Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

Precision Medicine in Urology: Molecular Mechanisms, Diagnostics and Therapeutic Targets - Report of the Society of Basic Urologic Research 2018 Annual Meeting Entitled

Magdalena Grabowska[1], Yan Dong[2], Christina AM Jamieson[3], Larisa Nonn[4], Scott Dehm[5], Allen Gao[6], Cindy K Miranti[7], Xue Li[8], Rosalyn M Adam[8], Douglas Strand[9], Marc B Cox[10]

[1]Case Western Reserve University School of Medicine; [2]Department of Structural and Cellular Biology, Tulane University School of Medicine; [3]Moores Cancer Center, University of California, San Diego; [4]University of Illinois at Chicago; [5]Department of Urology, Department of Laboratory Medicine and Pathology, Masonic Cancer Center, University of Minnesota; [6]University of California Davis School of Medicine; [7]Department of Cellular and Molecular Medicine, University of Arizona; [8]Department of Urology and Surgery, Boston Children's Hospital, Harvard Medical School; [9]University of Texas Southwestern Medical Center; [10]Border Biomedical Research Center and Department of Biological Sciences, University of Texas at El Paso

The 2018 Annual Meeting of the Society of Basic Urologic Research (SBUR) convened on November 8, 2018. This year's conference was held in sunny Rancho Mirage, CA and included a record-breaking number of participants. Over the course of four days and six plenary sessions, we attended talks describing basic mechanisms of steroid hormone receptor-mediated transcription; molecular pathways and molecules driving urologic disease pathogenesis and metastasis; alternative AR-targeted therapies and mechanisms of treatment resistance; emerging targeted therapies, prognostic and diagnostic markers in bladder, renal, and prostate cancer; phenotypic heterogeneity in benign urologic disease; genetic predisposition and health disparities in prostate cancer; and emerging technologies in urologic research including single cell genomics, metabolomics, and digital image analysis. Nestled between these talks were two spectacular poster sessions and a Trainee Affairs Symposium, continuing the SBUR's commitment to trainee development.

The conference opened with a plenary session focused on molecular mechanisms and histological phenotypes associated with advanced prostate cancer. Dr. Eva Corey, PhD began the session with an overview of the necropsy program at the University of Washington School of Medicine in Seattle. Metastases can be characterized as androgen receptor positive/Neuroendocrine marker negative. A number of cell lines and patient-derived xenograft (PDX) preclinical models have been established to study each phenotype and their responsiveness to different therapies. A PDX clinical trial set up using these LuCaP PDXs showed that multiple combinations display high efficacy in inhibition of tumor progression while monotherapies had generally limited effects. Finally, data were presented to demonstrate that supraphysiological testosterone therapy inhibits castration resistant prostate cancer (CRCP) progression with accompanied inhibition of ARV7, E2F, and DDR programs. Dr. Gail Prins, PhD from the University of Illinois at Chicago presented her work on the hierarchy of stem cells in the prostate. She showed that approximately 1% of primary human prostate epithelial cells grown in Matrigel as spheroids survive and replicate, serving as facultative progenitors. Treatment with estrogen increases the survival and size of spheroids and long-term BrdU retention labels $KRT13^{+}/KRT14^{-}/WNT10B^{+}/ECAD^{-}/ER\beta^{+}/ER\alpha^{lo}$ cells with stem-like characteristics. The stem and progenitor cells are AR^{-} and express estrogen receptor (ER) α and ERβ with unique functions. Knockdown of ERα increases ERβ expression and, conversely, estrogen decreases ERβ expression through ERα. ERα expression in the

stem-like cells is regulated by the ubiquitin-proteasome pathway by the selective ligases SPOP and BRCA1. Estrogen signaling in the stem-like and progenitor cells is mediated through genomic and non-genomic signaling pathways with membrane-initiated signaling by ERα primarily through PI3K-Akt signaling whereas ERβ preferentially activates the Ras-Raf pathways. Finally, Dr. Nora Navone, MD, PhD from the University of Texas MD Anderson Cancer Center closed the session with a presentation summarizing the progress on the prostate cancer PDX program at MD Anderson (the MDA PCa PDX series), and on the role of the FGF axis in metastatic prostate cancer progression. In a study of the first 51 PDX established in the MDA PCa PDX program, she reports that 65% of the tumors display an adenocarcinoma and 35% a neuroendocrine phenotype. Also, 81% of the adenocarcinomas are AR^+, 41% contain gene fusions, and 71% are PTEN null. To-date the program has established 18 PDXs derived from bone metastases, 90 derived from primary prostate cancers, and 32 from metastases. Using some of the available MDA PCa PDXs she discovered that prostate cancer cells induce FGF signaling in the bone, which then reciprocally signaled back through tumor cell FGFR1 to increase survival. FGFR1 expression in PC3 cells caused decreased survival of mice and accelerated metastases after intracardiac injection in mice. These data help explain why Dovitinib, an FGFR inhibitor, causes regression of bone metastasis in castrate resistant prostate cancer.

The highlight of this year's conference was the Leland W.K. Chung Keynote Lecture presented by Dr. Jason Carroll, PhD from the Cambridge Research Institute at Cambridge University. Dr. Carroll studies hormone dependent transcription factors with the goal of defining how AR and ER mediate gene expression and what the events are that culminate in drug resistance, including castration resistant prostate cancer (CRPC). In this context, the presentation focused on understanding the hormonal crosstalk between the Progesterone Receptor (PR) and ER. There is a long and controversial relationship between PR and ER, with much of the literature suggesting that progesterone activation of PR is a pro-proliferative event. However, this is based on extrapolation from studies in healthy tissue and it was shown that in ER-driven cancer, activation of PR can inhibit ER by sequestering the ER complex to different regions in the genome. In addition, they used CRISPR screening approaches to discover genes involved in endocrine response, revealing the BAF protein ARID1A, as a crucial factor that maintains a transcriptionally repressive environment. ARID1A is frequently mutated in cancer and data showing that this results in a shift to a BRD4-driven tumor context was shown, which reveals opportunities for exploiting epigenetic inhibitors. These findings demonstrate that activation of parallel endogenous Nuclear Receptor (NR) pathways can impinge on the driving NR, an area that Dr. Carroll is currently exploring in prostate cancer with the goal of reprogramming AR binding and blocking transcriptional activity. The findings also reveal important chromatin regulatory factors that contribute to gene regulation in prostate cancer cells.

Despite the challenges of anti-androgen resistance, AR remains a key transcriptional regulator and therapeutic target in prostate cancer. A number of presentations focused on alternative AR-targeting approaches and mechanisms of anti-androgen resistance in prostate cancer. Dr. Steven Balk, MD, PhD from Harvard Medical School highlighted mechanisms associated with resistance to AR-targeted therapies, focusing on enzalutamide/abiraterone. Several mechanisms including AR-V7, DPP4, ERBB2 (Her2), and Wnt signaling were overexpressed in a model of enzalutamide/abiraterone resistant-VCaP cells generated in VCaP cells treated with both enzalutamide and abiraterone. Strategies for targeting these overexpressed mechanisms and re-sensitizing the resistant cells to AR-targeted therapies were discussed. Dr. Artem Cherkasov, PhD from the Prostate Centre at Vancouver General Hospital and the University of British Columbia discussed the mutability of the AR ligand binding domain, and the use of cell free DNA profiling to monitor the emergence and divergence of AR ligand binding domain mutations in patients undergoing therapy with AR antagonists. As an alternative strategy to AR antagonists, targeting an alternative solvent-exposed surface near the AR ligand binding domain termed Binding Function 3 (BF3) was highlighted, in addition to the computer-assisted drug discovery strategies they have used to target BF3. Dr. Hannelore Hee-

mers, PhD from the Cleveland Clinic presented on the factors that contribute to transcriptional output of AR including the DNA sequence of the androgen response element, adjacent DNA binding sites for pioneer factors, and the repertoire of co-regulators that interact with AR. The results of a screen for co-regulator dependencies of AR focused on the mechanisms of a novel co-regulatory circuit wherein WDR77 bridges the transcriptional activities of AR and p53. These results indicate that WDR77 could represent an attractive therapeutic target to selectively disrupt an oncogenic transcriptional circuit in prostate cancer cells. Dr. Natasha Kyprianou, PhD from the University of Kentucky discussed mechanisms associated with resistance to taxanes and strategies to overcome resistance. Among the many mechanisms discussed, taxane-resistant cells exhibited an EMT phenotype mediated by TGF-beta. Data was presented showing that targeting the TGF-beta pathway using kinesins reversed EMT to MET, and re-sensitized resistant cells to taxane chemotherapy. Dr. Paul Rennie from the Prostate Centre at Vancouver General Hospital and the University of British Columbia presented on several newly discovered sites on the AR protein that can be targeted with small molecule inhibitors in order to inhibit both full length and truncated AR variants. Strategies to target the AR DNA binding domain via the P-Box site with VPC-14449 small molecule inhibitor, or the D-box site with the VPC-17005 small molecule inhibitor, were presented. These novel inhibitors were discovered using an *in silico* computer-guided screen followed by biochemical and cell-based testing and validation. It is anticipated that drugs based on these inhibitors will effectively treat many types of castration resistance. Alan Lombard from Dr. Allen Gao's laboratory at the University of California Davis gave the first travel award presentation focusing on mechanisms of prostate cancer treatment resistance that overlap between AR-targeted therapies, taxane chemotherapy, and the PARP inhibitor olaparib. Evidence that overexpression of the multidrug resistance protein 1 encoded by the ABCB1 gene may represent a mechanism that drives cross-resistance to docetaxel and olaparib in prostate cancer was presented. Finally, Megan Rice from Dr. Tanya Stoyanova's laboratory at Stanford University gave the second travel award presentation highlighting the results from the screening of a methoxychalcone chemical library to identify inhibitors of prostate cancer growth. A lead compound was identified that appeared to have a good pharmacokinetics and toxicity profile, and was able to synergize with enzalutamide and abiraterone to inhibit growth of prostate cancer cell lines. The use of proteomics screening to identify the cellular targets of this methoxychalcone compound was highlighted.

In addition to AR-targeted therapies for the treatment of prostate cancer, a number of presentations highlighted emerging novel targets, therapies, and prognostic/diagnostic indicators in a variety of urologic disease settings. This session opened with the American Urological Association (AUA) named lecturer, Dr. Claus Roehrborn, MD, from UT Southwestern in Dallas. Dr. Roehrborn provided a historical perspective on benign prostatic hyperplasia, including a discussion of the current state of understanding of the condition with an emphasis on phenotypic heterogeneity. Dr. Roehrborn discussed the challenges of treatment for BPH and associated lower urinary tract symptoms (LUTS) noting that medical therapies such as alpha-blockers and 5-alpha reductase inhibitors have a modest benefit in terms of symptomatic relief. Moreover, alpha-blockers and 5-alpha reductase inhibitors are administered to patients based primarily on size/volume of the prostate, in the absence of knowledge of the underlying BPH subtype displayed by a given patient. The BPH subtypes in both histopathological and molecular terms were described, identifying inflammation as an important determinant of phenotype. He concluded with recommendations for the clinical management of BPH based on knowledge of causative molecular pathways, identification of BPH phenotypes and associated biomarkers and evaluation of pathways driving resistance to current medical therapies. Dr. Timothy Meyer, MD, from Stanford University described colon-derived compounds that contribute to uremia in patients with kidney disease. In healthy individuals, organic compounds produced by the gut microbiota are absorbed in the colon and excreted by the kidneys. However, in patients with kidney disease, where excretion is diminished, these solutes can accumulate in plasma and may become toxic. Dr. Meyer discussed the application of mass spectrometric methods

to characterize of uremic solutes, as well as potential manipulation of the microbiome to modify the nature of organic compounds produced and minimize their toxicity in patients with kidney disease. Dr. Shu-Yuan Yeh, PhD from the University of Rochester presented her work on the role of estrogens and ER in bladder cancer pathogenesis, and how ER signaling may alter the immune microenvironment of bladder tumors. She presented data using ER isoform-specific knockout mice that ER-alpha protected against the development of bladder cancer whereas ER-beta promoted tumor formation. Dr. Yeh discussed the ER-alpha and ER-beta effectors INPP4B and MCM5, and their regulation of tumor cell growth. She also discussed co-culture of bladder cancer and mast cells that led to increased levels of the chemokine CCL2, and ultimately to enhanced invasion. Ongoing studies are exploring the role of ER-beta in regulation of PD-L1 in the tumor microenvironment and the implications of this for bladder cancer targeting. Dr. James Brooks, MD from Stanford University discussed benign prostatic hyperplasia from the perspective of prognostic indicators, and developed the concept that BPH arises as a result of 'cellular re-landscaping'. Using next-generation sequencing for transcriptional profiling, Dr. Brooks described at least two subtypes of BPH that suggested correlation with symptoms and the extent of bother to patients. One subtype was characterized by increased expression of BMP5 and CXCL13, both of which encode bioactive ligands that could participate in signaling to promote prostatic growth. He also described a shift in cell types within the stroma in BPH, described as cellular re-landscaping, reflected at both the molecular and histopathological levels. Dr. Kenneth Pienta, MD from Johns Hopkins University School of Medicine concluded the session with a provocative presentation on new ways of conceptualizing prostate cancer metastasis. He presented a model that incorporated features of the 'seed', namely the influence of dynamic cell behavior in the primary tumor leading to tumor cell escape and heterogeneity among cells comprising the primary tumor as well as the 'soil', namely the receptivity of the sites, such as lymph node or distant organs, in which disseminated cells would seed. In addition, he emphasized the use of PSMA imaging to characterize the dynamics of prostate cancer cell metastasis and how it could be deployed to stratify patients at risk of developing lethal disease.

African American (AA) men are diagnosed with aggressive prostate cancer more often, at a younger age and have more than twice the prostate cancer mortality rate as European Americans (EAs). A number of presentations focused on this disparity in prostate cancer. Dr. Rick Kittles, PhD from the City of Hope National Medical Center started off the session with a thorough background of the disparity of prostate cancer in AA men. Even after controlling for factors that are related to access to health care, the prostate cancer disparity in AA men remains, suggesting that ethnicity-related genetic and life style-affected epigenetic variations in prostate cancer tumors may contribute to the early onset, high incidence, and mortality rate in AA men. Dr. Kittles presented his research into genetic heterogeneity within the AA and other populations and how it contributed to biological diversity. His research using Ancestry Informative Markers (AIMS) facilitates rigorous analysis of prostate cancer within a genetically complex population. He also presented his findings that one of the widely used prostate cancer cell lines for racial disparity research, E006 cells, have <10% West African Ancestry. Dr. Anna Woloszynska, PhD from Roswell Park presented her work on epigenetic and genetic alterations as potential determinants of prostate cancer health disparities. She showed that DNA methylation patterns are different in tumor tissues from in AA and EA prostate cancer patients and correlate with poorer clinical outcomes in AA patients. Via integration of methylomic, transcriptomic, and mutation tumor data as well as expression of androgen receptor, Dr. Woloszynska identified different biological processes that may account for the disparity. Based on the multilevel analysis, she presented a model in which she showed how calcium pools, wnt signaling, GATA4 master transcription factor, and the cytoskeleton may contribute to androgen metabolism and AR protein levels, and impact different outcomes in AA and EA prostate cancer patients. Dr. Isaac Powel, MD, from Wayne State closed the session from the vantage point of a surgeon scientist. He presented his research to examine the ethnic differences in the biology and genetics of prostate cancer. His work focuses on inflammation as a driver of de-differ-

entiation of prostatic cells via cytokines. The pro-inflammatory genes are expressed at higher levels in AA men compared to EA men and may serve as a further therapeutic target to reduce the racial disparity in prostate cancer.

A variety of new technologies were highlighted for their application to urologic research. Dr. Gary Hon, PhD from UT Southwestern Medical Center presented work on using single-cell sequencing technology to unravel the complexity of transcriptional regulation. First, Dr. Hon described the development of mosaic single-cell analysis (Mosaic-seq), which uses the dCas9-KRAB system to perturb enhancers and then quantifies the effects on the transcriptome in single cells by indexed CRISPR sequencing. With Mosaic-seq, they measured the endogenous activities of 71 constituent enhancers from 15 super-enhancers and found that only a small number of constituent enhancers are main regulators of target-gene expression. It was also shown that simultaneous repression of several weak constituent enhancers synergistically modifies super-enhancer activity. Mosaic-seq allows interrogation of enhancer function in a high-throughput, unbiased, endogenous, and single-cell fashion. Dr. Hon talk highlighted work on the development of Reprogram-seq in which single-cell RNA-seq was performed on fibroblasts that are transduced with a library of transcription factors and are allowed to differentiate into other cell types. With Reprogram-seq, his group identified 3 transcription factors that reprogram mouse embryonic fibroblasts to epicardial cells. Further study of these transcription factors will have significant clinical applications. Dr. Arun Sreekumar, PhD from Baylor College of Medicine presented work on metabolic re-wiring in prostate cancer. Through metabolomics profiling, Dr. Sreekumar demonstrated sarcosine, an N-methyl derivative of the amino acid glycine, as an important metabolic intermediary of prostate cancer invasion and metastasis, and the role of androgen in activating amino acid metabolism in prostate cancer cells. Dr. Sreekumar also discussed the differences between AR and its splice variant 7 (AR-V7) in regulating metabolic pathways. While AR increases citrate levels, AR-V7 reduces citrate, which mirrors metabolic shifts observed in CRPC. The low citrate is a result of enhanced citrate metabolism rather than reduced synthesis of citrate. While both AR and AR-V7 enhance citrate production from glucose, AR-V7 exhibits increased dependence on glutaminolysis and reductive carboxylation to produce citrate. Dr. Sreekumar further described the role of steroid receptor coactivator 2 (SRC-2) in driving glutamine-dependent de novo lipogenesis to support prostate cancer cell survival and metastasis. Moreover, using a network-based integrative approach combining gene expression data with metabolomics data, Dr. Sreekumar showed downregulation of the hexosamine biosynthetic pathway (HBP) in castration-resistant prostate cancer compared to localized prostate cancer and the therapeutic potential of using HBP metabolites to treat castration-resistant prostate cancer. Lastly, Dr. Sreekumar presented the differences in methionine-homocysteine cycle in prostate cancers of European American and that of African American patients. Collectively, Dr. Sreekumar demonstrated that metabolic re-wiring in prostate cancers has significant therapeutic and biomarker implications. Dr. Stephen Hammes, MD, PhD from the University of Rochester Medical Center discussed the role of myeloid-derived suppressor cells (MDSCs) in prostate cancer progression. Dr. Hammes showed that increased number of MDSCs in prostate tumors is associated with poor clinical outcomes of the patients. In addition to suppressing T cell response, MDSCs can directly promote prostate tumor growth, invasion, and metastasis by producing an enzyme called neutrophil elastase that stimulates EFGR signaling. Prostate epithelial cells express an endogenous inhibitor of neutrophil elastase, SERPINB1. The expression of SERPINB1 is downregulated in prostate cancer via DNA methylation, and the downregulation is associated with shorter survival of the patients. Taken together, Dr. Hammes showed an important role of MDSCs in promoting prostate cancer progression. Dr. Beatrice Knudsen, MD, PhD from Cedars-Sinai Medical Center presented her work on developing two new methods that enhance the diagnostic and prognostic power of imaging in tumor pathology. The addition of deep learning, or machine learning, and artificial intelligence (AI) to the process cut the analysis time of histologic slides from 8 hours of computer time to 1 minute. First, Dr. Knudsen described their hypothesis-based, computational machine learning approaches to quantify tissue architecture which they combined with

gene expression signatures to stratify prognosis of renal cancer more accurately. This algorithm for automated outlining of the vasculature was applied to a cohort of 64 cases of clear cell renal carcinoma in the Cancer Genomics Atlas (TCGA) with high quality H&E images, gene expression data and clinical outcomes data for each patient. This discovery cohort was then used to identify an expression signature of 14 genes which were highly correlated with the 9VF vascular morphology features signature and could be used to separate patients into low and high risk DFS. Second, Dr. Knudsen highlighted the development of a combined IHC and image analysis workflow to quantitate the immune infiltrate using 5 chromogenic antibodies on 2 slides using the OPAL multiplex staining system. The slides are stained, de-stained, then re-stained with the next antibody in sequence on the same tissue section. Images are overlayed and an image analysis pipeline they developed is applied. New analysis software for accurate image registration between the two slides was developed. This allows for quantitative measurement of the specific immune sub-types in the TILs of tumor tissue sections for more accurate immunotyping of tumors for potential immunotherapy treatment planning. The third travel award presentation was given by Dr. Sanghee Lee, PhD from Dr. Christina Jamieson's laboratory at the University of California San Diego. Dr. Lee presented her work on a new model of patient-derived bone metastatic prostate cancer using samples obtained during orthopedic repair surgery of pathologic fractures or impending fractures caused by prostate cancer bone metastasis. The new PDX models are called the Prostate Cancer San Diego (PCSD) series. Usually, PCSD1 cells form tumors only at the site of injection and do not metastasize to distant sites in the mouse. However, in one mouse, in which PCSD1 cells had been injected into the femur, tumors were found at the neck and in the abdomen. As seen with in vivo bioluminescence, these tumors came from the intra-femorally injected PCSD1 which we have engineered to stably express GFP and luciferase. The aim of Dr. Lee's research was to characterize 3D/organoids derived from this spontaneous advanced metastasis (PCSD1_SAM), in which the PDX cells had metastasized from the femur to the neck and abdomen. This study showed that PCSD1_SAM organoids have acquired a more basal progenitor-like phenotype than the original PDX. The organoid-forming prostate cancer progenitor cells maintain cell-type representation and recapitulate in-vivo tumor structure. Thus, the PDX: PCSD1_SAM model represents an in vitro differentiation model of metastatic tumor formation. Dr. Ganesh Raj, MD, PhD from UT Southwestern concluded the session by highlighting work on developing and using a tumor tissue explant model using primary prostate tumor tissue cultured on sponge matrices in vitro to test therapies and understand tumor and microenvironment interactions. The advantage of this tumor explant sponge model over other patient derived models such as organoids and xenograft models is that primary prostate tumors with their original tumor architecture and microenvironment intact can be studied while all other model systems for prostate cancer are derived from metastatic cells that have been dissociated from their microenvironment. The model was validated by testing the therapeutic response to established agents such as bicalutamide and by measuring H&E histopathology compared to the original tumor, BrdU uptake, Ki67 positivity, qPCR for PSA and AR-response gene levels, AR, ER immunohistochemical staining. All of these showed that viability, proliferative capacity and tissue morphology were sustained for at least 6-8 days and that signal transduction such as endocrine signaling was also sustained. PELP1/AR interaction seen in PCa tumors was also seen in PDEs and a novel drug that blocks the interaction similarly blocked the interaction in the PDEs.

