1 suppresses ING4 by both transcriptional repression and induction of JFK. We compared the gene signature of differentiated cells to that of the tumorigenic EMPs. Surprisingly, 30% of the differentially expressed genes were CREB/ATF targets but there is less than 10% overlap, indicating CREB/ATF control distinct subsets of genes in differentiated luminal cells versus cancer cells. Some EMP-specific CREB/ATF targets (GATA2, TWIST1, Necdin, PPM1F) were further validated by qRT-PCR and immunoblotting. CREB1 and ATF1 were highly phosphorylated in EMP cells and knockdown of CREB1 restored ING4 expression and supra-basal formation. Conclusions: Our working model is that AKT activation upon PTEN loss in transiently differentiating cells results in premature and constitutive activation of CREB1/ATF1 bound to genes prior to induction of the ING4 chromatin switch. This prevents ING4 induction and the chromatin rearrangements required for terminal differentiation. In normal PrECs, CREB/ATF1 activation is tightly controlled by as of yet undetermined factors and is only permitted when the proper CRE binding sites are exposed. This model helps to explain how loss of PTEN disrupts luminal cell terminal differentiation to promote prostate cancer oncogenesis.

P52

Hormonal Modulation of DDX3 and its Implications on Translational Regulation in Prostate Cancer

Jordan Vellky

University of Wisconsin-Madison, Madison, WI, USA

Background: DDX3 is an ATP-dependent RNA helicase that can bind target mRNA to aid or prevent translation. Under normal conditions, DDX3 facilitates the translation of mRNA with a highly structured 5'UTR by resolving the secondary structure. Alternatively, under stress conditions DDX3 can form ribonucleoprotein (RNP) granules that prevent or delay translation of the sequestered mRNA. Recently, DDX3 expression has been implicated in several cancer types including prostate cancer (CaP), where it is overexpressed and correlated with high Gleason scores. Additionally, inhibiting DDX3 in CaP cell lines decreases proliferation and induces cell-cycle arrest *in vitro*. However, mechanisms for the regulation of DDX3 in prostate cancer progression and implications for DDX3 in translational regulation remain largely unstudied. Results: Using a novel series of cell lines derived from a single non-tumorigenic human prostate epithelial cell, we can study the progression of prostate cancer from benign through metastasis. DDX3 protein expression increases through CaP progression, concurrent with localization to puncta in the cytoplasm. To investigate potential mechanisms behind this change of expression and localization, we treated the progression lines with steroid hormones testosterone (T) and 17B-estradiol (E2). Results showed E2 alone, and co-treatment of T+E2 were sufficient to increase DDX3 expression at both the RNA and protein level, indicating DDX3 may be hormone-regulated. Promoter analysis of DDX3 showed binding sites for Sp1, Nkx3.1, and c-Myc upstream of the transcription start site. Additionally, treatment with clinically relevant anti-androgens was also sufficient to increase DDX3 RNA and protein expression. Because DDX3 is an RNA binding protein, we investigated potential target mRNA using our progression model; RNA-IP followed by qPCR shows DDX3 binds Rac1 and CCNE1 mRNA, and that modulation of this RNP complex correlates with protein expression changes. This supports a role for DDX3 as a translational regulator in CaP. Implications: DDX3-mediated translational regulation could have clinical implications as it provides an additional system of regulation in CaP. Based on preliminary data, DDX3 expression could be modulated in response to hormones or hormone therapy, and this could affect translation of target mRNA. Identification of DDX3 target mRNAs could reveal biomarkers of advanced disease, or markers to stratify patients that would benefit from single or combinatorial treatment with DDX3 inhibitors.

P53 - Travel Awardee

Protocadherin 7 is Overexpressed in Advanced Prostate Cancer and Promotes Prostate Cancer Progression by Modulating MEK and AKT Signaling

Gauri Shishodia, Prakash Srinivasan Timiri Shanmugam, Sweaty Koul, Hari Koul

LSUHSC-S, Shreveport, LA, USA

Introduction: Most of prostate cancer (PCa) deaths are a result of distant metastasis and due to emergence of castrate resistant PCa (CRPC). Protocadherins (PCDHs) are members of the cadherin superfamily that regulate cell adhesion. PCDH7, a cadherin superfamily transmembrane protein that is known to function in cell-cell recognition and adhesion, is reported to be overexpressed in breast cancer and non-small cell lung cancer (NSCLC). PCDH7 has been shown to mediate brain metastasis of breast cancer and its overexpression was reported to be associated with poor clinical outcome of NSCLC. However, there is no report on involvement of PCDH7 in prostate tumorigenesis and metastasis. In the present study, we evaluated expression and role of PCDH7 in PCa progression. Materials and Methods: PCa cells - LNCaP, C4-2b, DU145, PC3, 22Rv1 and RWPE1 were used in this study. PCDH7 protein and mRNA levels were checked by Western Blotting and quantitative real time PCR respectively. Immunohistochemistry was done to check expression in TRAMP mice FFPE prostate tissue sections. Publically available data set (Trento 2016) was used to analyze PCDH7 expression in NEPC patients. PCDH7 lentiviral knock down was performed using PCDH7 shRNA in PC3 cells. Cell migration and invasion were done using IncuCyte® Scratch Wound Cell Migration and Invasion System. Colony formation was assessed by staining with 0.4% crystal violet after 3 weeks of cell seeding. Results: PCDH7 mRNA and protein is overexpressed in CRPC cells (C4-2b, 22Rv1, DU145 and PC3 cells as compared to castrations sensitive LNCaP cells as well as normal prostate RWPE1 cells. We also observed highest levels for PCDH7 in enzalutamide refractory 22Rv1 cells and significant increased expression of PCDH7 in prostate tissues of TRAMP mice. Our results also show that PCDH7 is amplified in 9% of PCa patients and overexpressed in 43% patients. We observed that knocking down PCDH7 reduced ERK and AKT activities, decreased cell migration, reduced cell invasion, and decreased colony formation. Collectively, these data suggest PCDH7 plays a crucial role in advanced PCa. Conclusion: Our results are the first direct demonstration of increased PCDH7 expression in CRPC cells and in TRAMP mouse model of PCa, and that PCDH7 loss of function impairs PCa progression characteristics i.e. cell migration, invasion and colony formation by disrupting crucial signaling cascades (ERK and AKT pathways). Taken together, our results show that PCDH7 may be an attractive target for therapeutic intervention in PCa, in general, and CRPC in particular.

P54

A Prostate Tumor Neo-antigen Polyionic Virus-like Particle Vaccine Shows Immunogenicity and Efficacy in a Mouse Model of Advanced Prostate Cancer

Brian Simons, Fabiana Cannella, Ashley Ross, Raphael Viscidi

Johns Hopkins School of Medicine, Baltimore, MD, USA

Background & Methods: Vaccine-based strategies for prostate cancer immunotherapy to date have shown modest efficacy. Modified polyionic virus-like particle (VLP) vaccines can rapidly be generated by decorating the particles with peptides or full length proteins. VLP-based vaccines have significant advantages over conventional peptide or DNA-based vaccines. The goal of this project was to generate VLP vaccines using modified bovine papilloma virus decorated with prostate specific antigens and neo-antigens and test immunogenicity and efficacy of these vaccines in TRAMP mice. Results: VLP vaccines with peptide fragments of mouse PAP, PSCA, and the neo-antigen SPAS-1 generated strong and durable CD8+ T cell responses in wild type and TRAMP mice. Treatment of 20-week-old TRAMP mice with advanced prostate cancer by vaccination alone or by vaccination plus PD-1 immunotherapy induced a significant anti-tumor response with smaller tumor sizes than either therapy alone at 26 weeks of age. Conclusions: These results indicate combination therapy with VLP vaccine and checkpoint inhibitors could improve clinical efficacy of prostate cancer immunotherapy.

P55

Oncogenic Properties of Tumor Suppressor Protein STAG2 in Muscle-invasive Bladder Cancer

Lanni Aquila, Nithya Krishnan, Monika Rak, Swathi Ramakrishnan, Kristopher Atwood, Carl Morrison, Candace Johnson, Khurshid Guru, Jianmin Wang, Anna Woloszynska-Read

Roswell Park Cancer Institute, Buffalo, NY, USA

Background: Stromal antigen 2 (STAG2) is one of four conserved members of the sister chromatid cohesion and segregation (SCCS) complex. STAG2 is the most frequently mutated cohesin subunit in bladder cancer. We hypothesize that STAG2 has transcription factor properties independent of its function in SCCS. Methods: We utilized whole exome and targeted exome sequencing of over 150 human muscle-invasive bladder cancer (MIBC) patient specimens to determine the mutational landscape. We found STAG2 mutations in more than 10% of tumors, and examined protein expression of STAG2 using bladder tissue microarrays (TMAs). To study the functional role of STAG2 in MIBC, we generated stable shRNA knockdown and ectopic overexpression cell lines using T24 and UM-UC-3, respectively. We performed proliferation, wound-healing, and matrigel invasion assays to decipher phenotypes associated with differential STAG2 expression. To observe the effect STAG2 expression has *in vivo*, we subcutaneously injected the above cell lines into nude mice and measured end-point tumor weights. Integrated analysis of RNA and ChIP sequencing in T24 and UM-UC-3 using Cistrome algorithm led us to identify putative STAG2 targets. Results: By analyzing STAG2 protein expression through TMAs, we found that loss of STAG2 correlates with a significant increase in overall survival (77 vs. 137 median months) and progression free survival (13.5 vs. 23 median months) in patients with MIBC. *In vitro* knockdown of STAG2 in T24 did not induce any changes in proliferation, however we did find a significant decrease in cell motility and invasion ($p<.01$) compared to control cells. Tumors from mice injected with T24 shSTAG2 xenografts weighed less than control tumors (.184 vs. .322 g). Overexpression of STAG2 in UM-UC-3 resulted in an increase in both invasion and migration ($p<.001$). Mice injected with these cells developed tumors of greater weight than controls (1.34 vs. .78 g). Cistrome integration of RNA and ChIP sequencing revealed interaction between STAG2 and transcription factor NFIB. We identified genomic regions where these proteins co-localize and potentially regulate and are currently validating five targets, EI24, EZR, ABCA1, LPAR1, and WEE1 via qPCR. Conclusions: We found that STAG2 plays a role in bladder cancer biology through its invasion-promoting properties. In the absence of STAG2, we noted reversal of the invasive phenotype *in vitro* and formation of smaller tumors *in vivo*. Our results are consistent with the idea that loss of STAG2 may be beneficial in MIBC. We hypothesize that STAG2 cooperates with NFIB and regulates downstream targets to drive these phenotypes.