The conference concluded with a session highlighting basic molecular mechanisms in urologic disease. This session opened with our guest International Speaker, Dr. Shinichi Sakamoto, MD, PhD from Chiba University, who presented on the role of LAT1 in urologic cancers. He nicely demonstrated that LAT1 is dramatically elevated in prostate, bladder and renal cancers. LAT1, an amino acid transporter, is highly expressed in CRPC and blocking androgen increased, while androgen stimulation decreased LAT1 expression. Combined androgen deprivation therapy plus LAT1 siRNA synergistically suppressed proliferation, and high LAT1 expression was an independent predictor of CRPC. LAT1 is downstream of mTor signaling suggesting that it may also be a significant tar-

get of PI3K signaling in PTEN null tumors. Dr. Kenton Sanders, PhD from University of Nevada in Reno presented his work on identifying the markers and functions of interstitial cells (IC) in the bladder. He presented a model whereby Kit-negative, but PDGFRα-positive, ICs regulate contractility during bladder filling. Stretch-induced activation of channels in the bladder transmits a signal to activate TRPV4 Ca+ channels, which in turn activate Ca+-dependent K+ channel, SK3. Blocking the channels enhances contractility, while stimulating them limits it. Thus, the role of adjacent ICs is to stabilize bladder excitability and prevent contractility during filling. Dr. Douglas Strand, PhD from UT Southwestern in Dallas outlined his ongoing studies on the cellular pathogenesis of BPH. Attempts to treat BPH are challenged by the extensive heterogeneity of the disease. Pathologically, BPH can present as predominately stromal, basal, or luminal. To try to address the basis of this heterogeneity, Dr. Strand is using FACS sorting to identify distinct populations of cells within normal versus BPH tissues. In addition to the usual stroma, basal, and luminal populations, he has identified two new populations of AR-negative cells, one of which expresses Krt13 and may represent a luminal progenitor population. Future studies will include gene profiling of these populations and their functional contribution to the different BPH phenotypes. The session concluded with the final Travel Award Presentation of the conference presented by Fengtian Wang from Dr. Hari Koul's laboratory at Louisiana State University Health Sciences Center at Shreveport. The role of an Ets-like protein, SPDEF, on AR cistron modulation and luminal cell differentiation was highlighted. Loss of SPDEF is associated with metastasis of prostate cancer. Using microarray and gene set enrichment approaches, he determined that SPDEF upregulates luminal differentiation genes while suppressing stemness and EMT-related genes such as Twist1. In addition, he demonstrated that Twist1 suppresses SPDEF and SPDEF in turn induces the luminal marker Krt18 to suppress Twist1. Combined Low SPDEF and high Twist1 better predicted poor survival than either gene alone.

Address correspondence to: Marc B Cox, University of Texas at El Paso, 500 W. University Ave., El Paso, TX 79968. Tel: (915) 747-5429; E-mail: mbcox@utep.edu

Poster Session #1

PS1-01 A negative feedback loop of vitamin D import into African American prostate cells and tissues may have implications in hormonal carcinogenesis contributing to prostate cancer disparities

J Garcia[1], Z Richards[2], L Nonn[1]

[1]University of Illinois at Chicago; [2]Dana-Farber Cancer Institute, Harvard Medical School

Introduction & Objective: Prostate cancer (PCa) is a hormonally driven cancer and is currently the third most common cancer in the US. African American (AA) men are disproportionately at risk for both PCa and vitamin D (vitD) deficiency compared to white men. The numerous chemopreventative properties of vitD and epidemiological relationship of vitD status with PCa aggressiveness and mortality has led to the hypothesis that vitD deficiency is a biological contributor to the PCa disparity in AA men. Methods: Here we examine the mechanisms that regulate hormone import into the prostate to follow-up on unexpected relationships between serum and intraprostatic vitD metabolites in AA men that our lab recently reported. Results: We also found that Megalin, a multi-liganded endocytic membrane receptor encoded by the gene *LRP2*, is present on prostate epithelium and is regulated by vitD. Extra-renal activity of Megalin has not been well studied as the widely accepted Free Hormone Hypothesis assumes passive diffusion of circulating free hormones into tissues. However, the presence of megalin suggests that globulin bound hormones from the circulation, including 25D bound to vitamin D binding protein (DBP) and testosterone (T) bound to sex hormone binding globulin (SHBG), are imported into prostate in a regulated manner. Moreover, we found similar relationships between serum and intraprostatic testosterone metabolites, further supporting active megalin; AA men had higher levels of dihydrotestosterone (DHT) in prostate tissue compared to white men. Examination of megalin in vitro in primary human prostate cells and in tissue explants demonstrated that globulin-bound hormones are imported into the prostate and transcriptionally active. In vitro 25D deficiency increased expression of megalin protein and *LRP2* expression in cells and tissue slices. 25D decreased *LRP2* promoter activity in prostate cells. We also observed megalin-mediated internalization of DBP-bound 25D and SHBG-bound T into prostate cells. Ongoing studies are examining megalin-mediated import of estrogens, which are also implicated in carcinogenesis. Conclusions: In summary our findings support the presence of a negative feedback loop in which vitD deficiency increases hormone import into prostate epithelium via Megalin. Therefore the upregulation of megalin in the setting of vitamin D deficiency may facilitate increased import of circulating sex steroids into the prostate contributing to carcinogenesis in AA men.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-02 Bladder Cancer Rapid Autopsy: Emerging patient-derived xenograft models for metastatic diseases

H Lam[1], F Vakar-Lopez, H Cheng[1], M Schweizer[1], P Grivas[2], EY Yu[1], B Montgomery[1], A Hsieh[3], E Corey[4], J Wright

[1]University of Washington; [2]University of Washington/Seattle Cancer Care Alliance; [3]Fred Hutchinson Cancer Research Center; [4]University of Washington School of Medicine

Introduction & Objective: Patients with metastatic bladder cancer have a poor prognosis with a median survival of only 9-15 months. Understanding the biology of metastatic bladder cancer has been historically difficult due to a paucity of specimens and models that recapitulate human disease. To date, xenograft models of bladder cancer have been restricted to cell line or primary bladder cancer, models reflecting metastatic disease were not available. To this end, we established a bladder cancer rapid autopsy program to systemically acquire bladder cancer metastases, and build patient-derived xenograft (PDX) and organoid models for biological studies. Methods: Patients with metastatic bladder cancer were consented and a rapid autopsy was performed 2-6 hours after death to allow acquisition of normal and metastasis specimens. Frozen and formalin-fixed and paraffin embedded specimens were deposited into our biorepository and pathological evaluation was performed on each specimen. Additionally, tumors were implanted subcutaneously into SCID mice to establish PDXs. Once PDXs were developed, PDXs were dissociated for companion organoid culture. Clinical history and treatment information was documented for each participant. Results: We have performed 12 bladder cancer rapid autopsies to date, and have acquired 110 metastatic and 55 normal specimens. The pathological subtypes of these patients include urothelial cell carcinoma (4/12), and urothelial carcinoma with squamous differentiation (4/12) and plasmacytoid variant (4/12). The cohort has been treated BCG only (1/12), chemotherapy (5/12), and chemotherapy and immune checkpoint inhibitors (6/12). The leading site of metastasis was the liver (7/12) and lung (7/12), followed by lymph node (5/12), bone (5/12), intestine (4/12), and omentum (2/12). Approximately half (5/12) of the patients had extensive liver metastases that allow acquisition of multiple tumor foci. For the first time, PDX and organoids from three metastases (liver - CoCaB 10, omentum - CoCaB 14.1, liver - CoCaB 14.2) from the same patient were successfully developed for therapeutic studies. Conclusions: This bladder cancer rapid autopsy program provides an important volume of metastatic tumor specimens for study. Importantly, the first bladder cancer PDX derived from metastasis has been developed. The availability of metastatic bladder cancer specimens, PDXs, and organoids will allow biological studies of bladder cancer metastasis, heterogeneity, and treatment resistance.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-03 Carcinogen-induced bladder cancer in the FVB mouse strain is associated with glandular differentiation and increased Cd274/Pdl-1 expression

L Shuman[1], V Osei Amponsa, J Ellis[1], E Wang[1], V Walter[1], M Zaleski[2], J Warrick, JD Raman[1], D DeGraff[1]

[1]Penn State College of Medicine; [2]Penn State Health. MS Hershey Medical Center

Introduction & Objective: Creation of genetically engineered mouse models of bladder cancer often involves the use of several background strains in conjunction with the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN). However, carcinogen susceptibility in commonly used strains, as well as phenotypic differences, is not well characterized. Therefore, the objective of this study is to determine differences in susceptibility and phenotypic outcome following BBN exposure of C57BL/6 and FVB mice, two strains commonly used for model development. Methods: Male C57BL/6 and FVB mice were exposed to BBN (0.05%) in drinking water for 12 and 16 weeks. Dissected bladders were characterized by histological and immunohistochemical analyses. In addition, Gene Ontology analysis was performed to identify differences in gene expression across strains following BBN exposure. Results: While the C57BL/6 strain developed non-invasive (<pT1) tumors following 12 and 16 weeks of BBN exposure, FVB mice developed muscle invasive bladder cancer with squamous and/or glandular differentiation. Interestingly, glandular differentiation was exclusively observed in the FVB strain. FVB tumors were also highly immunogenic and inflamed as indicated by high expression of Cd274 (Pdl-1), murine histocompatibility complex (H2), and pro-inflammatory cytokines (Il-5 and Il-17). Conclusions: Following BBN exposure, FVB mice undergo rapid tumorigenesis and disease progression characterized by Pdl-1 expression and development of glandular differentiation. These studies identify a degree of tumor heterogeneity in the FVB tumors previously undescribed, and identify FVB mice as a potentially useful model for the study of bladder adenocarcinoma and the inflammatory tumor microenvironment.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-04 Characterization of Srd5a2creErt2 Expression in the Mouse Prostate: A Potential Source of Stromal Progenitors

K Wegner, J Guo, J McMahon, A McMahon, CM Vezina

University of Wisconsin-Madison

Introduction & Objective: Many genetic tools are available for studying prostate epithelial cells in mice, but surprisingly few have been validated for prostate stroma. The lack of genetic mouse lines targeting prostate stroma has made identification of potential stromal progenitors difficult. Pinpointing these progenitors is the first step in identifying mechanisms responsible for stromal disease causing behaviors, including proliferation, growth factor release, and extracellular matrix synthesis. Methods: This study uses the *Srd5a2*^{creErt2} mouse strain which expresses *cre* recombinase driven by the steroid 5 alpha-reductase type 2 (*Srd5a2)* promoter. Six-week-old male mice received four daily 200 mg/kg doses of tamoxifen to activate *cre*. We mapped *cre* expression in adult male *Srd5a2*^{creErt2} mice using a fluorescent reporter mouse strain (*tdTomato*^{fl}*).* To examine the regenerative capacity of these cells, mice were castrated seven days after *cre* activation, and subcutaneous testosterone (T) capsules were implanted two weeks later. T capsules were then removed and replaced at regular intervals a total of three times. To genetically ablate SRD5A2-expressing cells, *Srd5a2*^{creErt2} mice were crossed to mice which, upon *cre*-mediated recombination, express diphtheria toxin A subunit (DTA). Results: Fluorescent reporter experiments reveal that *Srd5a2*^{creErt2} is highly expressed in all prostate lobes and to a lesser degree in the seminal vesicle and testis. Cells expressing *cre* are largely localized to the fibromuscular tunica which surrounds prostate ducts and are occasionally found within the basal and luminal epithelial cell layers. Marked cells exhibit heterogenous androgen receptor (AR) expression, with the ratio of AR+ to AR- changing from the proximal to distal regions of the prostate. Following three rounds of castration and regeneration the relative abundance of *Srd5a2* lineage cells dramatically increases. Genetic ablation of these cells results in a loss of periductal smooth muscle layer integrity. Conclusions: Expression analysis of *Srd5a2*^{creErt2} mice highlight the novel utility of this strain for targeting prostate fibromuscular stroma. We observed that the Srd5a2 lineage is necessary for homeostasis of prostate fibromusclular stroma and our evidence indicating dramatic expansion of this lineage following repeated cycles of prostate involution and regrowth identify this population as a potential source of stromal progenitors.

PS1-05 Determining the Roles of DNA Repair Gene Aberrations in Driving the Development and Progression of Prostate Cancer

S Frank, O Klezovitch, V Vasioukhin, P Nelson

Fred Hutchinson Cancer Research Center

Introduction & Objective: Recent large-scale genomic analyses have revealed that mutations in DNA repair pathway genes (including *BRCA1* and *BRCA2*) occur in ~20% of metastatic castration-resistant prostate cancers (mCRPC). *CDK12* mutations are present in ~5% of mCRPC, which can cause transcriptional downregulation of multiple genes in the homologous recombination pathway. Interestingly, *CDK12* mutations tend to be exclusive with other DNA repair mutations. It is not known if loss of DNA repair genes alone is sufficient for prostate tumor initiation or progression, and research is needed to assess how these mutations affect tumor response to current anti-AR and DNA repair-deficient targeted therapies. Methods: To study the role of DNA repair gene loss in prostate cancer, a combination of models including genetic mouse models, patient derived xenografts (PDX), and engineered cell lines were utilized. Genetic mouse models with floxed *Brca1*, *Brca2*, or *Cdk12* (-/+ floxed *Pten*) were bred and crossed to Probasin-Cre. Floxed cells were isolated and cultured in 2D/3D conditions and recombined *in vitro* with adenoviral Cre to create knockout lines. Human PDX lines null for *BRCA2* (LuCaP 96CS) and *CDK12* (LuCaP 189.4) were cultured in 3D, and prostate cancer cell lines (LNCaP and PacMetUT1) were engineered with inducible shRNA knockdown of *BRCA1*, *BRCA2*, or *CDK12*. Results: Mice with conditional prostate-specific knockout of *Brca1*, *Brca2*, or *Cdk12* have been bred and are aging for tumor development. Floxed cells were isolated, recombined, and validated by PCR and immunoblot. LuCaP lines 96CS and 189.4 were successfully cultured and passaged in 3D conditions and verified by immunoblot for BRCA2 and CDK12 loss, respectively. LuCaP cells are being engineered to re-express *BRCA2* or *CDK12* with doxycycline-inducible vectors. Furthermore, LNCa P and PacMetUT1 lines were transduced with lentiviral inducible shRNA knockdown of *BRCA1*, *BRCA2*, and *CDK12* and validated by immunoblot and qPCR. Conclusions: These new models utilizing genetic mouse models, PDX lines, and traditional prostate cancer cell lines, have been established to enable future investigations into determining precisely how mutations in *BRCA1/2* and *CDK12* affect prostate tumor development and progression. Though these mutations only account for a subset of mCRPC patients, they are likely to have large implications for selecting effective therapeutic strategies, and these tools will provide a means to test new therapies and investigate mechanisms of resistance.

PS1-06 Ex vivo platform to determine treatment direction and metastatic potential for primary prostate cancer

KL Morel, AV Sheahan, DL Burkhart, MA Dorling, M Loda, CJ Sweeney, L Ellis

Dana-Farber Cancer Institute, Harvard Medical School

Introduction & Objective: While progress has been made to improve therapeutic approaches for prostate cancer (PCa), tumor heterogeneity significantly contributes to variability in cancer risk, drug response and disease progression. There is currently a lack of a functional clinical assay for tumors to examine individual patient response to therapy and metastatic potential of tumors. To gain insight into tumor cell behavior and drug response, we have established a three-dimensional (3D) PCa organoid platform to directly study tumor specific responses in tissue-like environments. Methods: As a proof of principal, we have established a 3D organoid platform utilizing murine organoids derived from the prostate tissue of genetically engineered mouse models (GEMM) of PCa. Pten loss (PbCre4:Pten$^{fl/fl}$) results in locally invasive androgen sensitive PCa tumors, but the combined loss of Pten and Rb1 (PbCre4:Pten$^{fl/fl}$:Rb1$^{fl/fl}$) results in decreased survival, reduced response to androgen deprivation therapy (ADT) and distant metastatic disease. For functional assays, organoids were embedded in a Matrigel/collagen 3D gel matrix in 96-well culture plates. In this format organoid behavior and response to therapy was observed and measured over short and long-term periods of time. Quantitative determination of therapeutic response was carried out in 3D using fluorescent cytotoxicity and proliferation assays. Protein and mRNA expression was measured in organoids by qPCR, IHC and flow cytometry. Results: Therapeutic response of the GEMM-derived organoids to enzalutamide reflected the *in vivo* response, whereby PbCre4:Pten$^{fl/fl}$ mice responded to ADT, while PbCre4:Pten$^{fl/fl}$:Rb1$^{fl/fl}$ mice displayed reduced androgen sensitivity. Characterization of the *ex vivo* models confirmed that the organoids matched *in vivo* tumor features. Rb1 deletion resulted in increased AR heterogeneity and reduced AR functional output both *in vivo* and *ex vivo*. The metastatic behavior of the organoids also echoed that observed *in vivo*. PbCre4:Pten$^{fl/fl}$:Rb1$^{fl/fl}$ organoids developed an invasive phenotype, while PbCre4:Pten$^{fl/fl}$ organoids remained indolent after several weeks in culture. Conclusions: We have shown that *ex vivo* organoid culture is able to faithfully recapitulate *in vivo* tumor behavior. It is anticipated that this platform can also be modified to utilize tumor organoids derived from patient samples. This would allow clinical screening for identification of effective therapeutics and prediction of metastatic potential *ex vivo*, with tailoring of patient-specific treatments. This will hopefully lead to enhanced clinical outcomes, and reduced morbidity and improve the quality of life in PCa patients.

PS1-07 N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer

NJ Brady[1], A Berger[1], E Dardenne[1], R Bareja[1], BD Robinson[1], V Conteduca[1], MA Augello[1], A Ahmed[1], I Hwang[1], AM Bagadion[1], L Puca[1], A Sboner[2], O Elemento[1], J Paik[1], CE Barbieri[1], N Dephoure[1], H Beltran[1], DS Rickman[1]

[1]Weill Cornell Medicine; [2]Englander Institute of Precision Medicine, Weill Cornell Medicine

Introduction & Objective: Despite recent advances in the development of highly effective androgen receptor (AR)-directed therapies for the treatment of prostate cancer, nearly 37% of patients develop resistance. A further third of these patients progress to develop aggressive neuroendocrine prostate cancer (NEPC) for which no effective therapies exist. Lineage plasticity, a process by which differentiated cells lose their identity and acquire an alternative lineage phenotype, has been proposed as a mechanism of resistance to targeted therapies in several cancer types, such as leukemias and epithelial tumors (including prostate cancer). However, the molecular programs underlying this lineage plasticity are poorly understood. Previously, we observed that the majority of NEPC and 20% of castration-resistant prostate cancer (CRPC) aberrantly overexpress the transcription factor *MYCN* (N-Myc). Despite this frequent occurrence, the role of N-Myc in driving lineage plasticity and the epigenetic mechanisms which regulate disease progression remain to be elucidated. Methods: We analyzed overall survival and whole transcriptome data from a cohort of over 200 prostate cancer patients, including the largest-to-date population of NEPC patients. We also analyzed epigenetic modifications along with the N-Myc transcriptome, cistrome and chromatin-bound interactome by performing ChIP-seq, RNA-seq and RIME in a combination of mouse models, human prostate cancer cell lines, and NEPC patient-derived organoids. Results: Expression of *MYCN* in CRPC and NEPC patients correlates with reduced overall survival. NEPC tumors are significantly enriched for stem cell genes associated with normal neuroendocrine cell precursors and embryonic stem cells as well as for neural lineage-defining genes from activated neural stem cells. The integration of next-generation sequencing data revealed that the N-Myc cistrome is androgen-dependent and drives a transcriptional program leading to epithelial plasticity and the acquisition of clinically relevant neuronal lineage markers. Interestingly, histone marks at lineage-defining genes are epigenetically reprogrammed by N-Myc. Finally, we demonstrated that N-Myc-induced gene expression and epigenetic changes can accurately classify our patient cohort. Conclusions: We describe a novel role for N-Myc in prostate cancer, characterized by changes in the N-Myc cistrome and interacting co-factors, as well as reprogramming of the epigenome in an androgen context-dependent manner. This reprogramming is associated with induction of a lineage plastic state and a switch towards a neural identity that favors the development of AR independence and NEPC.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-08 Myc Promotes Prostate Epithelial Differentiation through the in induction of ING4, and Loss of ING4 Leads to the Development of PIN lesions

C Miranti[1], S Frank[2], C Jensen[3], J Tran, P Berger, S Kim[3]

[1]University of Arizona College of Medicine; [2]Fred Hutchinson Cancer Research Center; [3]Collaborator

Introduction & Objective: Inhibitor of Growth Factor 4 (ING4) is a protein that binds to the trimethylated lysine 4 in Histone H3 to recruit the acetyltransferase HBO1. ING4 is recognized as a tumor suppressor gene in the context of breast cancer, which suppresses the oncogenic activities of Myc. Our previous work showed that overexpression of Myc or ING4 accelerates luminal prostate epithelial cell (PrEC) differentiation, and overexpression of Myc increases endogenous ING4 protein. Interestingly, ING4 overexpression does not affect Myc protein levels. Strikingly, by overexpressing ING4 in a tumorigenic cell line, iPEC37-EMP, we are able to rescue PrEC differentiation. We propose that Myc normally promotes PrEC differentiation through chromatin remodeling mediated by ING4, such that loss of ING4 is required for Myc oncogenesis, which leads to aggressive disease through suppression of differentiation. Methods: We generated a CRSPR KO ING4 mouse line, which will allow us to interrogate how loss of ING4 impacts Myc-driven prostate cancer develop *in-vivo*. IHC and PCR was used to validate ING4 loss in the mouse tissues. We used IHC to ascertain ING4 status in normal human prostate compared to prostate cancer. Results: ING4 expression is reduced or null in prostatic intra-epithelial neoplasia (PIN) lesions when compared to normal human tissue. Genotyping and IHC have validated successful generation of the ING4 KO mouse. We noted that ING KO alone was not sufficient to generate tumors in 1-year old mice. We intend to stain H&E for the collected organs of these mice to further evaluate tissue architecture. Additionally, we plan to cross ING4 KO mice with prostate-specific Myc overexpressing mice. Conclusions: Preliminary IHC staining showed low and null expression of ING4 in human disease prostate compared to normal human prostate tissue. We have successfully generated a CRSPR ING4 KO mouse line. Crossing these mice with our prostate-specific overexpressing mice will create a promising model, allowing us to ask *in-vivo* questions regarding the concerted mechanism involving Myc and ING4 observed in our *in-vitro* differentiation model.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-09 Patient Derived Models Reveal Impact of the Tumor Microenvironment on Therapeutic Response

A Shafi[1], MJ Schiewer[2], R de Leeuw, E Dylgjeri[1], P McCue[1], N Shah, L Gomella, C Lallas[1], E Trabulsi[3], M Centenera, T Hickey, L Butler[4], GV Raj, W Tilley, E Cukierman[5], KE Knudsen[1]

[1]Thomas Jefferson University; [2]Sidney Kimmel Medical College at Thomas Jefferson University; [3]Sidney Kimmel Medical College at Thomas Jefferson University; [4]University of Adelaide; [5]Fox Chase Cancer Center

Introduction & Objective: Prostate cancer (PCa) is the most common non-cutanenous cancer and the third leading cause of cancer-related death in American men. Androgen receptor (AR) is a hormone-activated transcription factor that plays an important role in both the development and progression of PCa. Androgen deprivation therapy is a common first-line therapy for disseminated disease. However, virtually all tumors become resistant to such therapy, and the tumor recurs and is termed castration resistant prostate cancer (CRPC). There is no durable cure for CRPC; thus, there is a vital need for the development of novel, more effective treatments. One major hurdle in this aspect is the lack of adequate preclinical models. Current models do not effectively recapitulate the heterogeneity and the microenvironment of human PCa tumors, significantly hindering the ability to accurately predict therapeutic response. Methods: Our collaborative group has utilized and characterized a method to culture patient tumors *ex vivo*, termed Patient Derived Explant (PDE). Fresh PCa tissue from patients who underwent radical prostatectomy was cultured as PDEs in order to examine therapeutic response. The impact of genomic and chemical perturbations in PDEs was assessed using numerous parameters (*e.g.* AR levels, Ki67 staining, and desmoplastic indices). Importantly, our PDE model can be manipulated both chemically (drugs/compounds) and genetically (shRNA) in order to determine specific reactions and mechanisms of response on individual tumor growth. Results: The PDE maintained the integrity of the native tumor microenvironment (TME), tumor tissue morphology, viability, and endogenous hormone signaling. Tumor cells in this model system exhibited *de novo* proliferative capacity. Examination of the native TME in the PDE revealed a first-in-field insight into patient-specific desmoplastic stromal indices and predicted responsiveness to AR-directed therapeutics. Conclusions: The PDE model allows for a comprehensive evaluation of individual tumors in their native TME to ultimately develop more effective therapeutic regimens tailored to individuals. Discernment of novel stromal markers may provide a basis for applying precision medicine in treating advanced PCa, which would have a transformative effect on patient outcomes. Thus, the PDE will be an asset for the development of novel metrics for the implementation of precision medicine in PCa.

PS1-10 Prostate cancer bone metastasis patient-derived three-dimensional organoids undergo a budding-like cell extrusion process to form cyst/gland-like structures: an in vitro model of metastatic tumor formation

S Lee[1], C Arreola[2], D Burner[3], M Muldong[3], T Mendoza, C Wu[4], C Jamieson, N Cacalano, A Kulidjian, C Kane, C Jamieson[1]

[1]University of California, San Diego; [2]Surgery-Urology, University of California, San Diego, Moores Cancer Center; [3]University of California San Diego Moores Cancer Center; [4]University of California, San Diego, Moores Cancer Center

Introduction & Objective: Bone metastatic prostate cancer (BMPC) patients usually develop androgen deprivation therapy resistance. Our group aimed to better understand the mechanism of tumorigenesis and metastasis of BMPC utilizing patient derived xenograft and 3D cultured organoid models. Organoids are three-dimensional (3D) ex vivo cellular structures which can closely recapitulate and maintain the in vivo condition of its original tissue. 3D cultured organoids from patient tumor tissues or patient derived xenograft (PDX) models provide invaluable opportunities to understand the mechanism of cellular signaling upon tumorigenesis and determine the effects of drug treatment on each cell population. While bone metastasis of prostate cancer cells is rare in mouse models, we successfully established our PDX models of BMPC by direct intra-femoral (IF) injection into male Rag2-/-gc-/- mice. We discovered a spontaneous advanced metastasis (SAM), in which the PDX cells had metastasized to the neck and abdomen. The cellular and molecular features of PDX SAM are characterized here in 3D/organoids and xenografts. Methods: SAM tumor was dissected from the neck of the mouse and used in 3D organoid cultures prepared as described in Gao *et al* (2014) plus 10% FBS. Time lapse video was recorded over 48 hours using confocal FV 10i microscope. SAM organoids were fixed with 4% paraformaldehyde, paraffin embedded and sectioned (10 mM). Immunohistochemistry was performed using antibodies specific to androgen receptor (AR), prostate specific antigen (PSA), Keratin 5 (K5) and Keratin 8 (K8). Results: 3D organoid cultures of the PDX SAM contained a mix of cyst and spheroid-like cell masses that had distinct growth and morphologic properties compared to the original PDX. The average diameter of cyst organoid lumen was 54.2 mM at 0 hours and 56.2 mM at 48 hours. The average thickness of epithelium-like walls of the cysts was 10.2 mM at 0 hours and 15 mM at 48 hours. Interestingly, 66.7% of organoids showed increased value in thickness of epithelium-like structure over 48 hours of observation. IHC revealed each population of AR+, PSA+, K5+ and K8+ cells. Strikingly, time lapse video captured the transition of several spheroid-like masses into thick-walled cysts via a budding-like extrusion of the central, dying cells. Conclusions: Our study provides evidence that PCSD1 SAM organoids differentiate from prostate cancer progenitor-like cells. Furthermore, the organoid-forming prostate cancer progenitor cells maintain cell-type representation and recapitulate in-vivo tumor structure. Thus, PDX SAM model represents an in vitro differentiation model of metastatic tumor formation.

PS1-11 Single cell analysis of prostate cancer bone metastases-mediated alteration of the bone microenvironment immune milieu

A Ahmed, P Schnepp, G Shelley, E Keller

University of Michigan

Introduction & Objective: Uncovering the complex relationship between prostate cancer (PCa) and the immune system is vital to investigate the immunogenic mechanisms behind bone metastasis. Our goal is to determine if the presence of PCa changes the bone marrow immune milieu. Methods: We implanted murine RM1 PCa cells (or phosphate-buffered saline (PBS) as control) into the tibiae of C57Bl/6 mice. After one week, we collected bone marrows of RM1- or PBS-injected tibiae and subjected them to either (1) mass cytometry (CyTOF) using a panel of 32 antibodies that allows for identification of immune cell types or (2) single cell-specific targeted amplification (STA) PCR using a panel that targets 96 genes. Manually gating and quality check were performed on Cytobank. Live singlet events from RM1 and PBS mice were then imported into Cytofkit, a R package that provides functions for data preprocessing, data visualization through linear or non-linear dimensionality reduction and automatic identification of cell subsets. PhenoGraph clustering method was used to stratify all events into subpopulations and then visualized on tSNE plots. tSNE provides each cell with a unique coordinate according to its expression of the 32 measured parameters and then displayed on the first two tSNE dimensions. Results: We identified over 15 types of immune cells in the bone marrow microenvironment. Our analysis revealed increases in monocytes, myeloid-derived suppressor cells (MDSCs), B cells and T regulatory cells in the presence of RM1 cells. An analysis of the $CD11b^+$ cells revealed a more diverse myeloid population in the RM1 bone marrow compared to control. tSNE plots displayed a population of monocytic and conventional dendritic cells in PBS control while RM1 bone marrow had eight distinct populations that featured eosinophils, neutrophils, inflammatory monocytes, and monocytic dendritic cells. Single-cell STA CR also revealed a similar trend in that the tumor containing marrow had an increase in myeloid population, B cells, and T cells versus nontumor marrow. Lastly, we found that RM1 implantation causes visible bone remodeling as seen in x-ray images. Conclusions: Single-cell analysis revealed that PCa modifies the immune milieu of the bone metastatic microenvironment. RM1 cells caused an increase in myeloid cells and an expansion of varied subsets of $CD11b^+$ cells. This data provides a granular view of the PCa-induced immune modifications and a rationale to explore how these alterations impact bone metastasis.

PS1-12 Single cell RNA sequencing of the normal human prostate reveals identities of novel stromal and epithelial cell types

GH Henry[1], A Malewska[1], D Binoy Joseph[2], V Malladi[1], J Lee[1], J Torrealba, R Mauck, J Gahan[1], GV Raj, C Roehrborn[1], G Hon[1], M MacConmara, J Reese, R Hutchinson[3], CM Vezina[2], DW Strand[1]

[1]UT Southwestern Medical Center; [2]University of Wisconsin-Madison; [3]University of Texas Southwestern Medical Center

Introduction & Objective: The cellular pathogenesis of human prostate disease is poorly understood. Designing drugs that target causative pathways requires a precise understanding of the molecular and cellular changes associated with disease progression. To understand the functional contribution of specific cell types in disease, a baseline molecular identification and anatomical distribution of each cell type in the normal organ is needed. Methods: 18-28 year old organ donor prostate specimens were dissected into anatomical zones fresh from surgery for digestion into a single cell suspension. Single cell RNA sequencing (scRNA-seq) was performed to derive a molecular profile of each cell type. Newly derived molecular markers were used to 1) improve the flow cytometry-based isolation of individual cell types for functional analysis and 2) provide immunohistochemical confirmation of anatomical position in whole mount specimens. Results: Five epithelial cell types were identified with scRNA-seq including classical KRT14$^+$ basal, KLK3$^+$ luminal, and CHGA$^+$ neuroendocrine cells. In addition, two novel epithelial cell types marked by KRT13 and SCGB1A1 were discovered and found to correlate strongly with hillock and club cells of the lung. Whole mount immunohistochemical analysis of KRT13$^+$ and SCGB1A1$^+$ epithelial cell types revealed their enrichment in the prostatic urethra and proximal prostatic ducts. Stromal cell types identified by scRNA-seq included smooth muscle and fibroblasts. Immunohistochemical analysis with newly derived molecular markers revealed that fibroblasts were enriched in the anterior fibromuscular stroma and peri-urethral zones while smooth muscle was enriched in the peripheral zone. Conclusions: An unbiased transcriptional identity of each cell type in the normal prostate is required for understanding functional interactions between cell types in homeostasis and disease. Our study provides the resources to identify, localize, and isolate every cell type in the human prostate. Our identification of novel epithelial cell types concentrated in the urethra and spread throughout the prostate could lead to a deeper understanding of zonal anatomy and cellular origin of human prostate diseases.

PS1-13 Voided Volumes Predict Degree of Partial Bladder Outlet Obstruction in a Murine Model

N Tassone[1], M Devine, B Li, R Dettman, E Gong[2]

[1]Ann & Robert H. Lurie Children's Hospital of Chicago; [2]Northwestern University

Introduction & Objective: The partial bladder outlet obstruction model (pBOO) is commonly used to mimic human disease. Unfortunately, pBOO demonstrates variable degrees of obstruction, requiring bladder weight (BW) or urodynamic studies to determine obstruction. Our objective is to identify extent of obstruction soon after surgery by utilizing Void Stain on Paper (VSOP) assays and correlating with late BW. Methods: pBOO was performed on 33 mice; 1- and 4-week VSOPs were quantified for mean voided volume (mVV). At 4 weeks, bladders were harvested and weighed. Correlation was evaluated using bivariate kernel density estimation (SAS). Single variable histogram of the data established groups based on BWs. mVV's and bladder weights within each group were averaged and plotted to render a non-linear regression model. Results: A significant correlation was found between 1-week mVVs and 4-week BWs with a correlation coefficient of -0.63 ($p=0.0001$) upon bivariate analysis. Conclusions: A non-linear regression of plotted data defined a statistically significant fit equation correlating 1-week mVV to 4-week BW. We demonstrate a novel method for forecasting degree of obstruction in pBOO based on 1-week post-operative VSOP mVV.

PS1-14 Postobstructive bladder smooth muscle remodeling is dependent on bladder stem cells

N Tassone[1], B Li, M Devine, R Dettman, E Gong[2]

[1]Ann & Robert H. Lurie Children's Hospital of Chicago; [2]Northwestern University

Introduction & Objective: Partial bladder outlet obstruction results from benign prostatic hypertrophy in men and posterior urethral valves in children, leading to detrusor remodeling and functional impairment. A Sca-1$^+$/CD34$^+$ coexpressing mesenchymal stem cell population was previously identified in the murine bladder which is altered after acute and partial outlet obstructions. We hypothesize that loss of Sca-1$^+$ will inhibit the physiologic detrusor hypertrophy that occurs after obstruction leading to decreased compensatory function. Methods: 6-8 week-old male Sca-1 null mice and wild-type mice both underwent surgical partial obstruction (PO) at the bladder neck. An additional cohort of Sca-1 null mice underwent sham surgery for comparison. Voiding stains on paper at 1, 2, and 4 weeks were analyzed for functional assessments of the bladder. Whole bladders were analyzed at 4 weeks by weight and distributed for histologic exam. Bladder thickness and circumference were measured. Masson's Trichome staining was used to calculate detrusor collagen-to-smooth muscle ratios and total smooth muscle content. Results: Comparisons were made between PO groups of Sca-1 null mice and C57/BL6 mice, as well as between Sca-1 null mice who either underwent PO or sham surgery. PO led to increased bladder circumference in Sca-1 null mice (2.06±0.42 cm) compared to the sham group (1.21±0.25 cm) (p=0.04). Mean bladder smooth muscle composition in C57/BL6 mice was 0.83±0.09 compared to 0.67±0.10 in Sca-1 null mice (p=0.02). Sca-1 null sham mice had a bladder smooth muscle composition of 0.88±0.05 (p=0.02). Lower collagen-to-smooth muscle ratios were also found in the C57/BL6 group at 0.23±0.14 compared to 0.55±0.25 in Sca-1 null mice (p=0.03). Conclusions: Our findings suggest that Sca-1$^+$/CD34$^+$ mesenchymal stem cells play a role in detrusor smooth muscle remodeling and hypertrophy after partial obstruction. Stem cell intact mice displayed increased bladder smooth muscle composition and decreased levels of collagen infiltration consistent with fibrosis compared to knockout comparisons.

PS1-15 Novel roles of DNA-PK in metabolic regulation

E Dylgjeri, J Goodwin, A Shafi, E Seifert, G Zadra, V Kothari, F Feng, KE Knudsen

Thomas Jefferson University

Introduction & Objective: DNA dependent protein kinase catalytic subunit (DNA-PK) is a multifunctional kinase involved in repairing double-strand DNA breaks through non-homologous end joining (NHEJ), with parallel roles as a master transcriptional regulator of gene networks supporting cell migration and invasion. In prostate cancer (PCa), DNA-PK was found to be highly upregulated and hyperactivated. Strikingly, DNA-PK is the most deregulated kinase in metastatic castration resistant prostate cancer (CRPC), and is independently predictive of metastasis and overall survival in patients with high-risk disease. Combined, these findings highlight the importance of understanding DNA-PK functions beyond DNA repair and transcriptional regulation, which promote the acquisition of aggressive tumor phenotypes. Methods: Rapid immunoprecipitation of endogenous proteins (RIME) identified the DNA-PK interactome. To assess the role of DNA-PK in regulation of metabolism, metabolic profiles of CRPC models were investigated upon DNA-PK inhibition, and validated in DNA-PK knockdown models, via steady-state metabolomics. Results: Key findings include the identification of the DNA-PK interactome, which uncovered novel DNA-PK interactors that play key roles in metabolism. Data presented will describe novel roles of DNA-PK in cancer metabolism and subsequent effects of DNA-PK modulation in key metabolic pathways. Conclusions: Preliminary studies strongly support the novel role of DNAPK in cancer metabolism through new DNA-PK interactions with various key metabolic enzymes impacting PCa survival.

PS1-16 Prostate cancer promotes bone metastasis progression through stimulating osteocytes to secrete GDF15 that activates EGR1 expression in prostate cancer cells

J Dai[1], W Wang, X Yang, Y Lu, J Zhang[2], E Keller[3]

[1]University of Michigan; [2]Southern University of Science and Technology School of Medicine; [3]University of Michigan

Introduction & Objective: Bone is the most frequent site of prostate cancer (PCa) metastasis; however, little is known about the role of the most common cell in bone, the osteocyte (OCy), in cancer biology. In this study we explored the crosstalk between PCa cells and OCys to determine if it contributes to PCa progression. Methods: A chemokine screening was performed to determine the chemokines and cytokines produced from OCys induced by PCa cell. Loss of function assays in OCys was performed to evaluate the effects of GDF15 produced by OCys on proliferation, migration and invasion of prostate cancer cells. Transcription factor array of PCa cells exposed to OCys with or without knockdown of GDF15 was performed to determine transcription factors expression in the PCa cells. Loss of function assays in PCa cells was performed to evaluate if OCy-derived GDF15-mediated induction of PCa cell proliferation, migration and invasion through EGR1. The subcutaneous or intraosseous co-injection of PCa cells and OCys with or without knockdown of GDF15 into mice to assess the growth of PCa cancer cells. Results: PCa cells induced OCys to promote PCa proliferation, migration and invasion. A chemokine screen revealed that PCa cell induced OCys to produce growth-derived factor 15 (GDF15). Knockdown of GDF15 in OCys demonstrated that PCa cells conferred the ability on OCys to promote PCa proliferation, migration and invasion through GDF15. Transcription factor array screening of PCa cells exposed to OCys with or without knockdown of GDF15 revealed that GDF15 in OCys promoted early growth response 1 (EGR1) expression in the PCa cells. Knockdown of EGR1 expression in PCa cells revealed it was required for the OCy-derived GDF15-mediated induction of PCa cell proliferation, migration and invasion. Subcutaneous co-injection of PCa cells and OCys into mice revealed that OCys promoted tumor growth *in vivo*, which was diminished by knockdown of GDF15 in the OCys. Knockdown of GDF15 in the tibiae diminished growth of PCa cancer cells injected into the tibiae, which was accompanied by decreased tumor cell proliferation and EGR1 expression. Conclusions: These results shed light on a novel mechanism through which PCa cell educate OCys to promote progression of PCa bone metastasis. They also suggest that targeting of GDF15-based and EGR1-based signaling pathways should be further explored for their potential to diminish progression of PCa bone metastasis.

PS1-17 Repression of TFAP2A by PPAR gamma Reveals a Novel Transcriptional Circuit in Basal Squamous Bladder Cancer

H Yamashita, Y Imamura, L Shuman, Z Zheng, T Tran, V Walter, J Warrick, H Al-Ahmadie, M Kaag, P Wong, JD Raman, T Williams, D DeGraff

Penn State College of Medicine

Introduction & Objective: The discovery of bladder cancer transcriptional subtypes provides an opportunity to identify high-risk patients, and tailor disease management. Recent studies suggest tumor heterogeneity contributes to "plasticity" of molecular subtype during progression and following treatment. Nonetheless, the transcriptional drivers of the aggressive Basal-Squamous subtype remain unidentified. As PPARɣ has been repeatedly implicated in the Luminal subtype of bladder cancer, we hypothesized inactivation of this transcriptional master regulator during progression results in increased expression of Basal-Squamous specific transcription factors (TFs) which act to drive aggressive behavior. Methods: In order to identify PPARɣ-repressed, Basal-Squamous specific TFs, we performed a pharmacologic and RNA-seq-based screen and hierarchical clustering of RNA-seq data following treatment of a panel of human bladder cancer cell lines with a PPARɣ agonist. Results: Hierarchical clustering identified a number of TFs regulated by PPARɣ activation, several of which are implicated in urothelial and squamous differentiation. One PPARɣ-repressed TF implicated in squamous differentiation identified is Transcription Factor Activating Protein 2 alpha (TFAP2A). We show TFAP2A and its paralog TFAP2C are overexpressed in Basal-Squamous bladder cancer and in squamous areas of cystectomy samples, and that overexpression is associated with increased lymph node metastasis and distant recurrence, respectively. Biochemical experiments confirmed the ability of PPARɣ activation to repress TFAP2A, while PPARɣ antagonist studies indicate the requirement of a functional receptor. *In vivo* tissue recombination studies show TFAP2A and TFAP2C promote tumor growth in line with the aggressive nature of Basal-Squamous bladder cancer. Conclusions: Our findings suggest PPARɣ inactivation, as well as TFAP2A and TFAP2C overexpression cooperate with other TFs in Basal-Squamous disease.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-18 Platelet-Tumor SCF-CD117 Tyrosine Kinase Signaling Axis Drives Prostate Cancer Progression and Metastasis

BM Foster[1], K Harris, P Elliott[2], L Shi[1], BA Kerr[1]

[1]Wake Forest University School of Medicine; [2]The University of North Carolina at Chapel Hill

Introduction & Objective: Platelets play an integral role in blood vessel stabilization of the primary tumor and survival of tumor cells in the bloodstream. Our prior data demonstrated that platelets and platelet alpha granule secretion control premetastatic changes in the bone microenvironment and bone marrow-derived cell mobilization to the primary tumor. The proteins and signaling pathways involved in this platelet-tumor communication have yet to be elucidated. In a preclinical patient study, we found stem cell factor (SCF) concentrations to be high in patients with advanced prostate cancer. In the same patient populations, the receptor for SCF, CD117 or c-kit, was measured on circulating tumor cells. Thus, we are examining how the platelet SCF interaction with tumor cell CD117 affects prostate cancer progression. Methods: RM1 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 SCF or CD117 inhibitors. Conditional knockout mice were generated to delete SCF in megakaryocytes/platelets. RM1 tumors from knockout mice were examined for changes in angiogenesis, tumor progression, and premetastatic changes in the bone structure. Results: Here, we examine the activation of CD117 by SCF in prostate cancer cell progression and migration. CD117 expression was associated with increased migration, sphere formation, and EMT marker expression but not proliferation. Treatment with the CD117 ligand SCF enhanced invasion indicating that CD117 activation may drive metastasis. Interestingly, SCF treatment increased EMT at low doses but stimulated MET at higher doses. In a syngeneic animal model, tumor size and angiogenesis were altered in tumors generated by CD117-expressing cells. Knockout of platelet-derived SCF significantly changed angiogenesis. Correspondingly, loss of platelet SCF affected changes in the bone structure otherwise stimulated by CD117-expressing tumor growth. Conclusions: Platelet-derived SCF activation of CD117 on cancer cells drives a more aggressive cell phenotype and may be involved in metastasis. This platelet-tumor SCF-CD117 axis represents one mechanism by which platelets assist in prostate cancer metastasis and is a potentially druggable target for the interference with the progression to lethal prostate cancer.

PS1-19 Primary stromal cells from BPH, but not normal adjacent prostate, stimulates benign prostatic epithelial cell growth in 3D culture

W Chen, LE Pascal, K Wang, R Dhir, D Stolz, Z Wang

University of Pittsburgh

Introduction & Objective: Benign prostatic hyperplasia (BPH) refers to nonmalignant growth of prostate, which is associated with lower urinary tracts symptoms (LUTS). Ageing and androgens are important factors in BPH development and progression. Stromal-epithelial interactions are also thought to play an important role in BPH pathogenesis. However, the underlying mechanisms of stromal-epithelial interactions in BPH are largely unknown. My objective is to elucidate the mechanisms of BPH stromal stimulation of prostatic epithelial cell growth compared to normal adjacent stromal cells. Methods: Primary cultures were prepared directly from prostate isolated from human in BPH. To determine the impact of stromal cells derived from BPH and normal adjacent on epithelial proliferation, matched pairs of primary BPH and normal adjacent stromal cells from 6 patients were separately co-cultured with the benign human prostate epithelial cell line BHPrE-1 in 3D Matrigel. To test the impact of androgen in the proliferation of epithelial cells in 3D co-culture, 1 nM synthetic androgen R1881 was added in 3D. In addition, conditioned medium from BPH and normal adjacent stromal cells were used to culture with epithelial cells as well. Results: The epithelial spheroids co-cultured in 3D with primary stromal cells derived from BPH were significantly larger than those co-cultured with normal adjacent. Furthermore, androgen significantly stimulated the proliferation of epithelial cells in the presence of stroma cells derived BPH tissue but did not stimulate epithelial proliferation in 3D co-cultures with normal adjacent stromal cells. The volume of BHPrE-1 sphere cultured in conditioned medium from BPH stromal cells was larger compared to that in conditioned medium from adjacent normal stroma. Conclusions: These findings suggest that the stromal cells in BPH tissues are different from normal adjacent stromal cells and could contribute to increased epithelial cell proliferations in the development of BPH. Furthermore, the androgen signaling in BPH stroma plays a significant role in mediating stromal-stimulation of the epithelial proliferation. Our results suggest that unlike the normal adult prostatic stromal cells, the BPH stromal cells seem to behave like embryonic prostatic stromal cells that can mediate androgen-stimulated epithelial cell growth. Also, it suggests that androgen signaling in BPH stromal cells is dysregulated and can contribute to epithelial growth.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-20 Obesity-associated inflammation induces androgenic to estrogenic switch in the prostate gland

B Xue[1], S Wu[1], L Hu[1], S Tabatabaei[1], C Wu[1], Z Cheng[2], DW Strand[3], A Olumi[1], Z Wang[1]

[1]Massachusetts General Hospital, Harvard Medical School; [2]Virginia Tech; [3]UT Southwestern Medical Center

Introduction & Objective: *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 have demonstrated that there is an "androgenic to estrogenic switch" when SRD5A2 is absent in the prostate gland. Here we wished to identify whether obesity-associated inflammation contributes to the androgenic to estrogenic switch in human prostate tissue. Methods: Primary prostatic stromal cells were isolated from patients undergoing prostate reduction therapy for BPH. Adipocytes and macrophages were differentiated and treated with saturated fatty acids (SFA) to induce inflammation, and SFA-free conditioned media were collected for stromal cell culturing. Protein and mRNA were extracted from stromal cells, the expression of aromatase, estrogen receptor alpha (ERα), G protein-coupled estrogen receptor 1 (GPER), and SRD5A2 was determined by Western Blot, ELISA and qPCR. *P*rostate specimens were collected from 35 patients who underwent transurethral resection of the prostate for symptomatic BPH at Massachusetts General Hospital. Prostatic levels of testosterone, dihydrotestosterone and estradiol were measured by HPLC-MS. Results: In primary cultured prostatic stromal cells, the levels of aromatase, ERα and GPER were significantly increased, the level of SRD5A2 was significantly decreased in a dose-dependent manner when cultured with conditioned media of adipocytes or macrophages. There were much higher levels of aromatase, ERα and GPER in the prostatic stromal cells when adipocytes and macrophages were pretreated with SFA. In BPH patients, BMI was significantly correlated with methylation of SRD5A2 gene promoter ($p<0.05$) and absence of SRD5A2 protein expression. Higher BMI was associated with higher prostatic estradiol levels ($p<0.05$). Treatment with 5ARIs dramatically increased the level of prostate testosterone levels and testosterone/estradiol ratio in the prostate specimens ($p<0.01$, $p=0.01$, respectively), and decreased the level of dihydrotestosterone ($p<0.05$). Conclusions: Our study demonstrates for the first time that there is an androgenic to estrogenic switch in the prostate glands of overweight patients. Associated with body weight, somatic epigenetic silencing of SRD5A2 changes the prostatic hormonal milieu, and may modulate prostatic homeostasis and growth. Targeting the estrogenic signaling may serve as an effective treatment strategy in subset of overweight BPH patients.