P56

DNA Methylation Alterations Contribute to Differences in Prostate Cancer Biology in African American and European American Men

Swathi Ramakrishnan, Xuan Peng, Qianya Qi, Qiang Hu, Emily Ellman, Gissou Azabdaftari, Elena Pop, James Mohler, Kristopher Attwood, Li Yan, Jianmin Wang, Anna Woloszynska-Read

Roswell Park Cancer Institute, Buffalo, NY, USA

Background: Prostate cancer health disparities between African American (AA) and European American (EA) men are attributed to socioeconomic as well as biological differences between the two groups. Despite lower frequency of genetic mutations, copy number alterations and TMPRSS2-ERG fusions, AA men are diagnosed with more aggressive prostate cancer than EA men. The purpose of our study is to investigate how epigenetic modifications, specifically DNA methylation, contribute to prostate cancer aggressiveness in AA men. Methods: We determined gene DNA methylation and expression of twelve radical prostatectomy specimens from AA and EA patients treated at Roswell Park (RPCI) by Infinium DNA methylation arrays and RNA sequencing, respectively. To integrate these data we used MethylMix algorithm to identify genes whose transcription is potentially regulated by DNA methylation. To increase analytical power, we analyzed additional AA (n=22)/EA (n=5) patients from RPCI and AA (n=22)/EA (n=172) patients from The Cancer Genome Atlas (TCGA). Results: Unsupervised hierarchical clustering of combined RPCI and TCGA DNA methylation data identified two large clusters Cluster1 (n=133) and Cluster3 (n=73). AA but not EA patients in Cluster1 had better overall survival (57 vs. 50 months, p=0.48) and disease free time (47 vs. 22 months, p=0.01) compared to Cluster3. Hypomethylated CG sites in Cluster3 belonged predominantly to neurogenesis development and the Wnt/Hedgehog signaling pathways. There was no difference in TMPRSS2-ERG fusion status between the two clusters. However, STK39, an apoptosis inducing gene, was differentially methylated between fusion positive and negative AA tumors, with STK39 mRNA expression negatively correlating with promoter DNA methylation (Pearson: -0.593). Furthermore, MethylMix analysis of tumors from AA patients revealed a number of epigenetically regulated genes involved in tumor invasion, glycosylation, calcium sensing and terminal differentiation. We observed significantly lower transcript levels of these genes GALNT5 (-0.23 vs. 0.15, $p<0.05$), STK39 (-0.30 vs. 0.03, $p<0.05$) and TMEM63C (46.68 vs. 22.65, $p<0.05$) in prostate tumors from AA compared to EA men. Conclusions: Our work reveals that clustering based on tumor DNA methylation may be developed as a prognostic indicator in AA patients. Functional studies are necessary to determine whether differential DNA methylation observed in the Wnt/Hedgehog pathway as well as gene transcription of GALNT5, STK39 and TMEM63C potentially regulated by DNA methylation are biological determinants of prostate cancer racial health disparities.

P57

CD117 Tyrosine Kinase Activation Drives Prostate Cancer Aggressiveness

Lihong Shi, Koran Harris, Brittni Foster, Aleksander Skardal, Bethany Kerr

Wake Forest School of Medicine, Winston Salem, NC, USA

Background: Tyrosine kinase receptors have been shown to drive prostate cancer progression and metastasis. In particular, CD117/c-kit expression is upregulated during tumor progression with the highest levels being expressed in bone metastases. In addition, the numbers of circulating CD117+ tumor cells were higher in advanced prostate cancer patients. The presence of these cells in patients' circulation after radical prostatectomy was also associated with biochemical recurrence. Further, the expression of CD117 can be upregulated on prostate cancer cells after repeated culturing in the bone microenvironment. These data indicated that CD117 expression and activation may be associated with prostate cancer aggressiveness. Methods: LNCaP-C4-2 cells were sorted into CD117+ and negative cell populations. Proliferation, matrigel invasion, sphere formation, and gene expression were compared in the two cell populations in the presence and absence of the ligand stem cell factor (SCF) or CD117 and Akt inhibitors. To track cells during coculture and competition experiments, sorted cells were infected to express mCherry (CD117+) or ZsGreen (negative). Using these cells, competition was tracked using an IncuCyte ZOOM live cell imager for *in vitro* studies and an IVIS imager for *in vivo* xenograft tumor initiation studies. To better visualize transendothelial migration, a 3D microfluidic metastasis-on-a-chip was developed to track tumor cell migration through the ECM and across an endothelial cell layer. Results: Here, we examine the activation of CD117 in prostate cancer cell progression and migration. CD117 expression was associated with increased proliferation, migration, and EMT marker expression. Interestingly, treatment with the CD117 ligand stem cell factor (SCF) reduced proliferation but further enhanced invasion indicating that CD117 activation may drive metastasis. CD117 activation stimulated Akt expression and drove the cancer stem cell phenotype as demonstrated by increased *Oct4* and *Sox2* expression. CD117 expression was associated with increased expression of cancer progression and EMT signaling pathways. Using live cell imaging, competition between CD117+ and negative cells was visualized in proliferation, scratch healing, invasion, trans-endothelial migration, and sphere formation. Xenograft models also demonstrated competition *in vivo* with CD117 expression increasing tumor initiation. Intravital imaging and sectioning of tumors demonstrated the localization and composition of co-injected cells. Using 3D microfluidics, we have modeled the competition between CD117+ and negative cell populations during transendothelial migration. Conclusions: CD117 expression on prostate cancer cells drives a more aggressive cell phenotype and may be involved in metastasis. Further, CD117+ cells represent a possible cancer stem cell population.

P58 - Travel Awardee

Stromal-epithelial Interactions Promote Resistance to Androgen-Targeted Therapy in the Context of Fatty Acid Oxidation

Krizia Rohena Rivera, Anisha Madhav, Veronica Placencio, Frank L Duong, Neil Bhowmick

Cedars-Sinai Medical Center, Los Angeles, CA, USA

Background: Prostate cancer (PCa) affects one in every six American men. Current treatment for advanced PCa with recurrent disease, includes androgen-targeted therapy. However, several tumors become resistant to such therapy and at least 25% undergo neuroendocrine differentiation (NED). Our lab and others have demonstrated the impact of the carcinoma associated fibroblasts (CAF) on the development of resistance. Further, the role of a high fat diet (HFD) on PCa progression, particularly associated with elevated circulating fatty acid metabolites, has been a point of interest. We have shown that mice fed a HFD have elevated circulating ß-hydroxybutyrate (ßHB) and develop PCa with a neuroendocrine phenotype when compared with those fed a normal diet. We tested the mechanism of how ßHB contributes to PCa resistance to androgen-targeted therapy. Methods: PCa cell lines and stromal fibroblasts were treated with different concentrations of ßHB in the context of enzalutamide (androgen receptor antagonist) under conditions of normoxia or hypoxia. Cell proliferation and death was evaluated, as were RNA and protein. To distinguish metabolic and signaling roles of ßHB, metabolically inactive enantiomer S-ßHB was used and compared with the metabolically active enantiomer R-ßHB. Results: We found that enzalutamide induced mitochondrial biogenesis and fatty acid oxidation in wild type mouse prostatic fibroblasts, leading to ßHB production. Interestingly, ßHB induced the expression of CAF markers: IL-6, FGF7, FAP and TNC, in support of a cell-autonomous mechanism for fibroblastic differentiation to promote epithelial progression. In the epithelia, we found that ßHB elevated PCa cell growth in a dose dependent fashion, as measured by MTT. Further studies with the different enantiomers of ßHB to distinguish the mechanism of action, showed that S-ßHB (metabolically-inactive) could reduce proliferation while R-ßHB (metabolically active) increased proliferation, both in the presence and absence of enzalutamide. This suggested, the ßHB from the stromal fibroblasts could serve as a source of energy for proliferation. To test the signaling role of ßHB on NED, we found that within 4h of treatment with ßHB and enzalutamide, FOXA2 and its downstream effectors NGN3 and NKX2.2 were upregulated, over enzalutamide or ßHB alone. Prolonged treatment of up to 72 h demonstrated ßHB and enzalutamide promoted N-MYC and CHGA expression, to suggest a progressive NED of PCa, over that induced by enzalutamide alone. Conclusions: Thus, resistance to androgen targeted therapy can be hastened by HFD due to stromal metabolism and activation promoting epithelial proliferation and NED.

P59

A Temporal and Spatial Map of Axons in the Developing Mouse Prostate

Anne E Turco[1], Mark T Cadena[1], Helen L Zhang[1], Richard E Peterson[1], Janet R Keast[2], Chad M Vezina[1]

[1]University of Wisconsin Madison, Madison, WI, USA; [2]University of Melbourne, Melbourne, Australia

Introduction: The autonomic nervous system (ANS) controls physiological features of the prostate, is functionally altered with age, and is structurally changed in benign prostatic hyperplasia and prostate cancer. Autonomic innervation of the prostate is composed of many axon subtypes including noradrenergic, cholinergic, and peptidergic. Despite their functional significance and potential role in prostate pathology, the organization and function of these axon subtypes have not been well characterized. This study sought to map autonomic (noradrenergic, cholinergic, peptidergic) and sensory (myelinated) axon subtypes in the mouse prostate throughout development to begin to understand their importance in the prostate. Methods: Immunohistochemical staining of mouse prostate sections at the time points E12, E14.5, E15.5, E16.5, E17.5, P9, and P50 (spanning the period of budding, branching, and glandular development) was used to identify axon subtype organization. Developmental axon patterning was elucidated, and axon density changes were quantified across developmental time and across mature dorsal and ventral prostate lobes. Results: All axon subtypes investigated were first identified in the prostate between E12 and E14.5 and remained present throughout sexual maturity. Adrenergic axons were localized to the smooth muscle in all developmental stages. Peptidergic axons were denser in the stromal compartment inside the rhabdosphincter compared to outside. They penetrated prostate ductal epithelium both inside and outside the rhabdosphincter and were often associated with neuroendocrine cells. Cholinergic axons were present in the stromal compartment at all developmental stages and were most abundant inside the rhabdosphincter. These axons also crossed the epithelium of prostate ducts and were frequently observed near neuroendocrine cells. Conclusions: Axon subtypes in the prostate were distributed throughout prostate lobes decreasing in density further from the rhabdosphincter. The functional targets of most axons were smooth muscle or neuroendocrine cells. Knowledge of organization and patterning is vital to determining the role of axons in prostate pathology and could make therapies or axon labeling or targeting experiments more specific. Work supported by NIH grant RO1ES001332 and T32 ES007015.

P60

Andrographolide Inhibits Epithelial to Mesenchymal Transition in Prostate Cancer

Hina Mir[1], Neeraj Kapur[1], Guru Sonpavde[2], Shailesh Singh[1]

[1]Morehouse School of Medicine, Atlanta, GA, USA; [2]Dana Farber Cancer Institute, Boston, MA, USA

Background: During cancer progression, particularly metastasis, cells undergo a characteristic transition from epithelial to mesenchymal cell type. Mesenchymal cells are metastatic and resistant to conventional chemotherapy posing a major challenge in prostate cancer (PCa) treatment. Robust therapeutic regimes that can either prevent this cell-type transition or curb the resistant mesenchymal cells could effectively control PCa progression. Natural compounds with their multitude of effects are gaining popularity in this regard over conventional anti-cancer agents. In this study, we have investigated the effects of Andrographolide (AG), a natural compound isolated from *Andrographis paniculata* on epithelial to mesenchymal transition (EMT) in prostate cancer (PCa). Methods: Cell viability assay was used to determine effective cytotoxic concentration of AG for PCa cells. Effect of AG on EMT was determined by FACS analysis of transition markers. Change in cytokine profile contributing to AG induced effect on EMT was determined by ELISA and RTqPCR. Results: Previously we have shown the mechanism of AG induced cell cycle cessation as well as reduced migration in PCa cells. Here, we demonstrate effects of this agent on key EMT markers of PCa cells. It disposes PCa cells towards therapeutically more responsive epithelial phenotype by up-regulating E-cadherin and inhibiting ZEB-1, SNAIL and TWIST. In addition to these, AG treatment resulted in significant reduction of IL-6, which is known as a poor prognostic indicator of PCa, as compared to untreated controls. Conclusion: Our result suggests that AG inhibits EMT by reducing IL-6, which in turn may be due to its effect on suppressor of cytokine signaling molecules. Therefore, our data underscores the ability of AG to impede cancer growth and rationalizes its application as a potent therapeutic agent.

P61 - Travel Awardee

A Proteomic Fingerprint of Hormone-induced Lower Urinary Tract Dysfunction in Mice

Samuel Thomas, Ling Hao, Seth Hyman, Laura Steinke, Kellen Delaney, William Ricke, Lingjun Li

University of Wisconsin-Madison, Madison, WI, USA

Background: Lower urinary tract symptoms impact roughly half of US men aged 50 and 70% of men over 70, with annual costs >$3 billion per year. Lack of preclinical models is a current challenge in the field. A promising mouse model has been developed, which uses subcutaneous implantation of slow release testosterone and estradiol (T+E2) pellets, mimicking the aging male hormone milieu. This model recapitulates an enlarged bladder phenotype accompanied by increased prostate duct number, implicating benign prostatic hyperplasia in the dysfunction. A comparative proteomics analysis was performed to explore molecular mechanisms and seek objective biomarkers for future patient stratification. Methods: Eight C57BL/6J mice were treated with slow-release subcutaneous T+E2 pellet implantation after initial urine collection. A repeated measures design was used for urine proteomics, with samples collected at weeks 0, 2, 4, and 8 of T+E2 treatment. Eight littermates were included alongside treated mice as controls in tissue proteomics and metrology. Treated and control mice were sacrificed after the week 8 urine collection. Bladder and prostate masses were recorded at necropsy. Prostates were homogenized for proteomics. Urine proteins were concentrated via 3 kDa MWCO centrifugal filters. Prostate and urine samples were normalized to total protein, digested via trypsin, and desalted using solid-phase extraction. Samples were analyzed using nanoUPLC-ESI coupled to a Q-Exactive HF mass spectrometer. Data were processed using MaxQuant and a paired t-test with FDR correction was used for initial statistical analysis. The present data were compared with our prior human patient analysis. Results: Bladder and prostate mass were significantly different (increased) in mice with 8 weeks of T+E2 treatment versus untreated controls (n=8 per group, Student's t-test $p<0.0001$ and $p=0.001$, respectively). For week 0 vs. week 8, 336 urinary proteins were identified and quantified (LFQ; MaxQuant). 47 were significantly dysregulated with T+E2 treatment ($q<0.05$; paired t-test, FDR correction). Urine proteomics showed dysregulation of proteins related to inflammation and other disease-relevant processes: growth of male reproductive organs, REDOX homeostasis, etc. Significantly overrepresented processes found in this model were also seen in our prior human analysis, including response to stimuli and metabolic processes ($p<0.05$; hypergeometric test, FDR correction; Cytoscape BiNGO). Data from weeks 2 and 4 are being processed for information regarding onset of dysfunction. Prostate tissue proteomics will provide information regarding the origin of urinary proteins. Conclusions: Discovery-phase urinary proteomics identified dysregulation of disease-relevant processes (e.g., inflammation) in this hormone-induced LUTD model. Prior human data showed similar process-level changes.

P62

Myokine Signaling Blockade Prevents Androgen Deprivation Therapy Induced Sarcopenia in a Mouse Model of Prostate Cancer

Chunliu Pan, Yanni Zulia, Kent L Nastiuk

Roswell Park Cancer Institute, Buffalo, NY, USA

Background: One of the most significant side effects of androgen deprivation therapy (ADT), the first line therapy for recurrent and metastatic prostate cancer (PrCa), is sarcopenia (loss of skeletal muscle mass and function). ADT produces a significant disability syndrome in PrCa patients known as obese frailty. Use of ADT has been increasing such that side effects increasingly impact patient quality of life, and result in nursing home placement. The sarcopenia and functional loss of strength is recapitulated following castration of 'middle-aged', but not younger, male mice. In this model all five muscle-regulating TGFß superfamily members, known as myokines, are up-regulated in distinctive temporal patterns during sarcopenia development. Complete myokine blockade fully restores muscle mass and strength in castrated mice. Methods: We castrated PbCre4 x $PTEN^{fl/fl}$ PrCa-bearing 6-9 month old mice and measured tumor volume (by high-frequency ultrasound), body composition (by NMR), muscle mass, fat mass, grip strength and expression of TGFß family members. Tumor-bearing mice were treated with ActRIIB-Fc (or vehicle) to block myokine signaling, additional tumor-bearing mice were sacrificed biweekly to monitor myokine levels. Results: In an autochthonous mouse model of PrCa, castration-induced changes closely resemble the loss of skeletal muscle strength and body composition that occurs in ADT treated patients. The reduction in muscle strength is comparable to that seen in patients. ADT reduces the mass of individual skeletal muscles, overall LBM, and reduces grip strength. We examined the expression of myokines following castration and found that muscle levels of free dimeric myostatin, the activins, and GDF11 all increase over time, but with distinctive kinetics. Functionally, ActIIRB-Fc, which binds and blocks myokines, inhibits sarcopenia in our model indicating that the myokines mediate ADT-induced sarcopenia. Surprisingly, myokine blockade also induced prostate tumor regression in the absence of ADT, suggesting a pro-tumorigenic effect of myokines. In the tumor tissue and in the circulation, as in the muscle tissue of these mice, some of the myokines also increase post-castration. Conclusions: Myokines mediate the induction of sarcopenia following castration, and may also mediate tumor growth. These results suggests that myokines might be both biomarkers and potential targets for therapy to reduce ADT-induced obese frailty in prostate cancer patients and may also control tumor growth directly.

P63 - Travel Awardee

FOXA2 Promotes Prostate Cancer Bone Colonization

Zachary M Connelly[1], Shu Yang[1], A Wayne Orr[1], Ranjie Jin[2], David DeGraff[3], Xiaotun Zhang[4], Colm Morrissey[4], Eva Corey[4], Robert J Matusik[2], Xiuping Yu[1]

[1]LSUHSC Shreveport, Shreveport, LA, USA; [2]Vanderbilt University Medical Center, Nashville, TN, USA; [3]Penn State College of Medicine and Milton S. Hershey Medical Center, Hershey, PA, USA; [4]University of Washington, Seattle, WA, USA

Background: It is estimated that 26,000 men will die from prostate cancer (PCa) in 2017. Androgen deprivation therapy (ADT) is the gold standard treatment for advanced PCa. Initially, patients respond to treatment, however, these tumors almost invariably progress to castrate-resistant PCa, for which there is no cure. Most PCa patients who fail ADT develop metastasis and preferentially relocate to the bones. Bone metastasis results in significant morbidity and mortality as the average time to death is approximately 3-5 years with no treatment available. Understanding the mechanisms by which PCa grow in the bone is critical for the development of novel therapeutics to treat and decrease tumor-mediated bone destruction. Methods: Gene expression profiling studies have found that FOXA2, a forkhead transcription factor that is expressed in embryonic prostate and neuroendocrine PCa, is present in a subset of metastatic PCa specimens. Our preliminary study found FOXA2 expression in a sample set of human PCa bone metastases, suggesting an involvement of FOXA2 in human PCa bone metastases. Also, we found high levels of FOXA2 in aggressive PCa PC3 cells, but not in PCa LNCaP cells. PC3 cells generate osteolytic lesions in bone, whereas LNCaP cells minimally grow following bone inoculation. To establish FOXA2's role in promoting PCa metastasis, FOXA2 was stably knocked down in PC3 cells and overexpressed in LNCaP cells. Results: We found that FOXA2 knockdown in PC3 cells resulted in a significant decrease in PC3 mediated *in vivo* bone destruction following intra-tibial injection. To understand how FOXA2 is facilitating these changes, we examined the expression of integrins and observed that FOXA2 knockdown decreased the expression of collagen-binding integrins α1 and αv in PC3 cells. Furthermore, we found FOXA2 knockdown decreased PC3 cells' adhesion and spreading on collagen I (a major component of bone ECM) coated surfaces. The Foxa2-controlled expression of integrins α1 and αv and the resulting changes in the adherence and spreading would provide a mechanism for PCa cells colonize bone and initiate the bone-destruction cycle. Additionally, FOXA2 knockdown in PC3 cells resulted in a significant decrease in expression of parathyroid hormone-related protein (PTHrP), a bone remodeling-associated protein. We observed that PTHrP mRNA was decreased in FOXA2 knockdown cells. When PC3 FOXA2 knockdown cells were co-cultured with osteoblast and osteoclast, osteoclast markers were decreased. Conclusions: Taken together, FOXA2 plays a major role in facilitating PCa's ability to colonize bone, and further, promote osteoclast activation.