PS1-21 Mouse prostatic neuroendocrine cells mediate smooth muscle contraction

MT Cadena[1], AE Turco[1], PZ Romero[2], HL Holmes[3], MF Romero[3], NR Tykocki[4], CM Vezina[1]

[1]University of Wisconsin-Madison; [2]Mayo Clinic College of Medicine & Science; [3]Mayo Clinic; [4]University of Vermont

Introduction & Objective: Prostatic neuroendocrine cells are present in all prostate-bearing species. While neuroendocrine cell differentiation has been examined as a mechanism of prostate cancer, little is known about prostatic, neuroendocrine cell function in benign, normal prostate. More specifically, factors triggering prostatic neuroendocrine cell secretion are unknown and biological responses to secreted peptides have not been resolved. Methods: To identify a signaling network initiated by prostatic neuroendocrine cells, we used immunohistochemistry and genetically-modified reporter mice to probe mouse prostatic tissue sections for nociceptors, neuropeptides and their receptors, and other markers of cell identity. To test whether prostatic neuroendocrine cells stimulate prostatic smooth muscle contraction, we deployed genetically encoded calcium sensors in mouse prostatic smooth muscle and designed an *ex vivo* perfusion system to visualize calcium transients associated with prostatic smooth muscle contraction in response to agonists of the nociceptor TRPV1 (capsaicin) and serotonin receptor 5-HT_{2B} (BW273C86). Results: We detected the nociceptor TRPV1 (mediates inflammatory responses in some cells) and serotonin in prostatic neuroendocrine cells and detected the serotonin receptor 5-HT_{2B} in a rare population of KIT+ stromal cells (putative Interstitial Cells of Cajal). We also found that capsaicin and BW273C86 stimulate mouse prostatic smooth muscle contraction. Conclusions: Our initial findings support the hypothesis that noxious stimuli activate prostatic neuroendocrine cell serotonin secretion, which then simulates 5-HT_{2B} on Interstitial Cells of Cajal to initiate prostatic smooth muscle contraction. This is a putative innate response mechanism to constrict prostatic ducts in the presence of prostate inflammation, thereby impeding access to ascending microbes.

PS1-22 Protease activated receptor 2 activates Ca2+ release activated channel mediated Ca2+ influx to cause prostate smooth muscle contraction

M Paul, C Hall, A Schaeffer, P Thumbikat

Northwestern University

Introduction & Objective: Protease activated receptor 2 (PAR2) is a G-protein coupled receptor that contributes to fibrosis in the prostate and lower urinary tract symptoms (LUTS). In addition to fibrosis, aberrant smooth muscle tone in the prostate has been hypothesized to play a role in pathology of LUTS. We therefore examined PAR2 expression in primary human prostate smooth muscle cells (PSMC) and studied the downstream signaling effects of PAR2 activation. Methods: We performed immunofluorescence staining on mice prostate, and reverse transcriptase PCR (RT-PCR) to determine PAR2 expression in PSMC. Involvement of PAR2 and signaling molecules in prostate smooth muscle contraction studied by *in vitro* collagen hydrogel contraction assay. Myosin light chain 20 (MLC20) phosphorylation studied using immunoblotting. Ratiometric calcium imaging was performed in PSMC with Fura-2AM. Quantitative RT-PCR (qRT-PCR) performed to determine expression of L-type and Ca^{2+} release activated channels (CRAC). Results: We show that PAR2 is expressed in PSMC. PAR2 activation mediates a biphasic elevation in intracellular Ca^{2+} and phosphorylation of MLC20, causing cellular contraction as assessed by *in vitro* collagen hydrogel contraction assay. Intracellular Ca^{2+} flux was inhibited by a phosphoinositide hydrolysis inhibitor, U73122, showing a requirement for phospholipase C β (PLCβ) activation. PSMC expressed mRNA for L-type voltage dependent Ca^{2+} channels (VDCC) as well as CRAC, a hitherto unreported finding. Secondary intracellular Ca^{2+} oscillations were abrogated only by BTP2, the CRAC channel inhibitor, but not by nifedipine, inhibitor of VDCC. Conclusions: Our study shows PAR2 is expressed in smooth muscles of the prostate and upon stimulation, PAR2 causes contraction of these cells by stimulating PLCβ/Ca^{2+} and activating surface CRAC. To our knowledge, this is the first report describing PAR2 and CRAC in prostate smooth muscle contraction. Activation of α-adrenergic receptors also activate PLCβ/Ca^{2+} in the prostate. However, drugs blocking α-adrenergic receptors (α-blockers) to treat LUTS associated with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) has a modest impact on relieving urinary symptoms. Based on our findings, we think activation of non-adrenergic receptors, such as PAR2, which can initiate smooth muscle contractions, limit α-blocker efficacy. Future studies aim to understand effects of simultaneous activation of PAR2 and α-adrenergic receptor on prostate smooth muscles and explore using PAR2 blockade, standalone or in combination, with α-blockers to manage LUTS associated with CP/CPPS.

PS1-23 Pro-tumorigenic function of GRM1 in the prostate cancer

G Lee, IY Kim

Rutgers Cancer Institute of New Jersey

Introduction & Objective: Prostate cancer (CaP) is the second leading cause of cancer death in men in the United States. Initially, most CaP-related androgen signalling involves androgen receptors. For this reason, the immediate results of androgen ablation are effective; however, hormone-resistant prostate cancer (HRPC) cells inevitably emerge. In the previous report, it was presented that serum glutamate levels were highly correlated with Gleason score in African American men. Glutamate is a non-essential amino acid, and its signalling is comprised of glutamate, glutamate transporters, and glutamate receptors (GluRs). Specifically, GRM1, a glutamate receptor subtype, is not expressed in luminal acinar epithelial cells of normal or hyperplastic glands. In this study, we examined the role of GRM1 in prostate cancer. Methods: After overexpression of GRM1 in the LNCaP prostate cancer cell line cell growth was measured. And cell proliferation assay after treatment of Bay36-7620 (GRM1 specific inhibitor) and Riluzole (potent inhibitor of glutamate release) was performed. GRM1 immunohistochemistry was also performed using prostate cancer tissue microarray. Results: Overexpression of GRM1 increased cell growth around 30% in the LNCaP cell line. Cell proliferation was decreased by Bay36-7620 or Riluzole treatment in 22Rv1 cells, which expressed GRM1 at the highest level compared to LNCaP and VCaP prostate cancer cell lines. In the TMA analysis (n=99, primary prostate cancer) with GRM1 IHC, Gleason score and GRM1 expression were positively correlated. Conclusions: GRM1 expression was correlated to tumor growth and can be a potential therapeutic target for prostate cancer.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-24 Modeling the Androgen Receptor in Bladder Cancer

M Mudryj[1], K Katleba[1], AP Lombard[2], H Baek[1], K Nishida[1], S Libertini[1], R Nakagawa[1], A Platero[1], J Tian[1]

[1]University of California, Davis; [2]University of California, Davis, School of Medicine

Introduction & Objective: Bladder cancer (BlCa) exhibits a striking gender disparity where men are three times more likely to develop the malignancy then women. The androgen receptor (AR) has been shown to have a role in bladder tumorigenesis, and clinical trials aimed at limiting AR activity by targeting the ligand binding domain (LBD) of the molecule are under way. However, AR target genes that are instrumental in modifying cell proliferation and apoptosis have not been identified. The objectives of the studies were to define AR-dependent mechansims that affect bladder tumor cell viabiltiy. Methods: Molecular, biochemical and RNA-seq studies were employed to investigate the role of AR in bladder tumor cell viability and to identify regulated genes, pathways and networks. Results: An analysis of AR expression in bladder tumor-derived cells showed that they harbor full length AR but also low molecular weight (LMW) isoforms that are missing the LBD. Depletion of all AR forms significantly reduces cell viability in all cells, inhibited cell cycle progression and induced apoptosis. The AR V7 splice variant frequently detected in prostate tumors is expressed in UM-UC-3 cells, and V7 depletion reduces cell viability. Additionally, we cloned a novel AR splice variant, AR-BI1, which localizes to the nucleus, and transactivated transcription from an AR-dependent promoter. siRNA mediated depletion of this isoform reduces cell viability. Using a combination of siRNA AR isoform ablation and variant AR overexpression, RNA seq studies were used to define AR regulated genes and pathways. Conclusions: This study suggests that AR regulates gene expression, and that targeting AR isoforms may be an effective strategy for limiting bladder tumorigenesis.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-25 Pro-differentiating Activity of Vitamin D on Patient-Derived Prostate Epithelial Organoids Involves DKK3

T McCray, L Nonn

University of Illinois at Chicago

Introduction & Objective: Prostate cancer is a leading cause of death in men and deficiency in vitamin D (1,25D) is associated with increased risk of lethal prostate cancer. 1,25D is a pleotropic hormone that regulates calcium homeostasis and plays a role in processes such as differentiation, apoptosis and proliferation. Vitamin D receptor (VDR) expression is positively correlated with tumor differentiation, further indicating its role in differentiation, but its mechanism of action in the prostate is not well defined. Using human primary prostate epithelial (PrE) organoids as a model, the differentiative properties of vitamin D were examined and identified DKK3 as a potential target. Dickkopf-3 (DKK3) has recently emerged as a regulator of prostate epithelial proliferation and terminal differentiation that may act through the non-canonical Wnt and/or TGF-β pathways. Because vitamin D is a known modulator of Wnt and TGF-β signaling in other cell types, we hypothesized it may mediate DKK3 in the prostate as a means to promote or maintain differentiation. Methods: To test this, PrE cells and prostate cell lines were grown as monolayers or organoids in the presence of low doses of 1,25-dihydrovitamin D (1,25D). Results: Both 2D and 3D conditions showed reduced *DKK3* mRNA, and reduced DKK3 protein expression and secretion. ChIP-sequencing for VDR-bound-DNA revealed a peak near the DKK3 promoter, suggesting direct 1,25D-regulation over *DKK3*. The PrE organoids grown in 1,25D conditioned media were strikingly larger than controls and showed altered basal and luminal cell populations via flow cytometry and whole-mount immunofluorescence. Addition of DKK3 to organoids attenuated these effects. 1,25D treatment altered p-Smad2 expression, consistent with findings of DKK3 as a regulator of TGF-β signaling. Ongoing studies are focused further quantifying downstream consequences of DKK3 in both the TGF-β and non-canonical Wnt pathway. Conclusions: Taken together our data show that vitamin D inhibits DKK3 to regulate TGF-β signaling, and support its role as mediator of differentiation in prostate epithelium. The TGF-β pathway is required during prostate development to regulate branching morphogenesis and this pathway is dysregulated in disease. Vitamin D may mediate this pathway through DKK3 to promote the expansion of intermediate cells towards a luminal phenotype. Future studies will explore the implications of this in disease and explore 1,25D activity in other developmental pathways such as the Wnt pathway.

PS1-26 Identification of a rare unannotated long noncoding RNA overlapping the LCK gene that regulates prostate cancer cell growth

H Ta, H Whitworth, S Bhadel, H Frierson, M Conaway, Y Yin, GV Raj, DG Gioeli

University of Virginia

Introduction & Objective: Virtually all patients with metastatic prostate cancer will relapse and develop lethal castration-resistant prostate cancer. Long noncoding RNAs (lncRNAs) are emerging as critical regulatory elements of many cellular biological processes, and there is increasing evidence demonstrating that dysregulation of lncRNAs is associated with many human cancers, including prostate cancer. We have discovered in a high-throughput RNAi screen a novel previously unannotated lncRNA that regulates prostate cancer growth and is associated with prostate cancer progression. Methods: LNCaP, C4-2, and CWR-22-Rv1 cells were used for *in vitro* studies. Rapid Amplification of cDNA Ends (RACE) and sequencing was used to define the previously unannotated lncRNA. shRNAs, qPCR, and cell fractionation was used to determine lncRNA regulation. Cyquant assay, which measures DNA content as a surrogate for cell number, was used to determine how modulation of the lncRNA affects cell growth. Results: RACE and sequencing has revealed that this previously unannotated lncRNA lies within exon six and the 3'; UTR of the *LCK* gene. This lncRNA is dramatically upregulated by androgen in a dose-dependent manner. Furthermore, enzalutamide completely blocked the hormone-induced increase in transcript levels of this lncRNA. Therefore, we have labeled this lncRNA "HULLK" for Hormone-Upregulated lncRNA within *LCK*. Strand-specific PCR reveals that HULLK is transcribed from the sense strand, and cellular fractionation shows that HULLK predominantly localizes to the cytoplasm. Remarkably, we discovered that there was a significant positive correlation between HULLK expression and high-grade prostate cancer in two independent patient cohorts. shRNAs targeting HULLK significantly decreased prostate cancer cell growth. Moreover, cells overexpressing HULLK were more sensitive to the stimulatory effects of androgens. A p300 binding site resides near the HULLK transcriptional start site, and HULLK is downregulated by the p300 inhibitor, A-485. HDAC1 is 3'; cis to HULLK, and knockdown of HULLK leads to an increase in HDAC1 levels. Conclusions: Our data suggests that there is an unannotated lncRNA situated within the *LCK* gene, which we have named HULLK. HULLK is a novel regulator of prostate cancer proliferation *in vitro* and is elevated in patient samples with increasing grade indicating an important role for HULLK in prostate cancer progression. The histone acetyltransferase p300 is critical for HULLK expression, and HULLK negatively regulates the histone deacetylase HDAC1; therefore, this work is the first to implicate HAT repression of a HDAC through a lncRNA.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-27 Human Prostate Stromal Heterogeneity Suggests a Role for Carcinoma-Associated Fibroblast Subpopulations in Myeloid Cell Recruitment

RE Vickman[1], MM Broman[2], NM Lanman[2], A Sudyanti[2], OE Franco[1], TL Ratliff[2], SW Hayward[1]

[1]North Shore University Health System; [2]Purdue University

Introduction & Objective: Carcinoma-associated fibroblasts (CAF) are a heterogeneous group of cells within the tumor microenvironment (TME) that can promote tumorigenesis in the prostate. A more detailed description of the prostate TME, specifically by understanding the mechanism(s) by which CAF contribute to tumor growth, may result in new therapeutic targets for management of this disease. The objective of these studies is to determine whether unique subpopulations of human prostate CAF can be identified and functionally characterized. Methods: These studies utilized the 10x Genomics Chromium system for single-cell mRNA sequencing (scRNA-seq) of primary human prostate CAF. Unsupervised clustering was used to generate cell clusters based on differentially expressed (DE) gene profiles for each cluster. Potential communication between CAF and immune cells was also analyzed using *in vivo* tissue recombination by combining CAF or normal fibroblasts with benign prostate epithelial cell line, BPH-1. Resultant grafts were assessed for inflammatory cell recruitment. Results: Clustering of 3,321 CAF allowed for visualization of at least six CAF sub-populations, each with distinct DE gene profiles. Further investigation into the DE genes for each cluster suggests that these subpopulations have unique functions within the TME, including a role in immune/inflammatory cell recruitment. For example, one large cluster demonstrates increased *CCL2* expression while another large cluster has significantly elevated *CXCL12* expression compared to other subpopulations. Migration of THP-1 monocyte cells in the presence of CAF or normal prostate fibroblast conditioned medium *in vitro* demonstrate that these cytokines may be involved in CAF-stimulated migration of THP-1 cells. Additionally, *in vivo* tissue recombination assays suggest that recruitment of myeloid cells to prostate tissues is increased in the presence of CAF *versus* normal fibroblasts. Conclusions: These studies suggest that specific subpopulations of CAF express elevated levels of extracellular mitotic and chemotactic signaling molecules compared to the bulk CAF population. Chemokines produced by CAF may be involved in the recruitment of inflammatory cells or direct growth regulation of the tumor. These studies also demonstrate that CAF contain a limited number of subpopulations, which allows for biological study and mathematical modeling. Further studies will aim to characterize the subpopulation(s) of CAF which promote immune cell recruitment to the TME and/or stimulate prostate cancer growth and progression, with the goal of identifying novel therapeutic targets to regulate reactive properties within the tumor stroma.

PS1-28 Emerin deregulation links nuclear shape instability to metastatic potential

M Reis-Sobreiro[1], J Chen[1], T Novitskya, S You[1], S Morley, K Steadman[1], N Kaur Gill[2], A Eskaros[2], M Rotinen[1], GC Chu[1], LW Chung[1], H Tanaka[1], W Yang[1], B Knudsen[1], H Tseng[2], AC Rowat[2], EM Posadas[2], A Zijlstra[3], D Di Vizio[1], MR Freeman[1]

[1]Cedars-Sinai Medical Center; [2]University of California, Los Angeles; [3]Vanderbilt University Medical Center

Introduction & Objective: Abnormalities in nuclear shape are a well-known feature of cancer, but their contribution to malignant progression remains poorly understood. Deformation of the nucleus is required for migrating cells to pass through interstitial tissue spaces. Cancer cells exhibiting an “amoeboid” phenotype migrate in a manner that resembles neutrophil movement, in which nuclear deformation plays a critical role. Amoeboid tumor cells are characterized by their plasticity, ability to rapidly move through extracellular matrixes and high rates of shedding of extracellular vesicles (EV). Methods: Flow cytometry, differential centrifugation, iodixanol gradient, confocal imaging, mass spectrometry, time lapse video microscopy, western blot, quantitative digital imaging, bioinformatics, NanoVelcro Chip. Results: Here we show that depletion of the cytoskeletal regulator Diaphanous related formin 3 (DIAPH3), or the nuclear membrane protein lamin A/C, in prostate and breast cancer cells, induces nuclear shape instability, with a corresponding gain in malignant properties, including secretion of extracellular vesicles (EV) with nuclear material. This transformation is characterized by a reduction and/or mislocalization of the inner nuclear envelope protein emerin. Consistent with this, depletion of emerin evokes nuclear shape instability and promotes metastasis. By visualizing emerin localization, evidence for nuclear shape instability was observed in cultured tumor cells, in experimental models of prostate cancer, in human prostate cancer tissues and in circulating tumor cells (CTCs) from patients with metastatic disease. Quantitation of emerin mislocalization discriminated cancer from benign tissue and correlated with disease progression in a prostate cancer cohort. Conclusions: These results identify emerin as a mediator of nuclear shape stability in cancer and show that destabilization of emerin can promote metastasis.

PS1-29 Androgen Receptor Interactome in Prostate Cancer Cell Cycle Progression

DN Ekoue[1], M Goodarzi, J Lim[2], R Sonavane, EB Blatt[1], GV Raj

[1]University of Texas Southwestern Medical Center; [2]Rice University

Introduction & Objective: The androgen receptor (AR) is required for the development and progression of prostate cancer. AR is a ligand driven transcription factor and the interaction of AR with specific protein coregulators modulates its transcriptional activity. Prior ChIP-seq studies have established that AR interacts with distinct promoters in various phases of the cell cycle. RNA-Seq studies indicate different AR-driven transcriptional programs across the cell cycle. We hypothesized that the changes in AR activity during the cell cycle is mediated by AR interaction with distinct coregulators during the various phases of the cell cycle. Towards this end, we sought to establish the AR interactome that regulates cell cycle specific AR activity. Methods: Using flow cytometry, multiple prostate cancer cell lines were optimized for cell cycle arrest in G1 and G2/M phases of the cell cycle using pharmacologic inhibitors PD-0332991, aphidicolin and nocodazole. The cells were evaluated with and without stimulation with DHT. Protein lysates from cells in different cell cycle phases were subject to immunoprecipitation with AR and the AR coregulator binding profile was evaluated using mass spectrometry. The interaction of the top AR binders with AR were validated using multiple methods. Results: We synchronized multiple prostate cancer cells with at least 80% in G1 and 50% in G2. Using unbiased mass spectroscopy-based approaches, we have stratified AR binding partners into three categories - i) coregulators that interact with AR throughout all phases of the cell cycle, ii) coregulators that interact with AR in the G1 phase and iii) coregulators that interact with AR in the G2/M phase. While there were some cell line specific differences, we have defined an AR interactome that is cell cycle phase specific. Validation and functional importance of these interactions is ongoing. Conclusions: The study defines for the first time the AR interactome during various phases of the cell cycle in prostate cancer. While evaluation of the functional importance of these interactions is ongoing, our expertise in blocking specific AR coregulator interactions will enable the development of novel therapeutics in prostate cancer.

PS1-30 Does size matter? Reduced prostate size alters urinary function in male mice

H Ruetten, H Zhang, K Wegner, P Wang, Z Wang, D Bjorling, P Marker, CM Vezina

University of Wisconsin-Madison

Introduction & Objective: Our current understanding of lower urinary tract symptoms (LUTS) in men typically attributes symptoms to an enlarged prostate gland. Research into mechanisms behind LUTS commonly uses mouse models to mimic these symptoms. Many studies have correlated prostate size with LUTS in humans, but mice and men have differing prostatic anatomy and the relationship of prostate size and urinary function has not been explored in mice. Methods: We reduced prostate size using three different methods, surgical castration, finasteride treatment, and genetic modification. We castrated mice (n=8) and sham castrated controls (n=7) at six weeks. Starting at seven weeks, mice were given 100 mg/kg of finasteride daily in corn oil via oral gavage (n=9) and control mice oil alone (n=7). Probasin4 is expressed in prostate luminal epithelial cells. Constitutive Probasin-Cre4/+ male mice were bred to R26R TdTomato (TdT)/Diphtheria toxin (DTA) female mice and selected male offspring expressed Probasin-Cre4/+ and either R26R TdT or R26R DTA. The Probasin-Cre4/+; R26R DTA/+ mice experienced cell death in their prostatic luminal cells while their control littermates Probasin-Cre4/+; R26R TdT/+ merely expressed florescent protein. All mice were on a C57BL/6J background. Cystometry (CMG) and spontaneous void spot assays (VSA) were used to assess urinary function. Mouse urinary function was assessed, mice were humanely euthanized, and tissues collected, weighed, fixed, and embedded for histologic sectioning at nine weeks. Results: Castration reduces overall body mass, prostate mass, seminal vesicle mass, and bladder mass compared to sham castration ($p=0.0019$, <0.0001, <0.0001, and 0.019 respectively). In castrated mice, CMG revealed decreased void duration, voided volume, and baseline bladder pressure paired with an increased urine area as shown via VSA ($p=0.0154$, 0.0494, 0.004, 0.0301 respectively). Finasteride treatment for two weeks reduces prostate mass and seminal vesicle mass compared to oil treated controls ($p=0.0009$ and <0.0001 respectively). CMG revealed an increase in time between voids and voided volume ($p=0.0394$ and 0.0168 respectively) and VSA detected no difference between groups. Probasin-Cre4 collection is ongoing. Conclusions: These results indicate that reduction of prostate size alters urinary function in male mice and with the results of the third group, we hope to provide an educated hypothesis of how mouse prostate size impacts urinary function. The culminated results of this study will inform researchers on the utility of altering mouse prostate as a means to study human LUTS, and help tailor future studies to make better use of prostatic LUTS mouse models.