P64

Vitamin D Promotes Differentiation of Human Prostate Organoids in a microRNA-dependent Manner

Tara McCray, Giovanni Lugli, Larisa Nonn

University of Illinois, Chicago, Chicago, IL, USA

Background: Vitamin D deficiency is prevalent and is associated with an increased risk of lethal prostate cancer (PCa) and less-differentiated, more aggressive tumors. While the pro-differentiating mechanisms of vitamin D are well described in keratinocytes, the capacity for vitamin D to influence prostatic epithelium needs to be defined. Our lab recently discovered that vitamin D treatment globally upregulates microRNAs (miRs) in benign and malignant prostate cell lines, primary cells and in patient tissues. Temporal regulation of miRs is critical for differentiation of all tissues and levels of mature miRs are globally lower in PCa. Given the relationship between vitamin D and miRs, and their influence over differentiation, we hypothesize that replete levels of vitamin D promote differentiation in the prostate through the regulation of miRs. Methods: To evaluate this, we examined prostate epithelial organoid differentiation in the presence of 1,25D, the active metabolite of vitamin D. Organoids were grown from fresh primary human prostatic epithelial progenitor cells that were seeded as single cells in matrigel. Differentiation was determined by inspecting organoid morphology, number of mature luminal cells and protein secretion. Analysis of epithelial markers was performed by qPCR, flow cytometry and immunofluorescence and the ability for the organoid to recapitulate a normal prostate was assessed by PSA secretion into the media. The role of miRs in vitamin D-driven differentiation was explored by reducing global miR levels via knockdown of Drosha, an essential miR-processing protein. Results: Organoids grown in the presence of 1,25D were strikingly larger and formed more complex branching structures than those treated with vehicle, and also showed differential expression levels and patterns of basal epithelial proteins by qPCR and flow. Knockdown of Drosha abrogated these effects of 1,25D, as evidenced by reduced size, round shape, and protein expression. Conclusions: Together our findings indicate a role of 1,25D in normal prostate development and branching, a phenotype that is lost when mature miR levels are globally knocked-down. This suggests miRs are critical for vitamin D to promote differentiation in the prostate. Ongoing studies are focused on examination of these phenotypes in PCa organoids.

P65 - Travel Awardee

Examination of the Small RNA Landscape in Prostate Epithelium Reveals Abundance of PIWI-interacting RNAs and Regulation by Vitamin D

Bethany Baumann, Shang Gao, Giovanni Lugli, Zachary Richards, Larisa Nonn

University of Illinois, Chicago, IL, USA

Background: Low serum vitamin D is a risk factor for aggressive prostate cancer. Vitamin D is a prohormone which alters gene expression via the vitamin D receptor transcription factor. Regulation of small non-coding RNAs by vitamin D is not well described. piRNA and their interacting partners, PIWI-like (PIWIL) proteins, are aberrantly expressed in many cancers, but few studies have focused on piRNAs in the prostate. Methods: We used laser-capture microdissected (LCM) human prostate epithelium from a clinical trial of high dose vitamin D to examine the small non-coding RNA population by next-generation sequencing. The 8 patients for this study were selected for having either high (avg=48.7 pg/ml) or low (avg=21.4 pg/ml) prostatic vitamin D ($1,25(OH)_2D$) levels. Illumina sequencing of the epithelial small RNA libraries resulted in ~12 million quality reads per patient. Reads were mapped to rRNA and miRNA sequences, then to tRNA, snRNA, snoRNA and piRNA. Small RNA-sequencing on human primary epithelial cells (PrE) was done for comparison. PIWIL expression was assessed by RT-qPCR, immunoblot and immunofluorescence on a tissue microarray containing paired benign and cancer tissues (N=23). Results: Twenty percent of the reads from the LCM prostate epithelium mapped as piRNAs and the expression profile was similar to the PrE cells. Of the small RNA species investigated, piRNAs were most affected by patient vitamin D status (p=0.036, t-test), and over a dozen individual piRNAs were differentially expressed. The LCM results were compared to small-RNA sequencing data from benign prostate whole tissues (no LCM) available from The Cancer Genome Atlas (TCGA). The LCM-collected samples had a distinct, more consistent profile of piRNAs than TCGA samples, which represent whole prostate tissue and may contain stromal bias. Of the 4 human PIWIL proteins, PIWIL1, 2 and 4 were present in normal prostate epithelial cells by PCR. PIWIL1 protein was also detected in PrE and RWPE-1 benign cells. The PC3 prostate cancer cell line only expressed PIWIL2 and 4. By immunofluorescence, PIWIL1 protein was enriched in basal cells of normal epithelium and significantly decreased in prostate cancer (p=0.016, paired t-test). Conclusions: The PIWI pathway is intact in prostate epithelium as evidenced by the presence of both PIWIL proteins and piRNAs. Vitamin D status associated with the expression of piRNA in prostate epithelium in clinical trial samples. PIWIL1 protein levels were lower in prostate cancer, suggesting a protective role for the PIWI pathway in prostate that may be affected in vitamin D-deficient patients.

P66

Collagen Architecture of Canine and Murine Prostate: Impact of Age and Androgen Deprivation

Hannah Ruetten, Kyle Wegner, Diya Joseph, Sara Colopy, Ruth Sullivan, Chad Vezina

University of Wisconsin-Madison, Madision, WI, USA

Background: The incidence of prostate-related urinary dysfunction increases as men age. An emerging paradigm for how this dysfunction occurs is prostatic fibrosis, which potentially reduces urethral opening and impedes urine flow. Like human men, aging intact male dogs also develop prostate-related urinary dysfunction, but whether collagen accumulates in the male canine prostate with advancing age and potentially contributes to canine voiding dysfunction has not been previously examined. This study compares the impacts of age and castration on collagen architecture in canine prostate to that of mice, which are increasingly used to model human benign prostatic disease. Methods: Prostates from euthanized young intact, young castrate, old intact, and old castrate dogs and mice were obtained. Prostates were fixed in formalin, embedded in paraffin, and sectioned. Sections were stained with picrosirius red, imaged under fluorescent light, and collagen fiber thickness, length, and density were analyzed. Hematoxylin and eosin-stained sections were also analyzed. Results: Preliminary results in canine suggest that collagen fiber thickness, length, and density vary between regions of the prostate: capsule, prostate ducts, and prostatic urethra. They also show a decrease in collagen with age and neutered animals have denser collagen than intact animals. More intact canine samples and mice are currently being collected. Conclusions: The collagen architecture of the canine prostate varies between regions of prostate and between neutered and intact animals. Pending functional voiding analysis, collagen may also contribute to symptoms experienced by canines with BPH.

P67

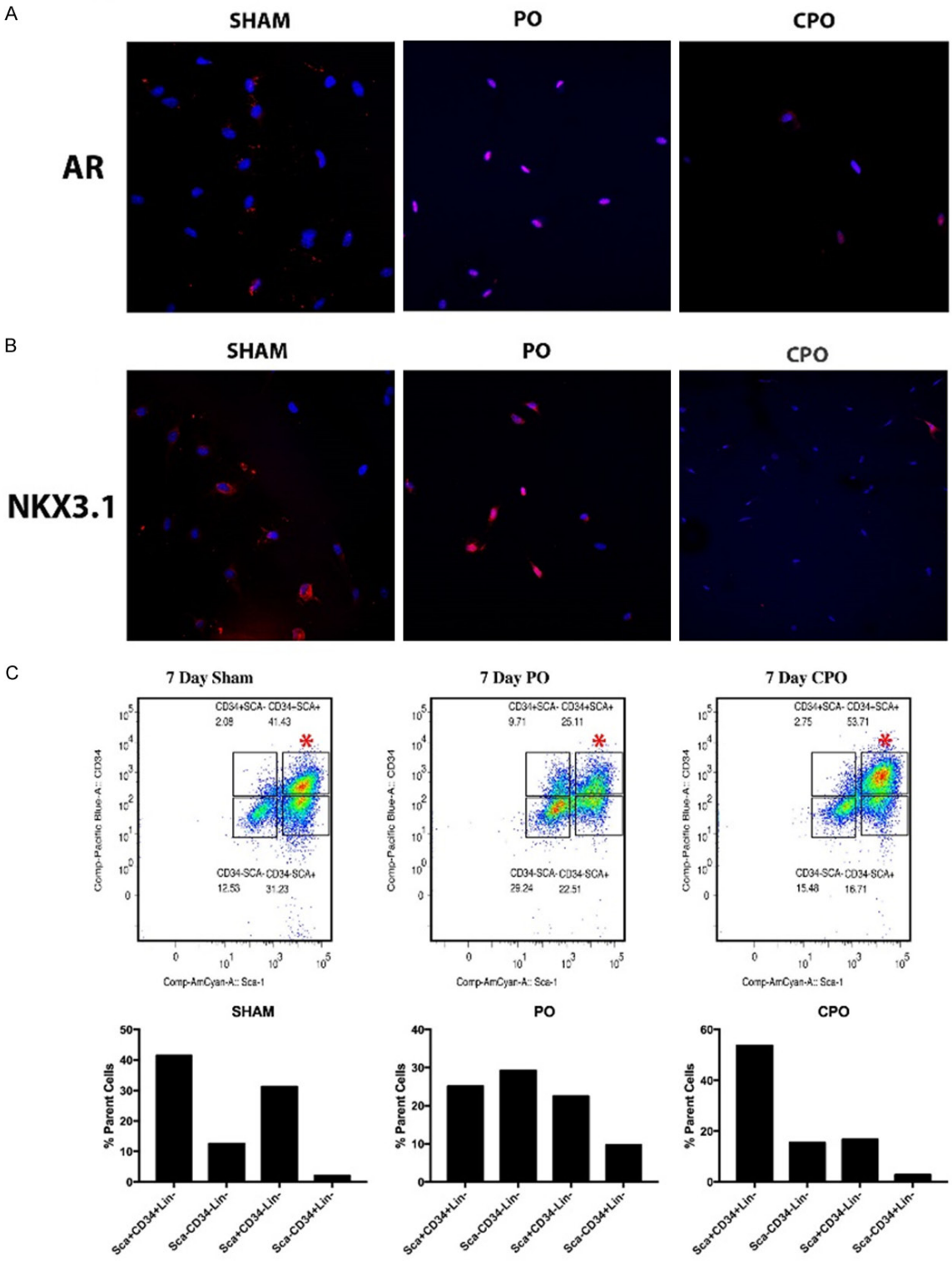
Can Androgens Have Significant Effects on Mesenchymal Stem Cells

Megan Y Devine[1], Paula R Firmiss[1], Diana K Bowen[2], Natalie A Kukulka[1], Robert W Dettman[2], Edward M Gong[2]

[1]Lurie Children's Hospital of Chicago, Chicago, IL, USA; [2]Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Background: We have identified a mesenchymal stem cell (MSC) population in the urinary bladder. In a model of fibrosis secondary to partial bladder outlet obstruction (PO) we observed that fibrosis was less severe in female mice as compared with male mice. We hypothesized that male androgen may function in the bladder in response to obstruction. Methods: 8-10 wk male CD-1 mice were surgically obstructed at the bladder neck to create a partial obstruction model (PO), with and without surgical castration (CPO). Fluorescence-activated cell sorting (FACS) was utilized to isolate MSCs (Sca-1$^+$/CD34$^+$/lin$^-$ (PECAM$^-$, CD45$^-$, Ter119$^-$)) in the sham, PO, and CPO bladders 7 days after obstruction. MSCs were cultured for 24 hours and expression of androgen receptor (AR) and NKX3.1 on the MSCs was determined with immunofluorescence. Quantitative PCR was performed to determine levels of gene expression. Testosterone was replaced in castrated mice with the administration of testosterone or dihydrotestosterone. Results: Bladder function decreased and fibrosis increased after PO. Function was normal and fibrosis reduced in CPO. Administration of testosterone or dihydrotestosterone to PO mice resulted in reduced function and increased fibrosis. The bladder MSC population was significantly decreased by PO. Castration restored this population as determined by FACS (Figure 1C). MSCs were found to express AR and twice as many sorted MSCs expressed AR (80%) by immunofluorescence after obstruction when compared to sham (40%) (Figure 1A). Microarray analysis identified the transcription factor NKX3.1 as an androgen response gene increased after PO. This was validated by qPCR and immunofluorescence staining of MSCs (Figure 1B). Conclusions: Our data suggest that testosterone directly effects bladder MSCs through AR and the AR target gene NKX3.1. NKX3.1 is a transcription factor that was previously thought to be expressed exclusively in the prostate gland. NKX3.1 is proposed to be expressed in and perform important functions in epithelial progenitor cells in the prostate. Activation of NKX3.1 in MSCs after obstruction may identify a maladaptive mechanism occurring in these cells that leads to fibrosis. It may identify novel therapeutic targets to prevent bladder deterioration. We are currently studying a knockout model of NKX3.1 to test these ideas.

Figure 1


A
SHAM
PO
CPO
AR
B
SHAM
PO
CPO
NKX3.1
C
7 Day Sham
7 Day PO
7 Day CPO
CD34+SCA- CD34-SCA+
2.08
41.43
CD34-SCA- CD34-SCA+
12.53
31.23
9.71
25.11
29.24
22.51
2.75
53.71
15.48
16.71
Comp-Pacific Blue-A:: CD34
Comp-AmCyan-A:: Sca-1
SHAM
PO
CPO
% Parent Cells
Sca+CD34+Lin-
Sca-CD34-Lin-
Sca+CD34-Lin-
Sca-CD34+Lin-

P68

Tracing Cell Movement in the Bladder During Regeneration after Subtotal Cystectomy

Megan Y Devine[1], Grace B Delos Santos[2], Jessica J Wetterlin[2], Paula R Firmiss[1], Natalie A Kukulka[1], Diana K Bowen[3], Robert W Dettman[3], Edward M Gong[3]

[1]Lurie Children's Hospital of Chicago, Chicago, IL, USA; [2]Loyola University Health System, Chicago, IL, USA; [3]Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Background: The bladder has significant regenerative capabilities, however little is known about how this is accomplished. Previous research indicates there are differences between regenerated organs and their original, healthy counterparts. This study explores the bladder regeneration pattern in mice with bladder resections. Methods: Subtotal cystectomy (STC) removed 50% of the bladder on 6-10 week female mice (Figure 1A). The surgical incision site was labeled with DiI. Ethynyl Deoxyuridine (EdU), a nucleotide analog, is taken up in proliferating cells and has been proven to persist in cells for long durations. To trace cell movement of early proliferating cells after STC, EdU was injected into mice 3 days following STC or sham surgery. Thymidine chase was performed 4 days after injection, labeling proliferating cells up to 1 week post-op. Bladders were harvested at 1, 4, and 8 weeks following STC and cryosectioned. After immunofluorescence (IF) staining, the bladders were divided into four cross-sections: suture site, middle bladder, and bladder neck. Within each cross-section, EdU labeled cells were quantified to determine the density of EdU per region. Results: Cytokeratin 5 (CK5) IF demonstrated complete restoration of the urothelial layer by two weeks following STC, whereas smooth muscle myosin (SMM) IF demonstrated incomplete regeneration of muscle at the surgical incision up to 8 weeks following STC (Figure 1B). The incision site demonstrated persistent collagen deposition by Masson's Trichrome at 8 weeks. Phospho-Histone H3 (PPH3) was seen at low levels throughout the bladder at all time-points. STC bladders demonstrated a high concentration of EdU labeled cells at the surgical incision 1 week post-STC. By 4 weeks post-op, there was a decrease in EdU at the surgical incision and an increase in the middle and bladder neck quadrants (Figure 2). This demonstrates movement of proliferating cells away from the surgical incision toward the bladder neck. Conclusions: We conclude that cell division is a response to STC. Within the 8 wk study, labeled cells have a net movement away from the incision site towards the bladder neck. This study provides insight about how the bladder repairs itself and may identify potential targets to enhance bladder restoration and function.

Figure 1

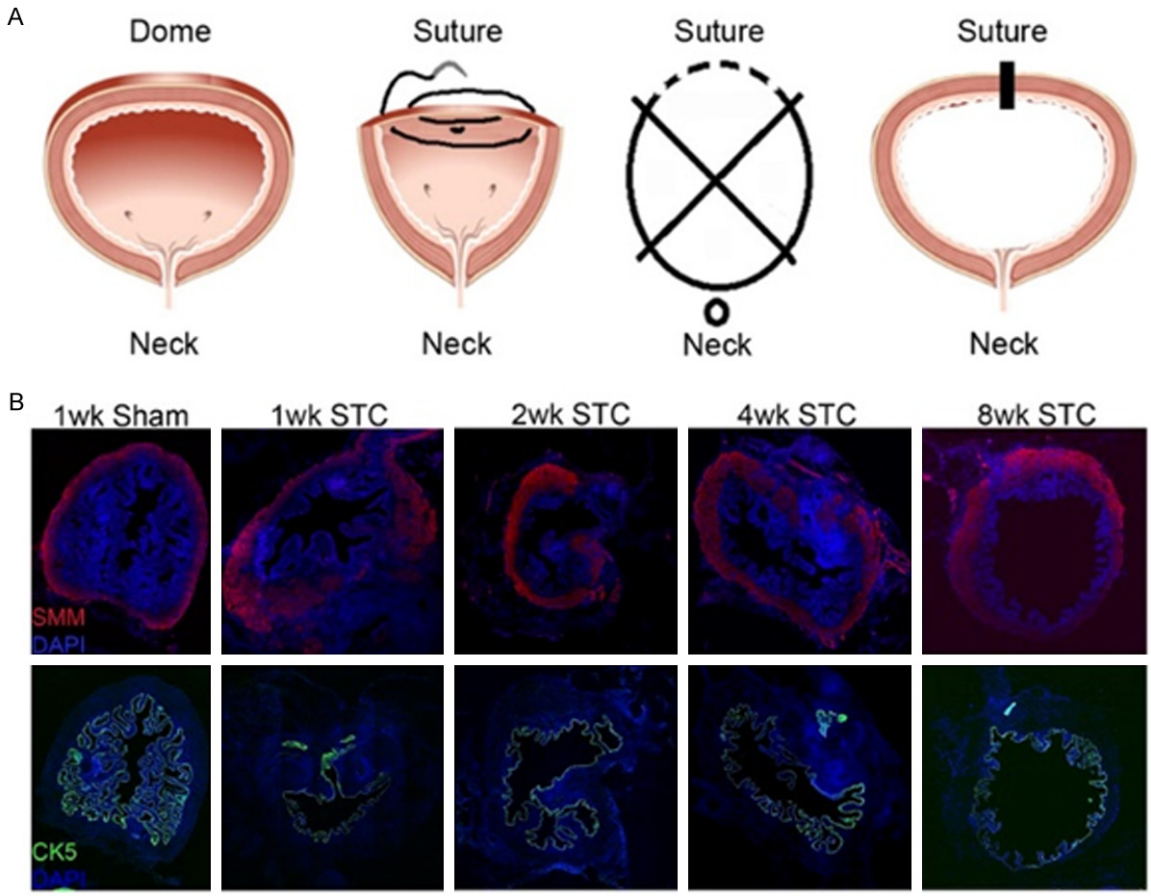
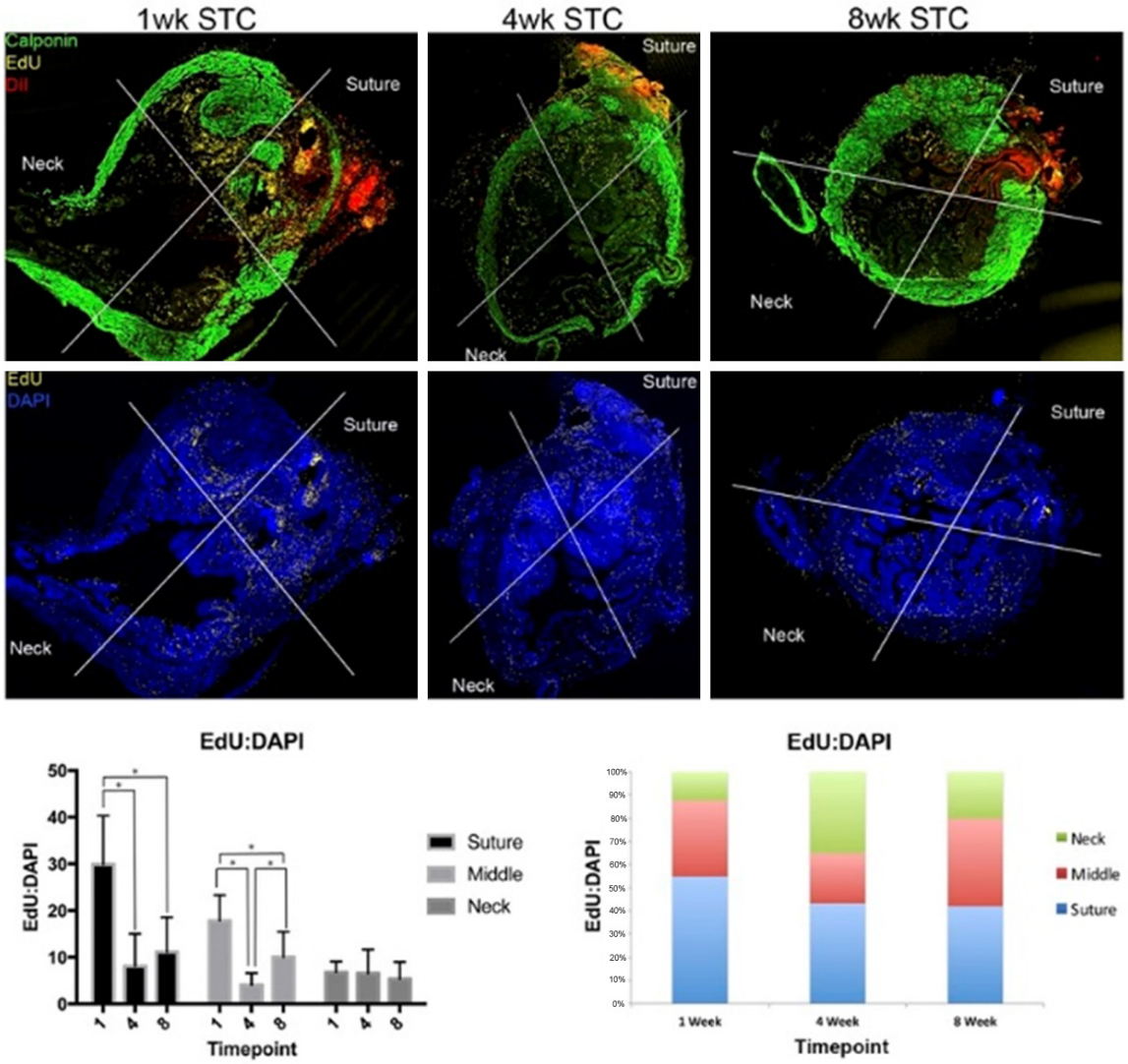