PS1-31 Hemizygous mutations in the X-chromosome Filamin A gene cause male Prune Belly Syndrome in familial and sporadic cases

N Iqbal[1], T Jascur, S Harrison, C Xing[1], EJ Sanchez[2], S Zhang, A Edwards, LA Baker[1]

[1]UT Southwestern Medical Center; [2]Children's Health

Introduction & Objective: The morbid Prune Belly Syndrome (PBS) includes congenitally hypoplastic abdominal muscle, megacystis/megaureter, and undescended testes. 95% male predominance and multiplex cases suggest X-linked recessive or sex-limited autosomal inheritance. While *CHRM3*, *ACTA2*, *ACTG2* and *HNF1β* have been implicated, their genomic variants only account for one consanguineous family or single cases. In this study, we report the results originating from genetic interrogation of two PBS affected maternally-shared half-brothers. Methods: Lymphocyte DNA was isolated from PBS patients and biological parents prospectively enrolled in our Pediatric Genitourinary DNA Repository. Paired-end, 150 bp Whole Exome Sequencing (WES) was performed and familial segregation of identified variants was assessed by Sanger Sequencing. Ratios of X-chromosome inactivation were measured using the androgen receptor methylation assay with methylation-sensitive restriction enzyme, HpaII. CHO cells were transfected with full-length wild type (WT) or mutant FLNA and lysates were used for pull down assays with immobilized β-integrin tails to assess binding. Effect of mutations on FLNA phosphorylation was assessed in HeLa cells by immunoprecipitation and western blot. Results: WES of a multiplex family with two maternally-shared PBS half-brothers identified 52 shared rare coding variants, including only one encoded on the X-chromosome - a novel, highly conserved, hemizygous missense mutation in *FLNA* (c.6722T>C; p.C2160R, PolyPhen-2 score =0.999, SIFT score =0.000, GERP score =5.58). The two half-brother's mother is the heterozygous carrier without PBS; she has highly skewed X inactivation ratios (95:5). Further WES of 102 unrelated, PBS probands identified two additional individuals with rare *FLNA* variants (c.4592C>T; p.A1448V and c.6956G>A; p.G2236E). Functional validation of all three variants was assessed by FLNA binding to the known interacting protein, ITGβ7. Both the p.C2160R and p.G2236E (but not p.A1448V) significantly enhanced FLNA binding to ITGβ7 tails compared to WT while only the p.C2160R mutation reduced phosphorylation of FLNA at the Serine 2152 position, a site known to be important for downstream FLNA function. Conclusions: *FLNA* is the first X-linked PBS candidate gene and it accounts for both familial and sporadic cases. FLNA is a regulatory versatile stretch mechanosensing actin-binding cytoskeletal scaffolding protein that is highly enriched in bladder and intestinal smooth muscle. Therefore, we propose *FLNA* to be a novel PBS candidate gene.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-32 Global Transcriptional Dynamics Identify Key Functional Pathways Involved in the Pathogenesis of Prune Belly Syndrome

N Iqbal[1], A Kern, T Jascur, GH Henry[1], A Edwards, D Wong, EJ Sanchez[2], L Baker[1]

[1]UT Southwestern Medical Center; [2]Children's Health

Introduction & Objective: Prune belly syndrome (PBS) is a morbid and phenotypically variable congenital disorder characterized by maldeveloped and malfunctioning abdominal wall skeletal and genitourinary smooth muscle. Despite a suggested genetic basis, the cause remains unexplained in the vast majority of human cases. In this study, we evaluated global mRNA changes in PBS affected bladders compared to unaffected controls (CTLs) to identify common molecular mechanisms that drive PBS pathogenesis. Methods: With IRB approval, bladder tissue was collected at surgery from PBS and unaffected CTL patients. mRNA profiles were assessed using the Affymetrix HTA 2.0 RNA expression array and analyzed with the Affymetrix Transcriptome Analysis Console (TAC) software. Differentially Expressed Genes (DEGs) were considered significant if >2 or <-2 fold change and $p<0.05$. Top DEGs were validated by quantitative PCR (qPCR). Gene set enrichment analysis was used to identify canonical pathways mis-regulated in PBS; these were compared to publically available microarray data from partial bladder outlet obstruction (pBOO) in adult rat. PBS and CTL bladders were further characterized using standard histology and electron microscopy (EM) methods. Results: 351 DEGs (183 up-regulated and 168 down-regulated) were identified from PBS (n=4) vs CTL (n=3) bladders. Canonical Pathway analysis confirmed extracellular matrix components (ECM) and integrin signaling as pathways highly enriched in PBS bladders. Histology (H&E and trichrome staining) and EM further validated the enhanced ECM gene expression observed in PBS bladders. Comparison of top PBS DEGs to the pBOO transcriptome identified a unique PBS gene signature including novel significantly mis-regulated genes *SYTL2, TBX20,* and *EDIL3*. Conclusions: PBS is a rare disease for which tissue availability is sparse. We, for the first time, have interrogated alterations in bladder global transcriptional networks associated with PBS pathology in conjunction with histological and ultrastructural analysis of affected tissues. Importantly, our data shed light on the etiology of PBS. While there has been much deliberation concerning the root cause of PBS in the past, we now present unbiased evidence that the bladder mRNA transcriptome of PBS is characterized by a unique signature of ECM deposition, mesenchymal determination, angiogenesis and integrin signaling molecules. Further study of these unique elements holds potential for identifying the molecular basis of PBS and may one day lead to improved therapies for dysfunctional bladders.

PS1-33 Characterization of histone deacetylases in in vitro and in vivo bladder cancer model systems

JM Buckwalter[1], J Ellis[2], JD Raman[2], D DeGraff[2]

[1]Penn State Hershey College of Medicine; [2]Penn State College of Medicine

Introduction & Objective: Bladder cancer (BLCa) is the second most common urologic malignancy with an estimated 81,000 new cases diagnosed in 2018. Epigenetic aberrations are prominent in BLCa with considerable evidence that epigenetic disturbances contribute to the pathogenesis of this disease. While DNA methylation has been extensively studied in BLCa, the role of other epigenetic mechanisms has not been well characterized such as histone deacetylases (HDACs). Histone modifiers are commonly mutated or deregulated in BLCa and play a pivotal role in the regulation of gene expression. In an effort to determine the involvement of HDACs in BLCa initiation and progression, we characterized the expression of HDACs in *in vitro* and *in vivo* BLCa model systems. Methods: We interrogated publically available expression data from The Cancer Genome Atlas (TCGA) bladder study to determine expression patterns of Class I and II HDACs. We used quantitative real-time PCR (qRT-PCR) and western blotting analyses to determine the expression status of Class I and II HDACs in human BLCa cell lines RT4, SW780, UMUC1, UMUC3, T24, TCCSUP, SCaBER, 5637, HT1376, and HT1197. In addition, we determined the expression of a subset of Class II HDACs in human tumor specimens via qRT-PCR. Results: Analysis of the TCGA Bladder study identified expression of HDAC4 and 9 as inversely correlated with expression of basal markers cytokeratins 14 (KRT14) and 5 (KRT5). These findings are in agreement with qRT-PCR results identifying increased expression of HDAC4, 7 and 9 in Basal BLCa cell lines and confirmed by western blotting analyses. Furthermore, qRT-PCR analysis of clinical specimens identified significant increases in the expression of HDAC4, 7, and 9 in invasive disease compared to non-invasive disease. Conclusions: Here, we identify suitable preclinical model systems for the study of HDACs, and show increased expression of Class IIa HDACs, specifically HDACs 4 and 9, in Basal BLCa cell lines and in invasive clinical specimens. These results suggest this class of HDACs may be best suited for targeted inhibition in patients with Basal BLCa. Foundational studies such as this are required to develop further understanding of HDAC mechanistic contributions to disease initiation and progression.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-34 Novel Regulation and Oncogenic Mechanisms of Fatty-Acid Synthase in Aggressive Prostate Cancer

K Ashcraft[1], R Swamidoss, D Bacich[1], B Pflug[2], D O'Keefe[3]

[1]University of Texas Health Science Center San Antonio; [2]Roswell Park Comprehensive Cancer Center; [3]UT Health San Antonio

Introduction & Objective: The mechanisms by which Prostate Cancer (PCa) becomes metastatic are largely unknown. Changes in energy pathways and cellular metabolism are associated with the ability of tumor cells to escape growth regulation and reliance on the microenvironment. Folate is a multifunctional vitamin that forms the methyl donor for methylation, and is responsible for synthesis of 3 of the 4 DNA bases. During development folate promotes energy pathway stability; however, folate induces dysregulated growth in tumor cells. Previous work in our lab showed that tumors from PCa patients with the highest serum folate levels grew 6x faster than the lowest group indicating increased folate may lead to a more aggressive phenotype. We have found that folate is a regulator of the lipid synthesizing enzyme, fatty acid synthase (FASN). In normal cells the lipid synthesis pathway is not active due to dietary fat. However, in PCa, it is highly upregulated and control is lost, mediated in part by Prostate-Specific Membrane Antigen (PSMA) which is upregulated in almost all metastatic PCa. Methods: Prostate tumors from patients were placed under kidney capsules of SCID mice. These mice were then fed diets with varying levels of folate (0, 2, and 10 mg/kg) for 16 weeks. Tumors were harvested and stained for FASN. LNCaP cells with a shRNA targeting PSMA were injected nude mice which were fed varying folate diets (0, 2, 5 mg/kg). Tumor growth was measured and RNA was isolated for RT-PCR. PC-3 cells were folate deprived for 24 hrs then treated with 100 nM folate for 240 minutes. Cells were lysed and AMP kinase was tested through western blot. Results: In the Patient derived xenografts, tumors from the higher folate diet mice exhibited an increase in FASN staining when compared to those on a low folate diet. A novel nuclear localized version of FASN was also found to be elevated high-folate diet tumors. Xenograft tumors from LNCaP cells knocked down for PSMA showed reduced growth when compared with tumors expressing PSMA as well as a reduction of FASN expression dependent on the presence of folate. The AMP-kinase pathway, a known negative-regulator of FASN, was shown to be activated through phosphorylation of AMPk by Folate deprivation in PC-3 cells but rapidly suppressed when folate was added back to the cells. Conclusions: Taken together, these data indicate a novel regulation of the FASN pathway by folate and PSMA. PSMA and FASN are both highly expressed in PCa and this study highlights the need to regulate the amount of folate intake in PCa patients. Men who consume fortified folate diets, or take multi-vitamins with folic acid may be exacerbating their PCa through upregulation of FASN, which acts as a metabolic oncogene in advanced PCa.

PS1-35 A Spatial and Temporal Map of Axons in Developing Mouse Prostate

AE Turco[1], MT Cadena[1], H Zhang[1], J Sandhu[1], R Peterson[1], J Keast[2], CM Vezina[1]

[1]University of Wisconsin-Madison; [2]University of Melbourne

Introduction & Objective: Axons in the prostate control glandular growth, fluid secretion, and smooth muscle contraction and are remodeled in prostate cancer or prostatitis. The causes of both are unknown, but it is hypothesized that a reawakening of developmental signaling pathways occurs. Reawakened developmental signaling pathways might be responsible for nerve remodeling in prostate diseases, but we do not understand when and how axons develop in the prostate. Methods: To address this gap, we used immunohistochemical staining of mouse prostate to map axon subtypes (TH+ (noradrenergic), VAChT+ (cholinergic), and CGRP+ (peptidergic sensory)) in the fetal, neonatal, and adult mouse prostate to determine when axons first innervate the prostate. We quantified axon density temporally and spatially within peri-ductal smooth muscle in dorsal and ventral prostate lobes. Lastly, we determined whether CGRP+, VAChT+, and TH+ axons innervated neuroendocrine cells visualized by immunoreactivity for synaptophysin. Results: We determined CGRP+, VAChT+, and TH+ axons begin to innervate the prostate between E14-15. The density of TH+ and VAChT+ axons did not differ in the developing prostate regions or in proximity to the urethra. TH+ axons were localized primarily in smooth muscle after E17. VAChT+ axons were associated adjacent to and within the epithelial compartment. CGRP+ axons are more dense in prostatic urethral stroma than in distal regions, become progressively more dense with age, and are present in prostatic stromal and urethral compartments. All axon subtypes studied innervated neuroendocrine cells. Conclusions: These results provide a foundation for understanding mouse prostatic axon development and organization, enabling future studies of axon changes caused by environmental factors, reawakening of developmental processes, or cancer. Supported in part by NIH awards R01ES001332 and T32ES007015.

PS1-36 FOXA2 Promotes Prostate Cancer Bone Colonization

ZM connelly[1], S Yang[1], A Orr[1], N Khater[1], R Jin[2], D DeGraff[3], C Morrissey[4], E Corey[4], R Matusik[2], X Yu[5]

[1]Louisiana State University Health Sciences Center-Shreveport; [2]Vanderbilt University Medical Center; [3]Penn State College of Medicine; [4]University of Washington School of Medicine; [5]LSU Health - Shreveport

Introduction & Objective: It is estimated that almost 30,000 men will die from prostate cancer (PCa) in 2018. Initially treatments are successful, however, some are not curative and these tumors almost invariably progress to lethal castrate-resistant PCa. While patients cancer progress, they frequently develop metastases that preferentially relocate to the bones. When this occurs, patient prognosis typically drops to 3-5 years due to lack of therapy available. Unveiling novel mechanisms to how PCa colonizes the bone provides innovative therapeutic targets to treat and, potentially prevent, bone metastases. Methods: Gene expression profiling studies revealed that the pioneer forkhead transcription factor, FOXA2, is expressed in a subset of metastatic PCa samples. Our preliminary study found FOXA2 expression in a sample set of human PCa bone metastases, which may suggest a functional role for FOXA2. Additionally, we found high levels of FOXA2 in aggressive PCa PC3 cells, which generate osteolytic lesions in bone. To establish FOXA2's role in PCa metastasis, FOXA2 was stably knocked down in PC3 cells. Results: FOXA2-knockdown (FOXA2-KD) in PC3 cells revealed a significant decrease of *in vivo* bone destruction following intra-tibial injection. To understand how FOXA2 is facilitating these changes, we examined the expression of all integrins and observed that FOXA2-KD decreased expression of the collagen-binding integrin α1. Furthermore, we found FOXA2-KD decreased PC3 cells' adhesion and spreading on collagen I (a major component of bone ECM) coated surfaces. We found strong evidence that FOXA2 directly regulates the ITGA1 promoter. Lastly, we overexpressed integrin α1 in these same PC3 FOXA2-KD cells and rescued the adherence phenotype. The FOXA2-controlled expression of integrin α1 resulted in changes in the adherence and spreading that would provide a mechanism for PCa cells colonize to the bone. Additionally, we wanted to attempt to understand how FOXA2 could regulate the bone microenvironment once adhered. The FOXA2-KD in PC3 cells resulted in a significant decrease in expression of the parathyroid hormone-related protein (PTHrP), a bone remodeling-associated protein. We observed that PTHrP mRNA and protein were decreased in FOXA2-KD cells. There is also strong evidence to support that FOXA2 directly regulates the PTHLH gene promoter. Furthermore, when PC3 FOXA2-KD cells were co-cultured with osteoblast and osteoclast, osteoclast differentiation markers were decreased. Conclusions: Taken together, FOXA2 plays a major role in orchestrating PCa's ability to colonize bone, and further, promote osteoclast activation.

PS1-37 Elucidating the role of nuclear Nrdp1 in mediating prostate cancer health disparities

S Krig[1], N Batra[1], TM Steele[2], S Evans[1], A Konda[1], A Sam[1], S Siddiqui[3], PM Ghosh[2], RL Vinall[1]

[1]California Northstate University; [2]University of California, Davis; [3]VA Northern California Health Care System

Introduction & Objective: We previously demonstrated that Nrdp1, an E3 ubiquitin ligase, is a transcriptional target of the androgen receptor (AR) and that Nrdp1 levels are reduced by ADT in Caucasian American (CA) CaP cell lines. Nrdp1 regulates levels of multiple targets including ErbB3, a molecule that is associated with ADT resistance. The goal of the current study was to determine whether differential expression and localization of Nrdp1 contributes to CaP health disparities. Methods: Nrdp1 and AR expression levels and localization in CaP patient biopsies and cell lines were assessed using immunohistochemistry (IHC) and subcellular fractionation/western blot, respectively. Immunoprecipitation was used to identify Nrdp1 binding partners. Manipulation of AR and/or Nrdp1 levels/localization was achieved via knockdown (siRNA), forced overexpression (Nrdp1-FLAG construct), and treatment with enzalutamide or synthetic androgen. Results: We demonstrate that nuclear Nrdp1 levels are significantly lower in African American (AA) CaP patients (n=19) versus CA CaP patients (n=121) with localized disease ($p=0.008$), and that AA CaP cell lines also express lower levels of nuclear Nrdp1. Nuclear localization of Nrdp1 has not previously been reported in CaP cells. Knockdown of AR or treatment with enzalutamide reduced levels of nuclear Nrdp1, while treatment with synthetic androgen increased nuclear Nrdp1 levels. Immunoprecipitation experiments verified Nrdp1 can bind to AR. In patients, a strong negative correlation exists between nuclear Nrdp1 and cytoplasmic (inactive) AR ($R=-0.64$, $p<0.001$) in AA tumors, but a weak negative correlation between cytoplasmic (inactive) AR and nuclear Nrdp1 in CA ($R=-0.37$, $p<0.001$). Forced overexpression of Nrdp1 in CaP cell lines results in increased levels of ubiquitination in both the nucleus and cytoplasm. Conclusions: Our patient and in vitro data demonstrate that AA cells express significantly lower levels of nuclear Nrdp1 compared to CA CaP cells and that a consequence of this is reduced ubiquitination of nuclear proteins in AA CaP cells. AR is involved in nuclear translocation of Nrdp1, and this can be inhibited by ADT resulting a further reduction in nuclear Nrdp1 levels. Next steps will be to identify the nuclear targets of Nrdp1 and to elucidate their role in CaP and contribution to CaP health disparities.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-38 Immunohistochemical comparison of novel epithelial and stromal cell types in the Human and Mouse prostate

S Sandhu[1], D Binoy Joseph[1], DW Strand[2], CM Vezina[1]

[1]University of Wisconsin-Madison; [2]UT Southwestern Medical Center

Introduction & Objective: Though mouse and human prostate differ in anatomy, most researchers have operated under the assumption they consist of orthologous cells. A recent single cell RNA sequencing study performed on young adult human prostates identified epithelial and stromal cell populations characterized by unique marker RNAs. These prostate cell populations are predicted to play important roles in prostatic cancer, benign growth and inflammation. The central question for this project is whether the newly discovered adult human prostate cells that are enriched in the developing prostate are also conserved in mice. Methods: We used immunostaining to identify prostate cells and compare them across four experimental groups: adult human (18-29 years) and adult mouse (12 weeks), as well as developing human (17-22 weeks post conception and developing mouse (postnatal day 9). The life stages selected for this study are sexual maturity in the young adult and the period of branching morphogenesis in the fetus/neonate. Prostates were embedded in paraffin, sectioned, placed onto slides, and stained by fluorescent immunohistochemistry. Results: Immunostaining included antibodies targeted against markers of newly identified human prostate cell types including KRT13 (basal epithelia), DHRS7 (luminal epithelia), SCGB1A1 (prostate club-like cells), SCG2 (neuroendocrine cells), Decorin (paracrine fibroblasts). Immunostaining also incorporated antibodies against traditional markers such as KRT5 and KRT14 (basal epithelia), KRT8 (luminal epithelia), CHGA (neuroendocrine cells), and MYH11 (smooth muscle myocytes). Conclusions: Similarities and differences were observed between human and mouse prostate. These results should be taken into consideration when using the mouse as a model for studying the prostate in humans.

PS1-39 Epithelial Dnmt1 regulates prostate bud formation and differentiation by maintaining survival of early prostate progenitors

D Binoy Joseph, A Chandrashekar, L Abler, L Chu, J Thomson, CM Vezina

University of Wisconsin-Madison

Introduction & Objective: The DNA methylating machinery is dynamically regulated during prostate development. Recent studies using pharmacological DNA methylation inhibitors have identified DNA methylation as an important regulatory mechanism during prostate budding morphogenesis. Endocrine disrupting chemicals alter prostate budding with accompanying changes in DNA methylation. To understand the mechanism of action of these chemicals, it is important to elucidate the role of DNA methylation in normal prostate development. The aim of this study was to use a targeted genetic approach to ablate the key maintenance methyltransferase, DNA methyltransferase-1 (*Dnmt1*), in the developing prostate epithelium. Methods: The Cre-lox approach was used to ablate *Dnmt1* in the urethral epithelium from which prostate buds arise. Cre-inducible lineage reporters were used to assess the fate of epithelial cells that have lost DNMT1 expression. In situ hybridization and fluorescent immunohistochemistry were used to investigate the molecular changes that occurred with *Dnmt1* ablation. RNA-seq analysis was performed to assess the transcriptional changes accompanying loss of DNMT1. Results: Conditional ablation of *Dnmt1* in mouse embryos resulted in neonatal lethality from lung defects and a reduction in prostate bud number. Renal grafts from *Dnmt1* mutant embryos showed a complete lack of prostate tissue. Further analyses showed that *Dnmt1* deleted prostate epithelial cells underwent DNA hypomethylation, DNA damage, P53-activation, cell cycle arrest and apoptosis. RNA-Seq analyses of prostate and urethral epithelium from *Dnmt1* mutants demonstrated reactivation of genes involved in germ cell development, activation of P53 target genes and differential expression of several genes involved in morphogenesis. Mosaic inactivation of *Dnmt1* in urethral epithelial cells showed that fast dividing DNMT1 expressing cells are preferentially located at the tips of prostate buds to the exclusion of DNMT1 ablated slow dividing cells. Conclusions: Our results establish a critical role for DNMT1 in regulating prostate epithelial budding by maintaining proliferation and survival of early prostate progenitors. RNA-Seq analysis of *Dnmt1* mutant epithelium identified several genes that are affected by loss of DNMT1 expression in the developing prostate. Further validation is required to identify whether the expression of these genes is regulated by promoter methylation. Changes in *Dnmt1* expression induced by endocrine disruptors might affect the prostate budding program by targeting these genes. Supported by R01DK099328.

PS1-40 A gene signature for CTCF, an epigenetic regulator, predicts adverse cancer outcomes and altered amino acid transport in prostate cancer

J Gawdzik, B Yang, R Machhi, TA Khemees, A Roopra, DF Jarrard

University of Wisconsin-Madison

Introduction & Objective: The cancer driver gene CCCTC-binding factor (CTCF) coordinates epigenetic signaling and interacts with cofactors such as BORIS and CHD8 that affect its function. Our objective is to generate a CTCF gene signature that reports loss of CTCF function regardless of how that function may be lost. This is important because CTCF function may be lost not just by mutation but by alterations in cofactor activity or levels. We then aimed to test whether the signature could inform disease aggression. Methods: CTCF was knocked-down by multiple shRNAs in cell lines and transcriptomes examined by RNA-seq. A shared gene expression signature was generated and used to query primary prostate cancer data in the TCGA and MSK databases. Clinicopathological parameters and gene expression values were compared between patients with this signature and those without. Ontological analysis on genes that are differentially expressed between the 2 tumor groups was performed and identified pathways were tested to determine if they were necessary for cell proliferation and survival in the absence of CTCF. Results: Transcriptome analysis following CTCF knock-down revealed a 52 gene signature comprised of 34 up-regulated and 18 down-regulated genes. Tumors with upregulated signature genes had higher Gleason scores (8 vs 7.5; $p<0.01$) and fewer disease-free months (36 vs 51 mos; $p=0.03$). Expression of known CTCF cofactors were also decreased in patients displaying this signature. When CTCF expression alone was analyzed in these tumors, worse outcomes were not identified-validating our 'signature'; approach. STRING analysis revealed that genes in this signature are involved in L-glutamine transmembrane transport, a well characterized coordinator of tumor growth and proliferation. Subsequently, we show that reduced L-glutamine levels decreases proliferation of CTCF deficient cells but not control cells. Conclusions: We have generated a unique 'broken CTCF'; signature in patients associated with worse tumor pathology, and fewer disease-free months than patients without this signature. These adverse outcomes could be due in part to alterations in amino acid transmembrane transport as CTCF deficient cells are sensitive to reductions in L-glutamine levels.