A
Dome
Suture
Suture
Suture
Neck
Neck
Neck
Neck
B
1wk Sham
1wk STC
2wk STC
4wk STC
8wk STC
SMM
DAPI
CK5

Figure 2


1wk STC
4wk STC
8wk STC
Calponin
EdU
DII
Suture
Neck
EdU
DAPI
EdU:DAPI
Timepoint
Suture
Middle
Neck
1 Week
4 Week
8 Week

P69 - Travel Awardee

Optogenetic Stimulation of Corticotropin-releasing Hormone Expressing Neurons in Barrington's Nucleus Recapitulates the Social Stress Voiding Phenotype in Mice

Jason Van Batavia, Stephan Butler, Eleanor Lewis, Joanna Fesi, Rita Valentino, Stephen Zderic

The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Introduction: Mice subjected to social stress show decreased voiding frequency and increased voided volumes along with increases in corticotropin-releasing hormone (CRH) expression in Barrington's nucleus (BN). Optogenetics is a technique to selectively stimulate cells or neurons of interest via light activated channel receptors (channel-2rhodopsin[Chr2]). Here we examined the effects of optogenetic manipulation of CRH BN neurons on the in vivo voiding phenotype in awake mice. Materials and Methods: Double transgenic mice expressing Chr2 in CRH cells were generated using the Cre-lox recombinase system and had fiberoptic probes implanted into BN. The mice also underwent simultaneous catheter placement into the bladder for in vivo cystometry in the unanesthetized state. In vivo cystometry before and during optogenetic stimulation at various frequencies (2, 25, and 50 Hz). was performed 3-4 days postoperatively. Saline was perfused at 10 µl/min and baseline stable voiding cycles were established. Bladder capacity, voiding pressure, and voided volume were recorded at baseline and at each optogenetic setting. In some mice, the protocol was repeated in the presence of CRH-antagonist. Results: Fiberoptic stimulation (470 nm at 25 and 50 Hz) produced a significant rise in the intermicturition interval, bladder capacities and increased void volumes (Figure 1 and Table 1). This effect was especially pronounced in females in whom bladder capacity and intermicturition interval more than doubled at 50 Hz stimulation. Fluoroscopic images confirmed complete bladder emptying with each void. The increased bladder capacity at higher frequencies (25 and 50 Hz) was CRH-dependent as injection of a CRH-antagonist blocked the optogenetic effect. Conclusions: Optogenetic stimulation of CRH BN neurons at higher frequency (25/50 Hz) inhibits micturition and recapitulates the voiding phenotype seen in socially stressed mice (large, infrequent voids). Further elucidation of the neurons in BN are warranted to understand micturition and how it may be manipulated in disease states such as infrequent voiding and acute urinary retention.

Figure 1

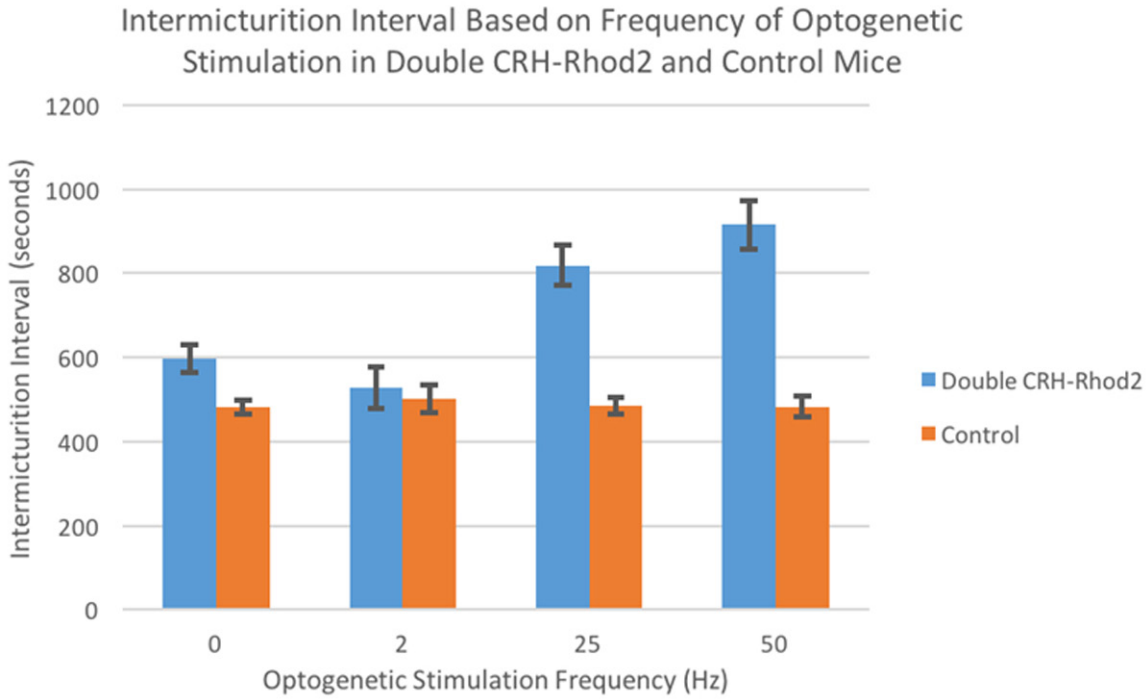


Table 1. Bladder capacities as a percentage of baseline bladder capacity at various optogenetic stimulation in male doubles (CRH-Rhod2), female doubles, and doubles in the presence of CRH-antagonist (NBI 30775). *P*-value for significance between stimulation versus baseline are shown ($p<0.05$ considered significant)

	Male Doubles (CRH-Rhod2) (n=6)		Female Doubles (CRH-Rhod2) (n=4)		Doubles + CRH-antagonist (n=3)	
	Percentage of Baseline Bladder Capacity (± SD)	*p*-value (compared to baseline)	Percentage of Baseline Bladder Capacity (± SD)	*p*-value (compared to baseline)	Percentage of Baseline Bladder Capacity (± SD)	*p*-value (compared to baseline)
Baseline	1.00		1.00		1.00	
2 Hz	0.96 ± 0.30	1.0	1.01 ± 0.06	0.49	0.93 ± 0.14	0.16
25 Hz	1.56 ± 0.75	0.002	1.97 ± 0.60	0.02	0.97 ± 0.08	0.16
50 Hz	1.59 ± 0.22	0.003	2.07 ± 0.31	0.01	0.91 ± 0.17	1.0

P70

Growth Hormone-releasing Hormone Antagonists Exert Multiple Beneficial Effects in a Rat Model of Non-Bacterial Prostatitis

Petra Popovics[1], Ferenc G Rick[2], Rhenzi Cai[1], Wei Sha[3], Andrew V Schally[2]

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Miller School of Medicine, University of Miami, Miami, FL, USA; [2]Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, Miami, FL, USA; [3]Interdisciplinary Stem Cell Institute, Miller School of Medicine, University of Miami, Miami, FL, USA

Introduction: The pathogenesis of benign prostatic hyperplasia (BPH) has been associated with various factors including hormonal imbalance, inflammation-induced cell proliferation and epithelial-to-mesenchymal transition (EMT). Our group previously demonstrated that prostatic levels of growth hormone-releasing hormone (GHRH) and its receptors are increased whereas GHRH antagonists possess anti-inflammatory action in a rat model of testosterone-induced BPH. Inspired by these studies, we recently utilized a mouse model of autoimmune prostatitis in which GHRH levels were also upregulated and GHRH antagonists reduced prostatic volumes. This indicates that GHRH may act as a local growth factor that is induced during inflammation and BPH and the blockade of its local action may confer therapeutic advantages in these conditions. Methods: A previously established model of prostatic inflammation was adjusted to demonstrate complications triggered by recurrent inflammation in Sprague-Dawley rats; 50 µl 3% carrageenan (sulphated polysaccharide) was injected into both right and left ventral lobes two times, three weeks apart. GHRH antagonist MIA-690 was administered 5 days after the second intraprostatic injection at 20 µg daily dose for 4 weeks. Results: Inflammation induced a 73.2% increase ($p<0.001$) in weights of the ventral prostate lobes which was reduced by 19.2% ($p<0.05$) in rats treated with MIA-690. Fluorescent staining of vimentin revealed that carrageenan injections increased the ratio of the stromal compartment which effect was downregulated by GHRH antagonists (2.85-fold vs. 1.79-fold elevation in total density). Western blot analysis showed that GHRH levels were increased to 264% ($p<0.01$) in inflamed prostates compared to controls. The inflammatory marker COX2 was also elevated to 376% ($p<0.05$) in carrageenan-induced prostatitis (CIP) but was reduced to 175% (ns. vs. control) in rats treated with MIA-690. CIP was also presented with elevated prostatic TGF-β1 levels (196% $p<0.05$) which was suppressed to 110% by GHRH antagonists. Prostatic IGF-1 levels measured by ELISA were increased by 65% ($p<0.01$) in CIP and were suppressed to control level by GHRH antagonist treatment. Analysis of transcript levels of genes related to EMT revealed an upregulation in several genes including collagens, matrix metalloproteinase 9, Snail1, TGF-β1 and vimentin. All of these genes were downregulated by treatment with MIA-690. Conclusions: Our current findings strongly indicate that GHRH is a key factor in prostatic inflammation-induced prostate enlargement. GHRH antagonists possess multiple actions by downregulating inflammation-related genes and by inhibiting EMT. Accordingly, GHRH antagonists could be clinically useful to treat early and advanced stages of BPH due to their anti-proliferative and anti-inflammatory activity.

P71

Developmental Origins of Prostate Neuroendocrine Cells

Mark T Cadena, BS Biology[1], Ryan L Trevena[1], Adam Gottschalk[1], Diya B Joseph[1], Michelle Southard-Smith[2], Chad M Vezina[1]

[1]University of Wisconsin-Madison, Madison, WI, USA; [2]Vanderbilt University, Nashville, TN, USA

Background: Prostate neuroendocrine cells are a rare population of neuromodulatory cells and their origin and function are not fully understood. This study tested whether prostate neuroendocrine cells derive from endoderm or neural crest, whether they harbor the transient receptor cation channel subfamily V member 1 (TRPV1) that is known to be expressed by, and activates, neuroendocrine cells outside of the prostate, and whether TRPV1-expressing neuroendocrine cell ablation changes prostate cell composition. Methods: Mice carrying Cre drivers for lineage labeling of the endoderm, neural crest, or sensory neurons were bred with lines harboring fluorescent reporter alleles. Additional mice harboring *Trpv1*^{cre} and an inducible form of diphtheria toxin were generated to examine the consequence of depleting prostate neuroendocrine and other TRPV1 expressing cells. Prostate sections from the resulting mice were immunostained to visualize lineage labeled cells and examine whether neuroendocrine cell depletion changes prostate stromal cell distribution. Results: We found evidence that prostate neuroendocrine cells arise from at least two progenitors (endoderm and neural crest) and potentially exist as two unique subpopulations. Further, we found that some prostate neuroendocrine cells express TRPV1 and that ablation of TRPV1+ cells leads to an abnormally thickened stroma characterized by an influx of CD45+ immune cells and vimentin+ fibroblasts. Conclusions: Together, these data support a dual origin of prostate neuroendocrine cells (endoderm and neural crest) and raise the hypothesis that at least some prostate neuroendocrine cells are responsible for prostate organ homeostasis. Elucidating differences in neuroendocrine cell subtypes is important when considering pathologies related to neuroendocrine cell biology. Funding provided by NIH U54 DK104310, U01 DK110807, U01 DK110804 (EMS).

P72

Heterogeneity and Drug Resistance in 3D Cultures of Patient Prostate Cancer Bone Metastases and Primagrafts

Christina AM Jamieson, Theresa Mendoza, Michelle Muldong, Abigail Gallegos, Christina N Wu, Danielle Burner, William Zhu, Olga Miakicheva, Elana Godebu, Omer Raheem, Jason R Woo, Nicholas A Cacalano, Christopher J Kane, Anna A Kulidjian, Terry Gaasterland

University of California, San Diego, La Jolla, CA, USA

Background: One in six men will be diagnosed with prostate cancer (PCa), making it one of the leading health problems affecting men in today's society. Patients diagnosed during the earlier stages are surviving longer due to improved therapies and the prevalence of prostate-specific antigen (PSA) testing. However, over 80% of advanced PCa patients develop bone metastatic prostate cancer for which there is no cure. Cell lines, widely used for the development of new drug treatments and therapies, fail to accurately recapitulate the heterogeneity of prostate cancer. Thus, it is important to establish new patient-derived cell culture models for bone metastatic prostate cancer which could better recapitulate the physiological processes that occur *in vivo*. Methods: Surgical prostate cancer bone metastasis samples were collected at the time of orthopaedic repair surgery and used to establish four novel patient-derived xenograft (PDX) models for advanced prostate cancer in the bone: PCSD1, PCSD4, PCSD5 and PCSD13. These PDX models closely reproduced bone metastatic disease in prostate cancer patients. In order to understand the changes that occur which may lead to progressive therapy resistance of the prostate cancer bone metastases we investigated and compared the genomic and transcriptomic variation in the longitudinal series of surgical bone metastasis prostate cancer patient samples and the xenografts derived from them. Through the incorporation of previously established methods into our own culturing methods, we optimized three-dimensional cell culture conditions which keep our patient-derived xenograft tumor cells viable *in vitro*. Results and Conclusions: Currently, we have established *in vitro* cultures for our patient-derived xenograft (PDX) and primary patient prostate cancer bone metastasis tumor cells which remain viable for more than 6 weeks without passaging. Furthermore, preliminary experiments showed that our three-dimensional cultures consist of heterogeneous cell populations that exhibit differential responses to hormone and drug treatments. In future experiments, we hope to further optimize our culturing conditions to improve the robustness and reproducibility of our three-dimensional cell cultures for patient-derived xenograft and primary prostate cancer tumor cells.

P73

Beta-arrestins Regulate Basal Cell and Cancer Stem Cell Phenotype in Muscle-invasive Bladder Cancer

Georgios Kallifatidis, Diandra K Smith, Jie Gao, James J Hoy, Richard Pearce, Jiemin Li, Vinata Lokeshwar, Bal L Lokeshwar; Assistant Research Scientist.

Augusta University, Augusta, GA, USA

Background: The muscle-invasive bladder cancer (MIBC) is the most aggressive form of bladder cancer with a five-year survival of less than 15%. High metastatic potential, poor response to chemotherapy and a largely basal cell molecular signature characterize MIBC. We identified two cytoplasmic proteins, β-arrestin 1 and β-arrestin 2 (BARR1 and BARR2) that are unevenly expressed in bladder cancer cell lines. The function of BARR1 and BARR2 is unknown in bladder cancer although these proteins are well-characterized in other systems, notably as regulators of G-protein coupled receptor activity in normal and abnormal cell physiology. We investigated the potential role of BARRs in established bladder cancer cells. Methods: Manipulations of expression of individual arrestins were performed by establishing stable cell lines with forced over expression by cDNA transfection, or depletion by shRNA or CRISPR-Cas9 mediated gene editing. The effect of altering BARRs in bladder cancer cells was investigated for changes in clonogenic survival, expression of basal cell markers, such as cytokeratins (CK), stem-cell associated markers, alteration in tumor cell motility and invasion. Results: Established cell lines from MIBC origin (e.g., 253J and HT-1376) had either high-level expression of BARR1 and BARR2 or only high expression of BARR1. Normal bladder cells predominantly expressed BARR2. Depletion of BARR2 in 253J cells resulted in increased basal cell markers (e.g. CK5, CK14). Depletion of BARR2 elevated many cancer stem cell (CSC) markers (e.g., ALDH2). BARR2 overexpression reduced expression of stem cell markers, and increased sensitivity towards gemcitabine. Furthermore, when cells were grown as spheroids - a technique used to enrich CSCs - we observed decreased expression of BARR2, whereas expression of BARR1 increased. In 253J cells, BARR2 depletion significantly increased motility, chemotaxis, and invasive potential. CRISPR-Cas9 mediated gene-knockout of BARR1 resulted in reversal of aggressive phenotype, including decrease in CD44, and phospho-STAT3. Conclusions: This is the first report of the role of BARR1 and BARR2 in MIBC. The results presented here strongly suggest a critical role of BARRs in bladder cancer metastasis and resistance to chemotherapy drugs; two key factors that determine the poor survival of bladder cancer patients. Further exploration of the roles of β-arrestins in bladder cancer has potential to improve therapy for patients with muscle invasive bladder cancer.

P74

Roles for Tumor-derived Matrix Metalloproteinase 3 (MMP-3) in Prostate Cancer Growth in Bone

Jeremy S Frieling[1], Leah M Cook[2], Marilena Tauro[1], Conor C Lynch[1]

[1]Moffitt Cancer Center, Tampa, FL, USA; [2]University of Nebraska Medical Center, Omaha, NE, USA

Background: The matrix metalloproteinases are a family of zinc-dependent proteases active in biological processes such as development, tissue repair, and tumorigenesis via their degradation of extracellular matrix and processing of bioactive proteins. MMPs have been shown to either promote or inhibit tumor growth depending on tissue context, therefore, it is necessary to study individual MMPs in specific cancers. Our lab has previously identified roles for MMP-2, -7, and -9 in bone metastatic prostate cancer, however, despite heightened expression in human prostate cancer specimens and datasets, roles for MMP-3 in the prostate cancer-bone microenvironment remain undefined. Methods: Publicly available datasets (Oncomine) and immunofluorescence staining of human bone metastatic prostate cancer tissue sections were used to assess MMP-3 expression levels. PCR was used to confirm MMP-3 expression in human and rodent-derived prostate cancer cell lines (LNCaP, C4-2B, PC3-M, and PaIII). Stable MMP-3 knockdown cell lines were generated with shRNA, and luminescence-based in vitro growth assays used to compared the growth of control and MMP-3 knockdown cell lines. An in vivo intratibial model of prostate to bone metastases was used to assess tumor growth in bone using bioluminescence as an indicator of growth. In vivo proliferative differences were confirmed with phospho-histone H3 immunofluorescence. Subsequent ex vivo analyses included X-ray (Faxitron) and microCT to measure tumor-induced effects on bone. Cytokine arrays were used to study differences in secreted protein content between control and MMP-3 knockdown cells. Signal transduction pathways were examined by immunoblotting. Results: Our analyses demonstrate strong expression of MMP-3 by tumor cells in both prostate cancer cell lines and human prostate to bone metastasis specimens. Ablation of tumor-derived MMP-3 significantly mitigated prostate cancer cell growth in vitro and in vivo ($p<0.05$), however no effects on associated bone disease were observed. A candidate approach using cytokine arrays revealed a 60% increase in levels of insulin growth factor binding protein 3 (IGFBP3) in MMP-3 knockdown cell conditioned media compared to control. The increased IGFBP3 levels corresponded with a reduction in IGF receptor 1 (IGF1R) phosphorylation. Further, we observed diminished ERK and AKT phosphorylation downstream of IGF1R, which could explain the decreased growth observed following MMP-3 knockdown. Conclusions: Our results suggest that tumor-derived MMP-3 contributes to prostate cancer growth in bone. These data indicate that selective inhibition of MMP-3 and/or targeting MMP generated neo-epitopes could be efficacious for the treatment of prostate to bone metastases.

P75

Mouse Bladder is a De Novo Organ

Liguang Xia, Nehizena S Aihie, Haochuan Zhang, Xue Sean Li

Boston Children's Hospital, Boston, MA, USA

The bladder is a hollow organ which serves as reservoir for urine produced by the kidneys. The bladder also serves as a sensory organ, sending signals to the brain that it is time to void. The bladder is also one of the most common sites of urinary tract infections. Despite its importance, the morphogenetic processes of urinary bladder and development remain largely unknown and speculative. This study attempts to shed light on this using the high resolution episcopic microscopy (HREM) to obtain detailed morphometric features of the developing bladder in wild type mice. Coupled with comprehensive 3-dimenstional reconstructions and molecular markers, we provide evidence suggesting that of the urethra and the bladder are embryonically distinct structures. Using Sox2 and Wnt2 markers, we show the bladder and the urethra have different molecular features. HREM 3D reconstructions were created from mouse embryonic day 8.5 to 15.5 to trace the development of the developing bladder. Morphometric data was collected to track the surface area, angle, and volume of the developing bladder. A critical point of bladder development was discovered during our observational study which we believe lends to the idea that the bladder may be a de novo structure. Additionally we have uncovered that the mouse cloaca has no association with the allantois, which argues against the notion that the bladder is formed from the ventral urogenital sinus that is continuous with the outside environment via the allantois in mammals. Collectively, these findings suggest that the bladder may not simply a derivative of the preexisting structure, eg., urogenital sinus of the cloaca but instead a de novo structure. The findings improve our understanding of normal urinary tract development, which may help to uncover embryonic basis of congenital urinary tract malformations such as bladder exstrophy, epispadias, as well as ureteral pathologies.