PS1-41 Gli activating properties of human nuclear steroid receptors

S Massah[1], N Li[1], S Truong[1], G Prins[2], R Buttyan[1]

[1]Vancouver Prostate Centre, University of British Columbia; [2]University of Illinois at Chicago

Introduction & Objective: Hedgehog (Hh) signaling is involved in cell growth and differentiation during embryonic development. This signaling pathway is hijacked in cancer cells to mediate proliferation, invasion and metastasis. The canonical activity of Hh is driven by *Smoothened,* which, when activated, suppresses the degradation of Gli transcription factors. Recently, we described a non-canonical pathway for Gli activation in prostate cancer cells mediated by the binding of transcriptionally active androgen receptor proteins (ARs) to Gli3. This binding protects Gli3 from degradation and maintains it in a transcriptionally-active form. Here, we tested two other human steroid receptors, estrogen receptor (ER) and glucocorticoid receptor (GR) for similar Gli-activating functions. Methods: Human ER-α or GR expression vectors were co-transfected, along with a Gli reporter vector (luciferase) into 293FT cells expressing Gli3. Transfected cells were then treated with vehicle, estradiol (E2-ER ligand); or dexamethasone (dex-GR ligand) and luciferase activity was measured. MCF7 (express ER) or LNCaP-AI cells (express GR) were transfected with reporter in the presence or absence of E2, dex or vehicle and luciferase activity was measured. ER- or GR-Gli3 complexes were detected in extracts of 293T cells by co-immunoprecipitation and by in situ proximity ligation assays in MCF7 cells (express ER-α). The effect of ER-α siRNA knockdown in MCF7 cells on Gli3 protein stability was measured on Western blots. Results: Gli reporter activity was increased by transfection with ER in the presence of vehicle but was further increased by 10 or 50 nM E2. Transfection with GR in the presence of vehicle did not change luciferase activity but the presence of 5 or 10 nM of dex tripled this activity. Gli-luciferase activity was significantly increased in E2-treated MCF7 cells and in dex-treated LNCaP-AI cells. Pulldown of ER co-immunoprecipitates with Gli3. PLA detects the presence of ER-Gli3 complexes in MCF7 cells. ER-α knockdown destablized Gli3 protein in MCF7 cells. Conclusions: Collectively our results have identified a secondary function (Gli activation) shared by an important evolutionary spectrum of human steroid receptors (ER, GR and AR). These observations may explain the growth promoting actions of steroid receptors in steroid-dependent tumour systems.

PS1-42 Cochaperones Cooperate With β-Catenin To Regulate The Androgen Receptor BF3 Surface

NR Ortiz, KE Rodarte, AN Payan, MB Cox

University of Texas at El Paso

Introduction & Objective: β-catenin is a well-known co-activator of AR-mediated transcription and has recently emerged as an important regulator of AR in prostate cancer. β-catenin is predicted to interact through the AR binding function 3 (BF3) surface, found on the AR ligand-binding domain. The tetratricopeptide repeat domain (TPR)-containing co-chaperone FKBP52 promotes β-catenin interaction with AR and is required for β-catenin co-activation of AR activity in prostate cancer cells. We previously demonstrated that FKBP52 and β-catenin interact directly *in vitro* and act in concert to promote a synergistic up-regulation of both hormone-independent and dependent AR signaling. Bag-1L is a co-chaperone known to play a role in AR nuclear translocation and is also predicted to exert its function on AR via the BF3 surface. Thus, our current hypothesis is that the BF3 surface is a promiscuous regulatory surface capable of binding diverse regulators, which then influence co-activator recruitment and promote distinct AR-regulated gene expression profiles. Methods: To assess the effects of Bag-1L on AR activity, AR-mediated luciferase assays were conducted in 22Rv1 cells. To further identify novel interacting proteins, co-immunoprecipitations using FLAG-tagged β-catenin were performed using lysates from 22Rv1 cells. Results: AR-mediated luciferase assays in which β-catenin and Bag-1L are overexpressed display increased AR activity analogous to the FKBP52/β-catenin synergy previously observed. Co-immunoprecipitations using FLAG-tagged β-catenin indicate the presence of a novel protein complex composed of AR, β-catenin, and Bag-1L. Conclusions: Given that Bag-1L, a co-chaperone known to bind AR BF3 and play a role in AR folding and maturation, interacts directly with β-catenin suggests that a larger complex, or multiple distinct complexes involving β-catenin regulate the AR BF3 surface. Future studies will be aimed at furthering our understanding of the mechanism by which the AR BF3 surface is regulated by diverse factors to influence AR co-activator recruitment, recruitment of AR to promoters, and the global expression of AR-dependent genes.

PS1-43 Gli2 Activation by Androgen Receptors and Effects on Prostate Cancer Cell Growth

J Foo[1], N Li[2], S Massah[2], R Buttyan[2]

[1]The Vancouver Prostate Centre; [2]Vancouver Prostate Centre, University of British Columbia

Introduction & Objective: Hedgehog (Hh) signaling regulates the activity of Gli transcription factors. Gli controls genes needed for development, cell growth and cell motility. Canonical Hh signaling through *Smoothened* [*Smo*] suppresses the proteolysis of Gli2 and Gli3, maintaining them in high molecular weight active forms. Previously, we showed that active androgen receptors ([AR] liganded AR full length and AR-V7) recognize and bind to Gli3 at its Protein Processing Domain. This binding overrides the need for *Smo* action and provides a non-canonical means for stabilization and activation of Gli3 in prostate cancer (PCa) cells. Studies on the effects of AR binding to Gli2 are more challenging because Gli2 is expressed at much lower levels than Gli3. Here, we report that AR affects Gli2 protein stability and show that Gli2, like Gli3, is a primary driver of PCa cell growth that is overexpressed in castration resistant disease. Methods: Immunohistochemistry (IHC) using Gli2- and Gli3-specific antibodies was used to assess expression of Gli2 and Gli3 in human PCa tumor microarrays. Western blots were used to identify and quantify levels of active Gli2 in androgen-treated PCa cells (LNCaP, VCaP, LAPC4). Proximity ligation assays (PLA) was used to visualize *in situ* and quantify AR-Gli2 complexes in PCa cells. Gli2- or Gli3-specific siRNAs were used to knock down Gli expression and the effects on cell growth were tested by the CyQuant DNA assay. Results: IHC outcomes showed that nuclear Gli2 and Gli3 protein expression was significantly higher in castration resistant tumors compared to primary disease. Androgens increased expression of active Gli2 in PCa cells and stimulated Gli reporter expression. PLA showed the presence of intranuclear complexes of Gli2-AR-Full-length in androgen-treated LNCaP and Gli2-AR-V7 complexes in CWR22rv1 cells. Knockdown of Gli2 with siRNA reduced the number of these intranuclear complexes. Knockdown of Gli2 or Gli3 inhibited growth of LNCaP-AI, CWR22Rv1 and LAPC4 cells and combined Gli2/Gli3 knockdown was a strong inhibitor of cell growth. Conclusions: AR activity affects Gli2 processing, as it does for Gli3. We can visualize and quantify AR-Gli2 complexes *in situ* in PCa cells. Gli2 suppression, like Gli3 suppression, has a profound effect on PCa cell growth but combined suppression has the strongest effects. Considering that Gli2 is expressed at much lower levels compared to Gli3 in most PCa cell lines, our data showing strong growth suppression by Gli2 knockdown suggests that Gli2 is a more potent transcriptional activator than Gli3. Finally, Gli2 and Gli3 protein expression is elevated in castration resistant disease.

PS1-44 Androgen receptor negative progenitor cells in the neonatal prostate

M Shibata[1], N Epsi[2], A Mitrofanova[2], M Shen[3]

[1]George Washington University; [2]Rutgers, The State University of New Jersey; [3]Columbia University Medical Center

Introduction & Objective: Many prostate cancers that initially respond to androgen deprivation therapy will ultimately develop castration-resistance. The development of more potent androgen receptor (AR) pathway inhibitors has modestly improved survival, but has led to an increase in the frequency of castration-resistant tumors that are AR-null. Thus, a better understanding of the biology of AR-null cells in the prostate is important for new treatment approaches. Because much of prostate organogenesis occurs when androgen levels are low, we hypothesized that progenitor cells in the developing prostate may be intrinsically "castration-resistant". The goal of our study was to determine whether AR signaling is required cell autonomously in prostate basal and luminal progenitor cells during postnatal stages of mouse prostate development. Methods: We deleted AR specifically in either basal or luminal epithelial cells using tamoxifen inducible *Cre* lines and a floxed *AR* allele. We simultaneously lineage-marked the AR-deleted cells with YFP, and lineage-traced the AR-deleted cells. We compared phenotypes of prostates with marked AR-deleted cells to prostates with marked control cells. Results: We analyzed AR-negative basal and luminal cells 4-weeks after AR-deletion in either luminal or basal cells, and found that neonatal basal and luminal progenitors were not affected by cell-autonomous AR deletion during prostate development. Our lineage-tracing results indicated that AR is not required for the differentiation of basal cells to luminal cells during prostate organogenesis. Surprisingly, we also found that wild type and AR-deleted luminal cells from the inner luminal epithelium are capable of generating basal cells. Conclusions: Our findings indicate that AR is not essential in epithelial progenitors during neonatal prostate organogenesis, and suggest that requirements for AR in early neonatal progenitor cells differ from adult progenitor cells. These findings have significant implications for prostate cancer treatment and understanding why hormone deprivation therapies fail, as they highlight the plasticity of cells during prostate development, and suggest that this cellular plasticity does not depend on cell autonomous AR signaling in epithelial cells.

PS1-45 A microRNA signature related to enlargement of prostate gland in benign hyperplasia

JM Cruz Burgos[1], G Morales Montor[2], I Salido Guadarrama[3], CD Cruz-Hernandez[1], S Cortés Ramírez, A Losada Garcia[1], M Rodríguez Dorantes[3]

[1]UNAM; [2]Hospital General Manuel Gea González; [3]INMEGEN

Introduction & Objective: Benign prostatic hyperplasia (BPH) refers to prostate enlargement due to the proliferation of epithelial and stromal cells. This condition affects about 50% of men over 60 years. It can lead to benign prostatic obstruction or lower urinary tract symptoms (LUTS) and it is difficult to determine what type of patients will develop large volume prostates. LUTS are the most common cause of decreased life quality in patients with BPH, treatment depends on gland size and volume which rarely correlate with prostate specific antigen (PSA) or age, but prostate volume is an excellent predictor of future prostate growth, clinical parameters of disease progression and treatment. Molecular signatures including miRNAS profiling have emerged as a promising prediction tools. They are chemically stable in tissues and fluids and have been described as biomarkers to distinguish prostate cancer from BPH. The differential expression and role of miRNAs in large volume BPH remains unclear. The aim of the study was to identify a microRNAs signature related to prostate gland size, using fresh-frozen large volume BPH tissue, considering size, PSA, and age as association covariates. Methods: RNA was extracted from 12 fresh-frozen samples of large volume prostates from "The General Hospital Manuel Gea González" and 6 controls from the medical forensic service using the trizol method. RNA integrity and quantification was assessed using the miRNA Small RNA chip. miRNA expression was analyzed using an Affymetrix Genechip miRNA 4.0 array and data was analyzed with the Affymetrix packages. Statistical significance was adjusted for multiple testing using the FDR method and Benjamini-Hochberg algorithm. miRNAs differentially expressed were validated for 20 selected samples by RT-qPCR. 5 BPH tissue samples, 10 urine, and 5 blood samples of patients with BPH and other 10 urine and blood control samples from healthy patients. Results were analyzed and normalized using the $2^{-\Delta\Delta CT}$ method. Differential expression was correlated with prostate gland size and age. Results: After analysis differential expression between sample groups was observed. Overexpression of miR-505-5p, miR-34a-5p, and miR-362-5p was determined. Bioinformatics analyses showed that these miRNAs were associated to steroid biosynthesis, cell cycle and adherent junction pathways. Conclusions: Our findings suggest that large volume prostates are associated with differential expression of miRNAs. The miRNA expression profiles together with another clinical tests as PSA, ultrasound, imaging; could be contribute to better understanding and clinical handle of patients with BPH.

PS1-47 Convergence of normal human prostate epithelial cells to a small cell neuroendocrine cancer

J Park[1], J Lee[2], KM Sheu[1], L Wang[1], NG Balanis[1], K Nguyen[1], B Smith[1], C Cheng[1], B Tsai[1], D Cheng[1], J Huang[3], S Kurdistani[1], T Graeber[1], ON Witte[1]

[1]University of California-Los Angeles; [2]Fred Hutchinson Cancer Research Center; [3]Duke University School of Medicine

Introduction & Objective: Genetically engineered animal studies and recent large-scale high-throughput sequencing efforts have provided insight into the molecular mechanisms regulating the development of extremely aggressive, neuroendocrine prostate cancer phenotypes including small cell neuroendocrine prostate cancer (SCNPC). SCNPC patients have a dismal prognosis due in part to a limited understanding of the molecular mechanisms driving this malignancy and the lack of effective treatments. Methods: We utilized normal human prostate epithelial cells in an organoid transformation assay to generate and investigate a genetically engineered SCNPC tumor that recapitulates the histological and molecular features of clinical SCNPC. Isolated primary human prostate epithelial cells were transduced with lentiviruses expressing a combination of dominant negative *TP53,* activated AKT1, shRNA targeting *RB1,* c-MYC and BCL2 (PARCB), cultured as organoids, and transplanted into immunodeficient mice. Results: Here, we found that the five-gene PARCB combination drove a SCNPC phenotype characterized by high expression of multiple neuroendocrine differentiation markers and neuroendocrine-specific transcription factors. We further established 6 cell lines from the PARCB tumors with each cell line showing uniform protein expression of defined neuroendocrine markers. High-throughput mRNA sequencing and bioinformatics analyses revealed that our PARCB tumor cell lines were transcriptionally similar to clinical samples of neuroendocrine prostate cancer. Application of a stem cell signature to gene expression datasets from the PARCB cell lines and diverse human prostate cancer phenotypes showed that the PARCB cell lines and human SCNPC were molecularly more stem-like than other prostate cancer variants. Furthermore, whole transcriptome profiling revealed that our PARCB cell lines have remarkably similar transcriptional features with small cell lung cancer cell lines and patient specimens. Conclusions: Our study provides a novel platform to study the biology that drives and promotes the small cell neuroendocrine phenotype. Further, our findings suggest that establishing the molecular mechanisms regulating human small cell cancer biology may provide insight into new therapeutic opportunities to treat this common lethal disease in the prostate and lung.

PS1-48 Mechanisms of MEIS1/2 tumor suppression in prostate cancer

C VanOpstall[1], H Brechka[2], M Gillard[1], B Zhu[1], S Lamperis[3], RM Brown[3], D Vander Griend[3]

[1]University of Chicago; [2]Cambridge University Hospitals; [3]University of Illinois at Chicago

Introduction & Objective: Our lab has previously identified MEIS1 and MEIS2 as novel, putative tumor suppressors in prostate cancer (PrCa). Recently, we observed a step-wise decrease in MEIS1/2 expression through progression of PrCa to metastasis. We also demonstrated that primary tumors with higher levels of MEIS1/2 had a more indolent gene signature and phenotype, while low MEIS1/2 expressing tumors were more likely to metastasize. These data implicate a functional role for MEIS1/2 in regulating PrCa progression, thus we sought a more mechanistic understanding of how MEIS1/2 exert their tumor suppressive functions in PrCa. Here we propose that the tumor suppressive capability of MEIS1/2 is linked to their role as the preferred co-factors for HOXB13 in the prostate. Methods: Using CWR-22rv1 cells exogenously expressing either MEIS1 or MEIS2 we performed cell proliferation/cell cycle assays, cell migration assays, RNAseq and ChIPseq of MEIS1 and HOXB13 *in vitro*. RNAseq data was analyzed with kallisto and edgeR, while ChIPseq was aligned with bowtie2 and analyzed with HOMER to call peaks and enriched motifs. We also used CRISPR with custom crRNAs to knock out HOXB13. Finally, we investigated the tumor suppressive capabilities *in vivo* with subcutaneous xenografts of the CWR-22rv1 cell line variants. Results: MEIS1- and MEIS2-overexpressing 22rv1 cells showed significantly reduced proliferation both *in vitro* and *in vivo* as compared to GFP controls, which can be attributed to a buildup of cells in the G1 phase. We also observed a significantly decreased capacity for migration in MEIS1-expressing cells compared to GFP controls. We have also shown that MEIS1 and HOXB13 interact with each other in the nucleus. Furthermore, the decreased proliferation and migration phenotypes observed with MEIS1 expression could not be recapitulated in HOXB13-knockout cells. Bioinformatic analysis identified regulation of TGFB and Cadherin signaling as potential mechanisms for the tumor suppressive phenotypes observed. Conclusions: Both MEIS1 and MEIS2 are independently capable of suppressing tumor growth, thus supporting prior reports from our lab that knockdown of both MEIS1 and MEIS2 is required for increased tumor growth *in vivo*. TGFB and Cadherin signaling have been identified as key factors in the mechanism of MEIS1's ability to suppress growth and metastasis. Additionally, the inability of MEIS1 to suppress proliferation and migration of CWR-22rv1 cells lacking HOXB13 highlights the critical importance of the MEIS1-HOXB13 interaction for suppressing PrCa.

PS1-49 Andrographolide Induces a DNA Damage Response in Prostate Cancer via DNA Double Strand Breaks

AR Lopez-Rivas[1], I Forestier-Roman[2], M Sanchez[2], H Ortiz-Zuazaga[1], C Torrez-Ramos[2], M Martinez-Ferrer[2]

[1]University of Puerto Rico - Rio Piedras Campus; [2]University of Puerto Rico Medical Science Campus

Introduction & Objective: Prostate cancer (PCa) is the second most common diagnosed cancer and is the fifth cause of cancer mortality in men worldwide. Although in United States, PCa is the most common diagnosed cancer and is the second cause of cancer mortality in men. Andrographolide has been found to inhibit prostate cancer progression but the mechanism of action remains unknown. Therefore, in this study, we aim to determine the mechanism of action of Andrographolide in PCa. Methods: Androgen-independent and androgen-dependent cell lines were treated with Andrographolide to determine the effect in cell motility, cell proliferation, apoptosis and the expression of DNA damage response genes in vitro. Tumor growth was evaluated using an orthotopic xenograft model in which the prostates of SCID mice were injected with 22RV1, and mice were treated three times per week with Andrographolide 10 mg/kg. Results: In this study, we report that Andrographolide decreased PCa cell migration, decreased invasion, and increased cell apoptosis in vitro. In vivo studies showed that Andrographolide decreased tumor volume, MMP11 expression and blood vessels formation. Gene expression analysis identified cellular compromise, cell cycle, and "DNA recombination, replication and repair" as the major molecular and cellular functions altered in tumors treated with Andrographolide. Flow cytometry analysis and immunoflourescence showed that Andrographolide increased significantly H2AX phosphorylation and double strand breaks in PCa cells. Conclusions: This study confirmed that Andrographolide increases significantly H2AX phosphorylation in PCa, and therefore induced DNA damage response through double strand break repair pathway.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-50 Low Testosterone Levels Cause Augmented Cistrome Binding and Leads to the "AR Malignancy Shift"

B Copeland, M Otto-Duessel, J Jones

City of Hope

Introduction & Objective: Prostate cancer (PC) is the second most diagnosed cancer and sixth leading cause of cancer mortality in men in the world. Interestingly, androgens decrease approximately 1% per year in men over the age of 40. Although the androgen receptor (AR) is the primary driver in PC and androgen depletion initially inhibits the growth of metastatic PC cells, there is no longer any doubt that low testosterone (low T) is strongly associated with increased risk and increased aggressiveness of PC. A majority of studies demonstrate an association between low T and poor prognostic features of PC, including high-grade disease, advanced pathologic stage and increased risk of biochemical recurrence following radical prostatectomy. In benign prostate epithelial cells, AR primarily binds consensus AR binding sites. In the tumor setting AR has altered DNA binding, associating with FOXA1 and HOXB13 binding sites, among others. We have termed this the AR malignancy shift. This shift leads to the transcription of genes associated with a cancerous phenotype. We hypothesize that low T causes increased PC risk and aggressiveness by altering AR activity. Methods: Using human frozen prostatectomy tissue we have used AR ChIP-seq and other techniques to characterize the AR shift. We have also developed mouse models of low T to analyze the effects of low T on the AR axis in the prostate. 40 male mice were divided into 4 groups; intact, castrate, castrate + low T and castrate + normal T. After 6 weeks of treatment, animals were euthanized and prostates harvested and AR ChIP-seq and other analyses were performed. Results: We have identified augmented AR binding patterns in human tumor tissue compared to benign. We also found that low T in benign wild-type mouse prostate tissue resulted in the AR malignancy shift and causes changes in AR signaling that recapitulates human PC. Conclusions: Our results show that low T alone in wild type mice was sufficient to cause the AR malignancy shift that recapitulates the human disease. There is lingering fear among clinicians that testosterone replacement therapy (TRT) could increase the risk of cancer due to the androgen dependent nature of metastatic PC cell growth. However, if we can clearly demonstrate that low T is contributing to PC development and define the mechanisms by which it does so, it would strongly support the call for appropriately powered studies in aging men to once and for all test the ability of TRT to reduce PC risk. A longer chronic low T study (52 weeks) is underway to investigate if low T alone can cause histopathological changes to the prostate in wild type mice.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-51 Differential Roles of Peroxisome Proliferator-Activated Receptor Gamma Isoforms in Prostate Cancer

M Salgia, C Elix, S Pal, J Jones

City of Hope

Introduction & Objective: Prostate cancer (PC) is the most commonly diagnosed cancer in males. Androgen receptor (AR) signaling is significant in the development and progression of PC, but other molecular pathways contribute as well. Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) has recently been implicated as an oncogene in PC, which may influence both the development and metastatic progression of the cancer. There are two isoforms of PPARγ, with PPARγ2 having an additional 30 amino acids at the N terminus. Here, we investigate the role of the two isoforms in PC. Methods: Immunohistochemistry (IHC) was used to quantify the expression of PPARγ1 and γ2 in a series of 35 localized and 8 metastatic PCs. Human and mouse cell lines were used to determine the influence of PPARγ isoforms on the growth of PC cells and on AR activity. Results: Using an antibody (Ab) that detects both isoforms, we found that PPARγ is expressed to varying degrees in cancerous prostate tissue. An Ab specific for PPARγ2 showed lower intensity and frequency of staining compared to the PPARγ1/2 Ab, but staining was still present, suggesting that both isoforms are expressed in some PCs. Interestingly, the PPARγ1/2 Ab, but not the γ2 Ab, also had very strong staining of prostate epithelial cells in select benign glands. The location of these cells and additional IHC markers suggest that these are intermediate, or transit amplifying (TA) cells. In the human prostate and PC cell lines examined, several had expression of PPARγ1, but none had expression of PPARγ2. The growth of cell lines expressing PPARγ1 was inhibited by PPARγ competitive antagonists. By transfecting the isoforms into PPARγ-negative PC cells, it was determined that PPARγ1 decreased AR activity while PPARγ2 increased its activity. We are now examining the differential effects of PPARγ1 and γ2 on growth and AR activity in additional human cell lines and in mouse primary prostate epithelial cells that endogenously express both isoforms. Conclusions: PPARγ is overexpressed in PC and relates to grade and stage of disease, suggesting that PPARγ inhibitors could have a role in treatment of the disease. PPARγ1 also appears to be expressed in TA cells, which could implicate it in PC initiation. Though expressed to a lesser extent, PPARγ2 is still relevant in the human prostate. Cell culture studies imply opposing roles for the two isoforms in terms of effects on AR activity and possibly PC cell growth. Thus, it is essential to dissect the roles of the two isoforms as this will impact the utility of PPARγ as a target in PC.