P76

Defining New Drivers of Castration-resistant Prostate Cancer

En-Chi Hsu[1], Meghan Rice[1], Rosalie Nolley[1], Abel Bermudez[1], Jiaoti Huang[2], Donna Peehl[1], Christian Kunder[1], Sharon Pitteri[1], James Brooks[1], Tanya Stoyanova[1]

[1]Stanford University, Palo Alto, CA, USA; [2]Duke University, Durham, NC, USA

Prostate cancer is the second most common cancer among men in the U.S. One of the major clinical challenges is treatment of advanced metastatic prostate cancer. The first line of treatment for metastatic prostate cancer is androgen ablation. Unfortunately, the disease commonly relapses in its lethal metastatic form referred to as castration resistant prostate cancer (CRPC). Current therapies have provided limited extension of the overall survival in patients with CRPC. Thus, there is an urgent need to identify new drivers and therapeutic targets for aggressive prostate cancer which will provide us with new insights into the development of novel therapeutic strategies for the advanced disease. Trop2 is a cell surface glycoprotein that is commonly altered in a broad range of epithelial cancers. Due to its overexpression in multiple epithelial cancers, Trop2 has emerged as a promising therapeutic target. An anti-Trop2 antibody conjugated with cytotoxic drug SN-38, Sacituzumab govitecan (IMMU-132), has been recently developed and has shown a favorable therapeutic effect in triple negative breast and other cancers. Currently, IMMU-132 is being evaluated as a single agent in patients with advanced epithelial cancers. Recently, we demonstrated that elevated expression of Trop2 is associated with high-risk prostate cancer and predicts disease recurrence. Trop2 is also highly expressed in CRPC and metastatic prostate cancer. To assess the functional role of Trop2 in prostate tumorigenesis we used CRISPR/Cas9 to achieve Trop2 gene deletion and lentiviral transduction to overexpress Trop2. Our study demonstrates that loss of Trop2 significantly delays growth, migration, and invasion of prostate cancer cells while overexpression of Trop2 enhances prostate cancer cell proliferation and invasion. Elevated expression of Trop2 induces androgen independent cell proliferation, and tumorsphere forming ability *in vitro*. We further demonstrate that overexpression of Trop2 enhances tumor growth and drives castration resistant phenotype *in vivo*. Tumors driven by Trop2 overexpression exhibit aggressive phenotype, loss of AR and neuroendocrine features. Furthermore, loss of Trop2 gene suppresses tumor growth *in vivo*. Our study identified Trop2 as a new driver of CRPC and neuroendocrine prostate cancer. Our results demonstrate that Trop2 plays a functional role in prostate cancer and CRPC. These findings provide a strong functional evidence that Trop2 may represent a novel rational therapeutic target for metastatic CRPC.

P77

Increased Tumor Induction by Prostate Fibroblasts from African American Men Compared to Caucasians

Marc Gillard[1], Rodrigo Javier[2], Susan Crawford[2], Donal Vander Griend[1], Omar Franco[2]

[1]University of Chicago, Chicago, IL, USA; [2]North Shore University Health System, Evanston, IL, USA

Introduction: Despite advances in the last 20 years in the diagnosis and treatment of prostate cancer (PCa), the incidence and death rate is still significantly higher for African Americans (AA) compared to Caucasian (CA) men. Although socioeconomic factors may be responsible to a certain extent, it is now appreciated that intrinsic differences in genetics and tumor biology make AA men more prone to aggressive PCa. Tumor evolution (from local carcinogenesis to distant metastasis) is strongly influenced by the microenvironmental conditions encountered by cancer cells. However the biological effects of fibroblasts in the TME of AA patients has not been intensively studied due to the lack of suitable models. Here we isolated fibroblasts from human prostate tissues from AA patients with PCa and compared the biological effects on several PCa cell lines to evaluate the similarities and/or differences to those from CA-derived fibroblasts. Method: Prostate fibroblasts were isolated from patients (AA and CA) undergoing robotic assisted laparoscopic prostatectomy (RALP) and cultured for less than 8 passages. In vitro characterization included proliferation assay and the expression of potential markers of carcinoma associated fibroblasts (CAF) including co-expression of αSMA and vimentin, FAP, and Tenascin-C. In addition, the effects of AA vs. CA fibroblasts on prostate cancer cell proliferation and motility were studied. In vivo pro-tumorigenic properties of fibroblasts were tested using a tissue recombination and subrenal xenograft model in SCID mice. Cytokine array analysis of potential paracrine mediators of tumorigenesis was performed. Results: Prostate fibroblasts from AA showed increased proliferation response to mitogens compared to CA. Expression of markers associated with myofibroblast activation (αSMA, vimentin and Tenascin-C) as well as AR were significantly elevated in while Caveolin 1 expression was lower in AA fibroblasts. AA fibroblasts significantly increased the tumorigenicity in vitro and in vivo of an AA patient-derived prostatic epithelial cell line E006AA compared to CA. Analysis of potential paracrine mediators identified a panel of pro-inflammatory cytokines notably interleukins (IL6, IL11, IL17, IL18BP), growth factors (VEGF, FGF, BDNF), and other mediators (DPPIV, CHI3L1, uPAR, Dkk1, CCL7 and Emmprin) to be enriched in AA fibroblasts. Conclusions: Prostate fibroblasts from African American men show enhanced secretion of pro-inflammatory mediators that can potentially increase the tumorigenicity of PCa cells through selective paracrine mechanisms.

P78

The Prostatic Inflammatory Environment of the NOD/ShiltJ Mouse

LaTayia Aaron[1], Susan Crawford[2], Simon Hayward[2]

[1]Meharry Medical College, Nashville, TN, USA; [2]North Shore University Health System Research Institute, Evanston, IL, USA

Background: Benign prostatic hyperplasia (BPH) has been associated with a number of morbidities related to metabolic syndrome. Metabolic syndrome includes diabetes mellitus, obesity and hyperlipidemia. It is not clear whether diabetes alone or in conjunction with obesity promotes a BPH phenotype. The non-obese diabetic (NOD) mouse is a model of autoimmune inflammation leading to type 1 diabetes in a proportion of mice. This model was used to determine the effects of diabetes and auto-immune inflammatory dysregulation on prostatic hyperplasia. Methods: Prostatic tissues harvested from NOD mice were divided, some were fixed and paraffin embedded, while other portions were wither processed for FACS analysis or frozen in OCT. Histological appearance of the prostate was evaluated by H&E staining while immunohistochemial staining determined the presence of inflammatory cell markers. The markers analyzed included CD4, a T-cell marker; CD19, a B-cell marker; and F4/80, a general macrophage marker. Microarray and cytokine array analysis were performed to determine which genes and cytokines are upregulated in the NOD mouse model. Results: Although dysregulation in the function of specific inflammatory cells in the NOD mouse model has been described in the literature, we were able to detect T-cells, B-cells and macrophages. Areas of inflammation were observed in both non-diabetic and diabetic mice. However, diabetic mice tended to exhibit more inflammation and in some areas the inflammatory cells migrated into the epithelial compartment. The microarray performed analyzed 22,207 total genes, of these 1,333 were upregulated in diabetic mice including c-myc binding protein and several TNF-α interacting proteins. Cytokine array analysis probed 111 cytokines, chemokines, and growth factors and approximately 20 of these analytes were upregulated in diabetic mice including AdipoQ, CD142 and PAP3. Conclusions: BPH is a complex disease and there are no animal models which replicate all of the components seen in human patients. Inflammation has been correlated with BPH pathogenesis in humans. Use of the NOD mouse model enabled us to examine the effects of inflammation and diabetes in a non-obese model. In this model, areas of focal and/or severe inflammation was observed in both non-diabetic and diabetic mice nonetheless diabetic mice tended to exhibit a more severe inflammatory phenotype.

P79

Targeting Androgen Receptor in Combination with Cisplatin: Effective Treatment Strategy for Muscle Invasive Bladder Cancer

Ashish Tyagi[1], Balaji Chandrasekaran[1], Samarpit Rai[1], Alatassi Houda[2], Ahmed Haddad[1], Murali K Ankem[1], Chendil Damodaran[1]

[1]Department of Urology, University of Louisville, KY 40202, USA; [2]Department of Pathology, University of Louisville, KY 40202, USA

Background: Bladder cancer is one of the major causes of cancer death in US and worldwide. Cisplatin is a key component of chemotherapeutic regimens employed in the treatment of advanced bladder cancer. The role of androgen and androgen receptor (AR) signaling in bladder cancer remains uncharacterized. Aim: The aim of the study is to delineate the role of AR in bladder cancer and to determine whether combination of AR inhibitor, enzalutamide (Enz) and cisplatin-based therapies effectively inhibit the growth of muscle invasive bladder cancer (MIBC). Methods: AR expression was determined in 75 human bladder cancer specimens and in a panel of bladder cancer cell lines. Cells grown in charcoal stripped media supplemented with dihydrotestosterone (DHT) were treated with cisplatin, enzalutamide (AR inhibitor), or a combination of both. Cellular/phenotypic analysis including MTT assay, apoptotic assay, migration as well as invasion assays and molecular analysis including western blotting, real time PCR analysis were performed. Isobologram analysis for the combination was performed and analyzed with CompuSyn. Experiments were repeated in triplicates and analyzed with unpaired Student's t-test and one way ANOVA *p≤0.05, **p≤0.01, ***p≤0.001. Results: AR expression was seen in around 40% of bladder cancer patients. Inhibition of AR signaling by enzalutamide effectively inhibited the growth of AR+ MIBC cells. Interestingly, enzalutamide in combination with cisplatin (Enz + Cis) synergistically inhibited the proliferation of MIBC cells, TCCSUP (CI: 0.42, 1.25 + 5 μM) and J82 (CI: 0.79, 2.5 + 5 μM) at low concentrations of enzalutamide and cisplatin resp.. The molecular studies revealed the induction of DNA damage markers (pATM, pATR, pChk1, pHis) and enhanced expression of the pro-apoptotic genes (Bax, caspases-3 and PARP) in Enz+Cis treated AR+ MIBC cells. In addition, we demonstrated abrogation of invasive and migratory potential with Enz + Cis treatment, by downregulation of the mesenchymal markers (N-cadherin, slug, β-catenin, and vimentin) in both cell lines. Our studies suggest combination of Enz + Cis may be effective in patients with AR+ MIBC. Conclusion: Combination of cisplatin and AR inhibition effectively inhibit bladder tumor growth and migration, and hold promise as synergetic therapies for AR+ bladder cancer patients.