PS1-52 Fluorescent-androgens can be used to track androgen metabolism in prostate cancer

M Fiandalo[1], V Sviripa[2], J Stocking[1], J Stocking[3], D Holbert[4], J Wilton[1], K Mantione[1], K Attwood, H Minderman[1], Y Wu[1], D Watt[2], J Mohler[1]

[1]Roswell Park Comprehensive Cancer Center; [2]University of Kentucky; [3]Massachusetts General Hospital; [4]University of West Virigina

Introduction & Objective: Men with advanced prostate cancer (CaP) receive androgen deprivation therapy (ADT), which lowers circulating testosterone (T) levels, impairs androgen receptor (AR) activation and CaP regresses. However, ADT is palliative and CaP recurs as lethal castration-recurrent/resistant CaP (CRPC). One mechanism that provides CaP resistance to ADT is intratumoral synthesis of dihydrotestosterone (DHT) that occurs via the frontdoor and primary and secondary backdoor androgen metabolism pathways. Androgen metabolism enzyme inhibitors, such as dutasteride or abiraterone, impair enzyme activity and lower CaP tissue T or DHT levels. CaP cells switch androgen metabolism pathways to overcome inhibition and maintain T or DHT levels sufficient to activate AR. When CaP cells change androgen metabolism and which pathways are preferred to overcome inhibition remains unknown. The objective was to characterize androgen metabolism using fluorescent-coumarin-labeled androgens. Methods: Androgen sensitive LAPC-4, VCaP and LNCaP, castration-recurrent CWR-R1 and 22rv1, and androgen-independent PC-3 cell lines were used. ImageStream, a hybrid technique of flow cytometry and fluorescent-microscopy, was used to assess CaP cell uptake of fluorescent-androgen derivatives of androsterone, androstanedione, androstanediol or dihydrotestosterone. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to measure androgen and fluorescent-androgen metabolism. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was used to assess cell growth and quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine if kallikrein-related peptidase 2 (KLK2) and prostate-specific antigen (PSA), were upregulated. Results: ImageStream revealed all four fluorescent-derivatives were taken up by all CaP cell lines. LC-MS/MS revealed fluorescent-androsterone and fluorescent-androstaneidol were metabolized to fluorescent-5α-dione or DHT, respectively. Fluorescent-DHT maintained CaP growth during androgen deprivation by serum-free complete media and stimulated AR-regulated gene transcription, as measured using MTT and qRT-PCR analysis, respectively. Conclusions: Fluorescent-androgens synthesized using coumarin produced functional fluorescent-androgens that were recognized by appropriate enzymes, maintained CaP survival during androgen deprivation, and stimulated AR activation. Fluorescent-androgens can be used to monitor cell line and probably CaP xenograft androgen metabolism.

PS1-53 Retinoid X Receptors Enhance Androgen Signaling and Promote Cell Cycle Progression in Prostate Cancer

LJ Brand, C McNair, KE Knudsen

Thomas Jefferson University

Introduction & Objective: Androgen Receptor (AR) is a master transcriptional regulator of the prostate, and a key driver of disease progression in both early- and late-stage prostate cancer (PCa). While initially effective, tumors eventually circumvent AR-targeted therapies, leading to the onset of lethal castration-resistant PCa (CRPC), for which no curative interventions currently exist. However, the majority of CRPC tumors continue to express AR and rely on AR signaling for survival and proliferation, indicating that the AR signaling axis remains a viable target in late-stage disease. Previous work by our lab identified enrichment of Retinoid X Receptor (RXR) response motifs near AR chromatin binding sites, suggesting that RXR may play a role in regulation of AR chromatin binding. Methods: PCa and CRPC cell models were treated with genetic and pharmacological agents to perturb RXR signaling, and then subjected to 1) whole transcriptome sequencing, 2) chromatin immunoprecipitation, and 3) cell proliferation assays to determine the role of RXR in PCa. Results: Inhibition of RXR expression and/or activity resulted in downregulation of androgen-regulated gene sets, including cell cycle progression and DNA damage response pathways. Moreover, stimulation with an RXR agonist resulted in altered AR chromatin binding and enhanced androgen-independent proliferation in multiple PCa cell models. Conclusions: These data support a novel role for RXR as a crucial AR-collaborating factor in PCa, particularly in response to acute androgen deprivation, and further suggest that co-targeting of the AR and RXR axes could lead to enhanced therapeutic benefit.

PS1-54 Role of Growth Hormone in Prostate Cancer Progression

CJ Unterberger, KA Wegner, EJ Avila, A Viteri, RL Malinowski, CM Vezina, SM Swanson, PC Marker

University of Wisconsin-Madison

Introduction & Objective: The growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis has been implicated in age-related diseases including prostate cancer (PCa). Humans with Laron syndrome lack functional growth hormone receptors (GHRs) and have reduced rates of cancer and diabetes. Mice with homozygous null mutations in *Ghr* are also protected from cancer in the C3(1)/SV40 TAg PCa model. However, available studies have not addressed the potential ongoing requirement for GH signaling at different stages of PCa progression, the requirement for GH/GHR signaling in prostate cancers with PI3K activation, the role of local GHR signaling in PCa cells, or the signal transduction mechanisms downstream of GHR activation important in PCa. This study aims to address these aspects of GH signaling in PCa using innovative genetic approaches in mice. Methods: Conditionally null *Ghr* mice with a floxed *Ghr* exon 4 will be combined with tamoxifen-regulated *cre* and prostate-specific *cre* transgenes to test the requirement for GHR signaling at different stages of prostate cancer progression and local GHR signaling in prostate cancer cells. A novel PCa model with prostate-specific PI3K activation will be bred onto a null *Ghr* background to test this model's dependence on the GH/IGF-1 axis. The roles of different signal transduction mechanisms of the GHR in PCa will be studied using two mutations in *Ghr*, a Box1 binding mutation and an m569 truncation, that selectively alter GHR signal transduction pathways. Results: We have characterized a new PCa mouse model with cre-dependent PI3K activation in the prostate under the control of probasin promoter. This model has more extensive fibrotic features and a shortened time course of neoplastic lesions compared to prostate-specific *Pten* deletion. Genetic crosses to evaluate the role of GH/GHR signaling in prostate cancer progression are in progress. Conclusions: We have characterized a new PCa model based on prostate-specific PI3K activation with unique features including a more extensive stromal reaction and a shortened time course of neoplastic lesions compared to prostate-specific *Pten* deletion. The temporally-controlled deletion of *Ghr* at different stage or PCa progression will determine if disrupting GH signaling can both *prevent* cancer progression and *regress* established lesions. Determining GH dependence in the PI3K PCa model will clarify if GH/GHR signaling is a viable therapeutic target for PCa driven by different genetic mechanisms.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-55 SFRP1 protein modulates the expression of TMPRSS2-ERG fusion gene in two models of prostate cancer

CD Cruz-Hernandez[1], S Cortes-Ramirez[2], M Cruz-Burgos[3], A Losada Garcia[1], M Rodríguez Dorantes[2]

[1]UNAM; [2]INMEGEN; [3]National Institute of Genomic Medicine

Introduction & Objective: Prostate cancer (PCa) is the leading cause of cancer deaths in men of western countries. Androgens play an important role in PCa progression they regulate several signaling pathways, including WNT and the expression of *TMPRSS2-ERG* gene fusion. TMPRSS2-ERG merges the TMPRSS2 to the ERG transcription factor. ERG protein belongs to the ETS family and it is associated with metastasis. *TMPRSS2-ERG* is present in more than 50% of PCa tumors. Interestingly, SFRP1 (protein over-expressed in stroma) also down-regulates AR activity in PCa cells, however is not clear it's effect on prostatce cancer cells positive to TMPRSS2-ERG fusion. The aim of this study was to determine the effect of SFRP1 protein on TMPRSS2-ERG expression using an *in vitro* and an *in vivo* models. Methods: Cell lines were treated with SFRP1 protein. *KLK3*, *AR*, *TMPRSS2*, *ERG*, *TMPRSS2-ERG*, *SFRP1*; *FZD4*, *WNT1*, *WNT3A, LEF1* and *GAPDH*, genes were analyzed using RT-qPCR. SFRP1, AR, ERG and GAPDH protein expression was analyzed by WB, and AR localization by Immunofluorescence. Cell viability, proliferation, and migration, assays were performed. Likewise tridimensional model of spheroids treated with SFRP1 protein was assayed, after 14 days, spheres were harvested and measured. Xenograft model: 3.5×10^6 cells were inoculated into balb/c nu/nu mice. Mice blood was collected to measure PSA by ELISA method. Castration surgery was made when volume of xenograft was around 300 mm^3. One week after castration, mice were randomized into two groups: control group (vehicle) and treated group (SFRP1 administration). treatments were subcutaneously administered once a week during 10 weeks. After mice were euthanized, tumor tissue was collected for mRNA, protein expression and IHC assays. Data were analyzed with Graph Pad Prism 6 statistical package. Results: The AR activity showed an increase after SFRP1 treatment in VCaP cells. Expression of KLK3 and TMPRSS2-ERG fusion gene were increased after SFRP1 treatment. SFRP1 treatment promoted cellular migration and expression of LEF-1. The spheroids treated with SFRP1 protein increased in size in a dose-dependent manner. Finally, SFRP1 protein administration in xenograft induced growth of tumor tissue compared to vehicle. Furthermore, levels of PSA in blood increased with SFRP1 administration. SFRP1 administration induces expression of TMPRSS2-ERG, KLK3 and LEF-1 genes and ERG. Conclusions: The over-expression of SFRP1 in the stroma of PCa cells enhances the expression of the TMPRSS2-ERG gene, promoting proliferation and migration. In PCa patients diagnosed TMPRSS2-ERG positive, the expression of SFRP1 could be important for a better prognostic of PCa progression.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-56 Sox2 Expression Marks Castration-Resistant Progenitor Cells in the Adult Murine Prostate

EM McAuley[1], D Moline[1], C VanOpstall[1], D Vander Griend[2]

[1]University of Chicago; [2]University of Illinois at Chicago

Introduction & Objective: The transcription factor Sox2 (SRY [sex determining region Y]-box 2) has recently been implicated in the maintenance of adult epithelial stem cells in multiple tissues. We and others have previously demonstrated that Sox2 expression is sufficient to promote *in vivo* castration resistance of tumor xenografts, and that androgen-mediated signaling influences Sox2 expression. Methods: To fate-map Sox2-expressing prostate epithelial cells, we generated transgenic Sox2-CreER; ROSA26-lox-STOP-lox-EYFP animals ("Sox2-LT") that express an enhanced yellow fluorescent protein reporter allele as well as a tamoxifen-inducible Cre allele inserted into the endogenous Sox2 locus. We used immunofluorescent staining to co-stain for putative lineage markers to fate-map lineage progenies of Sox2-expressing cells during prostatic development, homeostasis, and regeneration. Results: We observed expression of Sox2 during the formation of the embryonic urogenital sinus epithelium (UGSE). Sox2-LT mice pulsed *in utero* revealed YFP expression in both basal and luminal cell lineages of the UGSE. We found that Sox2 is predominantly expressed in the proximal adult murine prostate basal epithelial cells within intact males. Remarkably, Sox2 expression is present in a portion of proximal, but not distal, luminal cells. Sox2+ luminal cells were protected from castration-mediated apoptosis. Persisting luminal cells express Sox2 after castration. Cycling testosterone revealed YFP+ basal and luminal epithelial cells, some of which were castration-sensitive in a second testosterone cycle. Taken together, these data indicate Sox2+ cells are castration-resistant and contribute to prostatic regeneration. Conclusions: These data implicate Sox2 as a marker of and regulatory factor in the castration-resistance and self-renewal of adult prostate epithelial cells. As expression of SOX2 in human prostate cancer might indicate a phenotypic switch to a more therapy-resistant cancer, it is crucial to understand resultant changes in gene expression throughout tumor initiation and progression as a result of *SOX2* signaling.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-57 The Correlation Between RhoC Expression and Gleason Score in Prostate Cancer

A Nguyen[1], M Dhillon[1], H Abu-Hussain[1], PM Ghosh[2], N Hejazi[1]

[1]California Northstate University; [2]University of California, Davis

Introduction & Objective: RhoC is a member of the Ras-homologous family of genes, which has been implicated in tumorigenesis and tumor progression. However, the exact role of RhoC remains controversial and has yet to be clarified. Here, we investigated whether RhoC was upregulated in prostate cancer as compared to normal prostate, and whether it correlated with Gleason scores and Gleason grades. Methods: Immunohistochemistry (IHC) staining for RhoC using rabbit polyclonal antibody (Abcam ab204312, Statlab, McKinney, TX) was conducted on cross sections of normal prostate tissue and primary prostate cancer tissue, provided as tissue microarrays (TMA) (US Biomax PR1921b). Normal human small intestine tissue was used as positive and negative controls. Staining was categorized as either no expression, cytoplasmic staining, or membranous. Statistical analysis was conducted using GraphPad prism 6.0. Results: IHC analysis of a prostate cancer TMA containing 80 cases of adenocarcinoma and 16 normal prostate tissue (duplicate cores per case) matched for age and PSA, showed no staining for RhoC in normal prostate tissue. However, primary prostate cancer tissue stained positively for RhoC in the cytoplasm ($p=0.002$). There was no correlation between RhoC and age of the patient or PSA levels at the time of prostatectomy. We found significant strong negative correlation between RhoC and Gleason grade ($R=-0.29$, $p=0.0209$). Conclusions: Previous studies have shown that RhoC was upregulated in breast cancer as compared to normal breast tissue, but this is the first time RhoC has been shown to be upregulated in localized prostate cancer as compared to normal prostate. In contrast to a previous study, which showed no correlation between RhoC expression and Gleason grade, we observe significant negative correlation with low-grade tumors expressing higher RhoC compared to high grade ones. These results support previous reports showing that increased RhoC expression predicted a good outcome after radical prostatectomy.

PS1-58 The impact of age on the lower urinary tract of mice

T Liu, C Jeong

University of Wisconsin-Madison

Introduction & Objective: Benign prostatic hyperplasia (BPH) is a disease of aging that 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 rodent models have been invaluable in the study of lower urinary dysfunction (LUTD), many studies recapitulate aspects of aging, steroid hormones, and/or inflammation without using aged mice that would better correspond to the age range of human patients. In this study, we examine the changes within the lower urinary tract that occur under normal aging conditions in mice so we can better understand the contribution of age to disease phenotype. Methods: To examine the effect of aging on the lower urinary tract of male mice, we examined young (3 months) and old (24 months) cohorts. Using ultrasound, we examined the urinary flow velocity throughout the entire urethra. To examine the impact of proliferation on urinary changes, we examined BrdU incorporation by IHC. Using picrosirius red staining, we examined collagen deposition in the prostate and urethra. Using LC-MS, we quantified the circulating steroid hormone levels in young and old mice. Results: The old cohort of mice exhibited changes in bladder mass and volume consistent with young mice with LUTD. Using ultrasound, we observed a significant narrowing of the urethra and a corresponding increase in flow velocity with age; this narrowing and change in velocity occurs most prominently in the prostatic urethra area. Examination of the prostate lobes and urethra show a decrease in proliferation in the aged mice over the young mice. However, this change in proliferation is only indicative of a four week period and not throughout aging. Examination of collagen around the urethra showed a significant increase in collagen deposition with age. Additionally, there is a significant increase in thick collagen bundles around the urethral lumen in older mice with no difference in thinner collagen bundles. Examination of circulating levels of steroid hormones by LC-MS show changes correlating with age potentially contributing to LUTD. Conclusions: Our study shows that age can contribute to lower urinary tract changes leading to urethral lumen narrowing and increased collagen deposition. Although rodents do not develop prostate disease, these changes in proliferation, collagen deposition, and circulating hormone levels with age could give more insight into human disease. Our study also suggests the importance of age in the disease process, and studies examining disease progression and treatment efficacy using exclusively young mice could be incomplete.

PS1-59 The inductive mechanism of interferon-γ in prostate cancer progression via unique microRNA processing machinery

U Lo[1], R Pong, S Ma, C Lin[2], R Sonavane, GV Raj, E Hernandez, J Hsieh

[1]University of Texas Southwestern Medical Center; [2]UT Southwestern Medical Center

Introduction & Objective: Interferon-γ (IFNγ) is first characterized as a cytokine associated with anti-virus and anti-tumor activities as a potent cytotoxic agent used in several cancers. In contrast, several studies indicated that IFNγ has pro-tumorigenic activities such as eliciting several immune checkpoint proteins. In prostate cancer (PCa), IFNγ is highly elevated in patients'; serum after radiation; however, its role is largely unknown. Thus, we investigate the role of IFNγ in PCa, particularly, its impact on cancer progression. Methods: We employed a variety of model system: in vitro tissue culture cell (from immortalized normal cell to androgen receptor-positive and -negative cells), ex vivo human cancer explant culture, and in vivo xenograft as well as several discovery platforms such as microRNA (miR) polymerase chain reaction (PCR) array, RNA pull down-mass spectrometry. Subsequently, the candidate was confirmed by PCR, luciferase reporter gene, western blot, and functional assays for cell invasion and cancer stem cell (CSC). Results: We demonstrated that non-cytotoxic concentrations of IFNγ could increase PCa cell invasion by eliciting epithelial-to-mesenchymal transition (EMT) and CSC phenotypes in vitro, which is inversely correlated with several tumor suppressor miRs. We further identified the miRs'; targets (such as Slug, ZEB1, Bim1, Lin28) critical for EMT and CSC development. We further identified IFIT5 (an IFN-induced gene) complex containing ribonuclease (XRN1) responsible for degrading this group of miRs in their precursor form. Noticeably, this group of miRs exhibits a similar 5';-end structure with protrusion sequence that is specifically recognized by IFIT5. Ex vivo, IFIT5 can be induced in IFN-γ-treated tumor explants, which also causes the turnover of these tumor suppressor miRs. In addition, IFN-γ as well as IFIT5 can promote cancer metastasis in xenograft model. Clinically, IFIT5 is significantly elevated in high-grade PCa and its expression is inversely correlated with this group of miRs in PCa patients from TCGA data analyses. Conclusions: This study unveils a pro-tumorigenic role of IFNγ and its downstream gene-IFIT5 in PCa progression via a new mechanism of action. Particularly, IFIT5 complex represents unique miR turnover machinery that can selectively recognize a group of tumor suppressor miRs with a similar 5';-end structure. Overall, these data also suggest a close regulatory network between EMT and CSC underlying PCa progression.

PS1-60 The regulation of nuclear ErbB3 by the androgen receptor in prostate cancer

MK Jathal[1], S Siddiqui[2], FU Melgoza[2], TM Steele[1], BP Durbin-Johnson[1], PM Ghosh[1]

[1]University of California, Davis; [2]VA Northern California Health Care System

Introduction & Objective: Nuclear ErbB3 increases in castration-resistant prostate cancer (CRPC) and may be associated with disease progression. Low nuclear ErbB3 levels may predict biochemical recurrence in patients. Nuclear ErbB3 localization appeared negatively-correlated with androgen receptor (AR) in an animal model of prostate cancer (PCa) progression. Recent studies implicate an 80 kDa isoform of nuclear ErbB3 that transcriptionally controls some genetic targets. Here, we investigate the mechanism and scope of nuclear ErbB3 in PCa progression and metastasis. Methods: Prostate tissue was obtained from 232 patients (VA Northern California Health Care System, VANCHCS). Appropriate statistical methods were used to analyse marker expression, race, overall survival and time to PSA failure. ErbB3 and AR localization were investigated *in vitro* (cell lines used LNCaP/C4-2/PC3/PC3 stably-transfected with wild-type AR, 'PC3-wt-AR';). AR/ErbB3 expression, activity and subcellular localization were analyzed by high-magnification microscopy/immunoblot/fractionation/immunohistochemistry/reporter gene assays. Results: Radical prostatectomy samples showed higher nuclear and cytoplasmic ErbB3 staining in tumor tissue vs non-tumor tissue ($P<0.001$). Perinuclear ErbB3 was observed in prostate tumor tissues and cell lines and correlated strongly with the actin-binding protein RhoA (pin vitro showed that AR and ErbB3 localization were altered by dihydrotestosterone (DHT, activates AR) and heregulin-1β (HRG, activates ErbB3). Nuclear ErbB3 increased with AR inactivation and decreased with DHT addition, verifying their negative correlation. HRG induced nuclear ErbB3 in CRPC cells. AR-null PC3, but not PC3-wt-AR, demonstrated increased full-length nuclear ErbB3 (we did not detect the 80 kDa isoform seen by others). PC3-wt-AR (but not PC3) cells displayed cytoplasmic ErbB3 whose levels were more susceptible to HRG than DHT. Nuclear ErbB3 modestly decreased cell viability and reporter gene readout in AR-null cells but increased cell viability and reporter gene readouts in cells with active AR protein. Conclusions: Our results support a role for perinuclear ErbB3 expression in PCa progression. The metastasis-related role of increased perinuclear ErbB3 may be mediated via with RhoA (previously implicated in metastasis) and inactive AR. This points to a role for the observed increases in nuclear and cytoplasmic ErbB3 protein as a survival mechanism for the tumor, following androgen deprivation therapy (ADT), and supports observations of a role of nuclear ErbB3 as a predictor of biochemical recurrence, especially in African American men.

PS1-61 The Role of Prostate Derived Ets Factor on AR Cistrom Modulation and Luminal Differentiation

F Wang[1], HK Koul[2]

[1]Louisiana State University Health Sciences Center-Shreveport; [2]LSU Health - Shreveport

Introduction & Objective: Transcription regulation, governed by the presence of transcription factors and the accessibility of regulatory elements such as enhancers is crucial in restricting lineage plasticity. Deregulated expression of differentiation-related transcription factors leads to cancer progression and poor prognosis. Prostate-derived ETS factor (PDEF) is one of the cell identity-related transcription factors characterized by the abundance of H3K27ac near its genomic locus. Highly expressed in prostate luminal cells, PDEF has been shown to promote the transcription of PSA in collaboration with AR. Our previous studies showed loss of PDEF is associated with higher Gleason score and tumor metastasis through MMP9. However, the role of PDEF, as a transcription factor, remains poorly understood. In this study, we evaluated the effects of PDEF on modulation of AR cistrome and luminal/epithelial differentiation. Methods: TCGA data were extracted from cBioportal and analyzed using R. Gene set enrichment analysis (GSEA) was performed using default settings and gene sets were downloaded from MySigDB. Genomic coverage analysis was performed using SeqPlots. ChIP-seq analysis was performed using GALAXY. PC3 cells were grown in DMEM/F12 medium and retroviral expression was used to generate cells with stable PDEF expression. ChIP experiments were performed using Chromatrap ChIP-seq kit followed by standard qPCR with SYBR-Green. Gene track was visualized with IGV. Results: Despite the oncogenic role of AR signaling, it plays a pivotal part in prostate luminal differentiation. We observed loss of PDEF accompanied a transcription switch from canonical AR cistrome to noncanonical AR cistrome in TCGA database with prostate cancer progression. Especially, AR genomic coverage in prostate luminal cell-specific enhancer regions is decreased in CRPC tissues compared with the paired adjacent normal tissue. Moreover, stable-expression of PDEF in PC3 cells restored the expression of canonical AR targets. PDEF ChIP-seq analysis followed by ChIP-qPCR confirmed that PDEF colocalized with AR at the PSA enhancer region. We also observed that PDEF increased expression of prostate differentiation-related genes and decreased expression of stemness-related genes using qPCR. We discovered that PDEF binds to the promoter region of CK18 independent of AR. Moreover, a positive correlation was observed between PDEF and CK18 in cell culture and prostate cancer samples in the TCGA database. Conclusions: These results showed that PDEF plays an important role in the maintenance of canonical AR signature and luminal cell differentiation in prostate cancer in-part by rewiring the transcriptional program.

PS1-62 The role of Stathmin and CAMKII in promotion of EMT in Prostate Cancer

P Deford, S Kasper

University of Cincinnati

Introduction & Objective: The oncoprotein Stathmin is over-expressed in many cancers, including prostate cancer (PCa). Furthermore, increased expression is correlated with disease progression and poor prognostic outcome; however, the mechanisms by which Stathmin regulates cancer progression is poorly understood. Our previous work showed that Stmn1 was only over-expressed in undifferentiated PCa. In addition, Stathmin was differentially phosphorylated on Serine 16 (S16) in DU-145 and PC-3 cells compared to LNCaP cells, suggesting that S16 was associated with increased metastatic potential. Since Ca2+/calmodulin-dependent protein kinase II (CaMKII) is reported to phosphorylate S16, this study investigates the effects of CaMKII-mediated phosphorylation of Stathmin in regulating PCa cell proliferation and/or metastatic potential. Methods: Cell proliferation and migration assays were performed in DU145/WT and DU145/shStmn1 cells treated with increasing concentrations of: (a) the CaMKII small molecule inhibitor KN93, (b) KN92, an inactive derivative of KN-93, and (c) oleic acid which activates CaMKII activity. In addition, a CRISPR/Cas9 approach is being used to generate DU145 cell lines in which Ser16 is substituted with alanine (S16A) to mimic dephosphorylation/inactivation, or glutamic acid (S16E) to mimic phosphorylation/activation. Results: Inhibition of CaMKII activity by KN93 treatment decreased DU145/WT cell proliferation in a dose-dependent manner and this decrease was significantly greater than that observed by knocking down Stathmin protein expression. Treatment with KN92 and oleic acid had little effect on DU145/WT and/or DU145/shStmn1 cell proliferation at any concentration tested. In addition, treatment with KN93, KN92 or oleic acid did not alter basal levels of DU-145 cell migration. One unexpected observation was that proliferation of DU145/shStmn1 cells decreased with KN93 treatment, suggesting that KN93 might have off-target effects. Therefore, DU145/S16A and DU145/S16E cell lines are being generated and cell proliferation and migration assays will be performed and compared to DU145/WT and DU145/shStmn1 cell lines. Conclusions: Our preliminary work suggests that inhibiting S16 phosphorylation with KN-93 greatly inhibits cell proliferation without activating cell motility. Furthermore, targeting post-translational processing, e.g., phosphorylation, may be a more effective therapy for killing PCa cells than simply attempting to remove total protein expression.

PS1-63 Tight junction protein claudin 1 is down-regulated in benign prostatic hyperplasia and by TGF-beta 1 via ERK phosphorylation

K Wang[1], F Li, LE Pascal[2], R Dhir[2], W Chen, D Stolz, D Defranco[3], N Yoshimura, D He, Z Wang

[1]The First Affiliated Hospital of Xi'an Jiaotong University; [2]University of Pittsburgh; [3]University of Pittsburgh School of Medicine

Introduction & Objective: Benign prostatic hyperplasia (BPH) is arguably the most common disease in aging men. Chronic prostate inflammation is thought to play an important role in BPH initiation and progression. Our recent studies suggested that the prostatic epithelial permeability barrier is compromised in BPH specimens. Others have suggested that inflammation can impact tight junction formation and enhance epithelial barrier permeability. Thus, inflammation may be a potential cause of the compromised prostatic epithelial permeability barrier in BPH. However, the relationship between inflammation and tight junctions and their potential roles in BPH pathogenesis are not clear. This study investigated the expression of an important tight junction protein, claudin 1, in clinical BPH specimens, its role in prostatic epithelial monolayer permeability barrier and its regulation by inflammation-associated cytokine TGF-β1 in two human benign prostatic epithelial cell lines. Methods: The expression of claudin 1 was analyzed in 16 clinical BPH specimens by immunohistochemistry. BPH-1 and BHPrE-1 cells were treated with TGF-β1 and transfected with siRNA specific to claudin 1. Permeability changes in the treated cells were measured by transepithelium electrical resistant (TER) and FITC-dextran diffusion assays. The expression of claudin 1, E-cadherin, N-cadherin, snail, slug, ERK, MAPKs and AKT was measured by western blot (WB) underlying TGF-β1 treatment and the expression of claudin 1, E-cadherin, N-cadherin, ZO-1, ZO-2 and ZO-3 were analyzed by WB in the treated cells. Results: Claudin 1 expression was decreased in BPH tissue compared to adjacent normal prostatic tissue from patient specimens. TGF-β1 treatment or claudin 1 knockdown increased monolayer permeability in prostatic epithelial cell lines. TGF-β1 treatment led to decreased levels of claudin 1 and increased levels of snail and slug as well as increased phosphorylation of ERK. Although overexpression of snail or slug had no effect on claudin 1 expression, inhibition of ERK phosphorylation could restore claudin expression level as well as cell permeability barrier. Conclusions: Our findings suggest that TGF-β1 can increase prostatic epithelial monolayer permeability, possibly by down-regulating claudin 1 expression through the ERK pathway. These findings also suggest that inflammation and elevated TGF-β1 may be a major cause of claudin 1 down-regulation and compromised epithelial permeability barrier in clinical BPH specimens.

PS1-64 Transcription factor associations of the lncRNA HOTAIR in prostate cancer

M Kobelev, A Zoubeidi

University of British Columbia

Introduction & Objective: Long non-coding RNA (lncRNA) are a class of non-coding RNA that are greater than 200 bp in length. We have identified the lncRNA HOTAIR as being highly over expressed in castration resistant prostate cancer (CRPC), neuroendocrine prostate cancer (NEPC) patients as well as androgen indifferent cell lines. HOTAIR guides the PRC2 complex to target loci to supress gene expression and promote aggressiveness and treatment resistance. It has also been shown to promote androgen (AR) receptor transcriptional program in CRPC, however it is unclear what role it plays in NEPC. Computational analysis has identified various transcription factor (TF) binding sites along the HOTAIR transcript which we aimed to validate. Methods: We performed RNA associated transcription factor array (RATA) protocol to investigate HOTAIR-TF interactions. The HOTAIR gene was tagged with a 3'; MS2 RNA aptamer sequence in an expression plasmid. This tagged plasmid was overexpressed along with an MS2-FLAG expression plasmid in 42D cells and subject to immunoprecipitation (IP) using anti-FLAG antibody. The precipitate was then applied to a TF activation array and analyzed by 96 well plate reader. RNA-IP was used to validate some of the top RATA results. Luciferase assay and public sequencing data was used to measure changes in transcription factor activity in response to HOTAIR. Results: HOTAIR pulled down many TFs at a significant level. The strongest pulldown was for AR which has been previously shown to be an interacting partner. There is also a strong pull down of basic leucine zipper (bZIP) domain proteins such as AP1 (FOS, JUN) and ATF2. HOTAIR was also enriched by pulldown of AR, cJUN, and cFOS as measure by RNA-IP. HOTAIR overexpression in LNCaP cells produced an increase in AP1 transcriptional activity as measured by luciferase activity and RNA-seq. Conclusions: The results suggest that HOTAIR is capable of binding various TFs in neuroendocrine like cells, especially AR despite an AR-indifferent context. It is unknown if HOTAIR binds all of these TFs directly or indirectly and what the transcriptomic consequences are of these interactions. HOTAIR interaction with AR has previously been established but here we show that HOTAIR can bind AP1 and promote its transcriptional activity as well.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS1-65 Using Metabolic Pathways to Improve Diagnosis and Risk-Stratification of Prostate Cancer

M Siddiqui, H Bell, A Alqazzaz, D Fu, A Karimi, G Ferreira, MC McKenna

University of Maryland School of Medicine

Introduction & Objective: Prostate cancer cell metabolism has been well characterized, including disinhibition of aconitase and resulting decreased levels of citrate. [1-^{13}C]pyruvate can trace important metabolic steps in carcinogenesis, however is limited by the loss of the labeled carbon to CO_2 (Figure 1, yellow carbon). We have proposed that using [2-^{13}C]pyruvate as a biomarker metabolic substrate would better characterize these metabolic changes due to its labeling of downstream metabolites (Figure 1, red carbon). We intend to elucidate the differences in metabolic profiles among aggressive and non-aggressive prostate cancer cell lines (Table 1). Methods: Tumorigenic LNCaP and PC3 prostate cancer cells were exposed to medium containing [2-^{13}C]pyruvate for 4 hours. The metabolites of PC3 were then extracted from the incubation medium and subsequently frozen, lyophilized (to remove H_2O), desalted with Chelex 100, and reconstituted with D_2O and Dioxane (as an internal standard) for NMR chemical shift analysis in a BRUKER 950 MHz NMR. The same procedure was used to extract the metabolites from the LNCaP cell pellet, without use of Chelex 100. Chemical shifts were analyzed with focus on peaks for glutamate, lactate and alanine. Results: NMR quantification data is shown in Table 1. Of note are the differences in quantity of lactate between LNCaP cell (low lactate producing) extract and PC3 (high lactate producing) medium samples. Alanine was not quantifiable for PC3. Further, as expected, there was no quantifiable amount of citrate present in PC3 or LNCaP samples. Conclusions: [2-^{13}C]pyruvate can be successfully utilized as a marker to analyze prostate cancer cell metabolism among non-aggressive and aggressive cell lines via cell extract and medium. Further research will focus on risk-stratification and diagnosis frameworks in both animal and human models.

PS1-66 Vitamin D Deficiency Alters Mitochondrial Health and Metabolism in Primary Prostate Epithelium and Prostatic Tissue

CC Blajszczak, L Nonn

University of Illinois at Chicago

Introduction & Objective: Epidemiological data is mounting showing that vitamin D (vitD), specifically the predominant circulating form, $25(OH)D_3$, decreases the risk of prostate cancer (PCa) mortality. The activity of VitD is dependent on mitochondrial enzymes that convert the circulating pro-hormone, $25(OH)D_3$, to active hormone, $1,25(OH)_2D_3$ which can impact transcription. The metabolism of prostate cells is distinct from most other tissues in that they preferentially utilize glycolytic metabolism consequently decreasing the efficiency of energy production by up to 60%. Instead of energy, high levels of citrate, a vital component of for the prostatic fluid, are produced. Whether vitD primes prostate mitochondria to promote healthy prostatic mitochondria is unclear. Methods: Primary patient-derived epithelial cells and tissues were obtained from radical prostatectomy cores and characterized as cancer or non-cancer by histological analysis before culturing or dissociating. Extracellular flux analysis was done through assessing changes in oxygen consumption and extracellular acidification rates. Citrate levels from cells and tissue assessed through colorimetric analysis and mitochondrial health measured by immunocytochemistry. Results: Our data utilizing extracellular flux analysis has shown in 2- and 3-D culture that vitD increases mitochondrial capacity shortly after treatment and suppresses it after a day. Similarly, quantitative assessment of mitochondrial health in prostate cell lines and primary patient-derived epithelial cells (PrE) supports this dichotomy with early treatments increasing the oxidative potential and citrate production of PrE mitochondria and extended treatment subsequently decreasing them. Similar results were also seen in patient-derived tissues with citrate production increasing with vitD treatment. Additionally, PrEs and prostate cell lines cultured continuously in vitD show a decreased mitochondrial potential and decreased citrate production when vitD is decreased to trace amounts. Conclusions: Therefore, vitD plays an import role in mitochondrial modulation, reshaping cells to increase vitD activation and maintain prostate metabolism, a phenotype likely lost in the context of metabolic disease or metabolic stress. Ongoing experiments are looking at understanding the mechanism employed in these processes and understanding vitD deficiency as it pertains to prostate cell metabolism.

PS1-67 Identification and characterization of prostate cancer stem cells as therapeutic targets

W Hu[1], D Hu[1], L Xie, Y Li, H Hu, M Abern, L Nonn[1], T Shioda[2], G Prins[1]

[1]University of Illinois at Chicago; [2]Massachusetts General Hospital, Harvard Medical School

Introduction & Objective: Prostate cancer (PCa) progression, metastasis and tumor repopulation is increasingly attributed, in part, to prostate cancer stem-like cells (cSC) which are resistant to conventional therapies. Thus discovery of cSC biomarkers and therapeutic targets is essential for effective combined treatment for local and advanced disease, including castrate-resistant PCa (CRPC). We herein developed novel cSC assays and utilize them to efficiently identify and characterize prostate cSC for therapeutic purposes. Methods: PCa prostatectomy specimens collected at UIC Hospital were punched biopsied for primary cell culture and confirmed as >80% adenocarcinoma by pathology. Primary cultured PCa cells prelabeled with BrdU or CFSE were transferred to label-free 3D cultures for 5-7 days to form PCa spheres. Long-term label-retaining cSC were FACS isolated and subject to RNA-seq analysis. Rapidly proliferating non-labeled progeny cells (scPC) were analyzed for direct comparisons. Results: Similar to normal prostate SC, label-retaining prostate cSC undergo both symmetric and asymmetric cell division, a stem cells property. ICC confirmed increased levels of stemness proteins (KRT13, WNT10B) and autophagy (LC3) with decreased E-cadherin in prostate cSC compared to scPC. Knockdown of KRT13 significantly inhibited PCa sphere formation and cSC self-renewal implicating a critical role in cSC survival. RNA-seq identified 916 differentially expressed genes ($P<0.05$) enriched in cSC including cancer associated genes (CRISP3, SEPW1, SMCP) and autophagy gene (GABARAPL1) and 394 genes enriched in scPC. MetaCore analysis revealed the top cSC enriched pathways as Cytoskeletal keratin filaments, Ligand-independent activation of AR, Transcription HIF-1 targets, IGF-1 signaling and Autophagy. Pathways involved in cell cycle and NF-kB activation were enriched in scPC. Gene clustering analysis found 16 KRT genes increasingly expressed in cSC, including KRT13, 23, 80, 78 and 4 common to disease-free prostate SC, plus cancer unique KRT10, 19, 6, 75, 16, 79, 3 and 82, confirmed by ICC. Surprisingly, stem-like cells from patient-matched benign regions exhibit a similar 16 KRT gene profile as cSC, suggesting a cancer field effect. Conclusions: Through use of novel approaches, we have characterized prostate cSC and identified unique genes/pathways enriched in these unique cells. This clarification and gene profiling of human prostate cSC may provide enhanced opportunities for translational studies that target therapeutic-resistant prostate cancer.

PS1-68 Targeting prostate cancer cells with enzalutamide-HDAC inhibitor hybrid 2-75

W Hu[1], L Xu, B Chen, S Ou[2], D Hu[1], Y Li, D Vander Griend[1], G Prins[1], Z Qin[3]

[1]University of Illinois at Chicago; [2]Chemily, LLC; [3]Wayne State University

Introduction & Objective: The progression of castration-resistant prostate cancer (CRPC) still requires androgen receptor (AR) function by evolving mechanisms to reactivate AR signaling. Histone deacetylase inhibitors (HDACis) modulate cytosolic AR chaperone heat shock protein 90 (Hsp90) and disrupt the cancer cells growth. Methods: We designed hybrid molecules containing partial chemical scaffolds of enzalutamide (Enz) and suberoylanilide hydroxamic acid (SAHA). We have shown that Enz-HDACi hybrid 2-75 targets both HSP90 and AR that inhibits the growth of Enz-resistant C4-2 cells. In the current study, we further investigated the detail molecular and cellular actions of 2-75 and tested its anti-cancer effects *in vivo*. Results: Our results showed that compared to Enz, 2-75 showed greater AR antagonistic effects by decreasing the stability, transcriptional activity and nuclear translocation of intracellular AR. In addition to the full length AR, 2-75 also downregulated AR-V7 variant in multiple PCa cell lines. Interestingly, our mechanistic studies indicated that AR affinity retains 2-75 in the cytoplasm of AR^+ PCa cells and subsequently directs 2-75 to AR-associated protein complex, resulting in higher impact on AR-associated Hsp90. Further, different from pan-HDACi SAHA, 2-75 displayed significant cytoplasmic effects on HDAC6 and relatively weaker impact on nuclear HDACs. These novel unique cytoplasmic effects of 2-75 overcome the unfavorable resistance and toxicity properties associated with classical AR antagonists, HDACis and Hsp90 inhibitors. Finally, 2-75 showed greater anti-tumor effect than Enz *in vivo* on subcutaneous xenografts derived from both LNCaP cells and LNCaP-Enz resistant cells. Conclusions: Our data suggest that the newly designed Enz-HDACi hybrid 2-75 and their related AR antagonist-HDACi hybrids may become potential effective anti-cancer drugs in managing CRPC.

Poster Session #2

PS2-01 Identifying Novel Gene Deletions as Predicting Biomarkers of Antiandrogen Resistance in Advanced Prostate Cancer

P Mu[1], J Zhou[1], Z Zhang[2], E Hoover[2], S Barnes[1], L Metang[1], D Yun[1], N Johnson[1]

[1]UT Southwestern Medical Center; [2]Memorial Sloan Kettering Cancer Center

Introduction & Objective: Patients with advanced prostate cancer (PCa) are frequently treated with antiandrogens such as enzalutamide. Unfortunately, nearly 60% of patients exhibit early resistance to antiandrogen, with most initial responders eventually becoming resistant to treatment. Given this, there is an urgent need to understand the mechanism of resistance and identify novel biomarkers for predicting antiandrogen response. The highly heterogeneous nature of advanced PCa strongly suggests that this genetic heterogeneity may be the main determinant of sensitivity and resistance to antiandrogen therapy. Methods: Our previous work revealed that advanced prostate cancer with *TP53/RB1* alterations could acquire lineage plasticity and use it as a means to escape luminal-specific drug therapy targeting AR, through up-regulation of *SOX2*. However, patients carrying alterations in these two loci only account for less than half (40%) of patients who ultimately develop resistance. Therefore, additional genomic alterations must be responsible for resistance in these other CRPC tumors. Results: To identify the genetic alterations that lead to antiandrogen resistance, we successfully conducted an *in vivo* shRNA-based library screen and identified several prime candidate genes which confer antiandrogen resistance when deleted or mutated, with *CHD1* as one of the top candidates. Using both the CRISPR/Cas9 and shRNA system, I have generated LNCaP/AR prostate cancer model carrying knockdown or complete deletions of *CHD1* and demonstrated that both the shRNA-mediated knockdown and complete deletions of this gene can confer significant enzalutamide resistance both *in vitro* and *in vivo*. Notably, sustained inhibition of Androgen Receptor (AR) signaling was observed in *CHD1* deleted tumors, suggesting an AR-independent resistance mechanism, despite robust AR expression. We are now working on elucidating the molecular mechanisms of this resistance, in effort to develop novel therapies to overcome the resistance. Conclusions: The genetic heterogeneity in advanced PCa could be the major reason of various responses to antiandrogen resistance. Given that there are ~10% patients carrying *CHD1* deletion based on several PCa cohorts, the completion of this project will not only add clarity to the underlying mechanism of antiandrogen resistance but may also lead to the development of novel predicting biomarker, as well as combination therapy that would overcome resistance, consequently providing greater clinical benefit to patients with advanced PCa.

PS2-02 Role of beta hydroxybutyrate in androgen receptor signaling inhibition therapy resistance

K Rohena Rivera, A Madhav, V Placencio-Hickok, F Duong, B Angara, N Bhowmick

Cedars-Sinai Medical Center

Introduction & Objective: Prostate cancer (PCa) affects one in every six American men. Current treatment for advanced PCa with recurrent disease, includes androgen-targeted therapy. Our lab and others have demonstrated the impact of the carcinoma associated fibroblasts (CAF) on the development of resistance. We found that enzalutamide caused prostatic fibroblasts to express a tumor-inductive CAF-like genetic signature. Regulation of fatty acid oxidation (FAO) has been identified as a hallmark of progenitor cells and cancer as a significant energy source. Moreover, there is clinical evidence that enzymes involved in the ketogenic pathway are increased in PCa progression. Beta-hydroxybutyrate (βHB), is a product of FAO that can that can be further metabolized, a recognized signaling molecule, HDAC inhibitor, and histone modifier. Given the growing evidence that βHB can have multiple roles in cancer, we tested the mechanism of how βHB contributes to PCa resistance. Methods: PCa cell lines and stromal fibroblasts were treated with different concentrations of βHB in the context of enzalutamide (androgen receptor antagonist). Cell proliferation and death was evaluated, as were RNA and protein. To distinguish metabolic and signaling roles of βHB, metabolically inactive enantiomer S-βHB was used and compared with the metabolically active enantiomer R-βHB. Results: We found that enzalutamide induced mitochondrial biogenesis and fatty acid oxidation in wild type mouse prostatic fibroblasts, leading to βHB production. Interestingly, βHB induced the expression of CAF markers: IL-6, FGF7, FAP and TNC. In the epithelia, we found that βHB elevated PCa cell growth in a dose dependent fashion, as measured by MTT. To determine if βHB mediated castrate resistance due to its metabolic capacity, a metabolically-inactive, S-βHB enantiomer was tested. The S-βHB was found to sensitize PCa to enzalutamide, but the R-βHB (metabolically active) caused proliferation, in the presence of enzalutamide. To test the signaling role of βHB on, we found that within 4 h of treatment with βHB and enzalutamide, FOXA2 and its downstream effectors NGN3 and NKX2.2 were upregulated, over enzalutamide or βHB alone. Prolonged treatment of up to 72 h demonstrated βHB and enzalutamide further promoted N-MYC and CHGA expression, to suggest a progressive neuroendocrine phenotype. Conclusions: Thus, resistance to androgen targeted therapy can be promoted by to stromal FAO and βHB metabolism by PCa epithelia. Further, S-βHB can have a pharmacologic effect in enzalutamide sensitization.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-03 SOX2 is an AR Repressed Gene That Drives Castration-Resistant Prostate Cancer Progression and Mediates AR-Targeted Therapeutic Resistance

A Williams[1], L de Wet[1], S Kregel[2], E McAuley[1], M Gillard[1], R Szmulewitz[1], D Vander Griend[3]

[1]University of Chicago; [2]University of Michigan; [3]University of Illinois at Chicago

Introduction & Objective: Prostate cancer is driven by androgen signaling mediated by the Androgen Receptor (AR) and can be treated by androgen deprivation therapy (ADT). Although initially effective, recurrence of disease following ADT is virtually inevitable, and the development to castration-resistant prostate cancer (CRPC) marks transition to the lethal form of the disease, for which current therapeutic modalities are only palliative. Work by our group and others has identified overexpression of SOX2 [sex determining region Y-box 2], an essential transcription factor for maintaining the survival and pluripotency of undifferentiated embryonic stem cells, to be associated with aggressive prostate cancer. We have demonstrated that *SOX2* is an AR-repressed gene, and that the constitutive over-expression of SOX2 in a hormone-sensitive, SOX2-negative prostate cancer cell line is sufficient to generate a castration-resistant phenotype *in vitro* and *in vivo*. Methods: To elucidate novel SOX2 gene targets in CRPC, we performed SOX2 chromatin immunoprecipitation and sequencing (ChIP-Seq) in CWRR1 prostate cancer cells, normal prostate epithelial cells (PrECs) and human ES cells (WA01) for comparative analyses. To assess SOX2 activity in prostate cancer under androgen deprivation, we perfomed RNA-sequencing (RNA-Seq) in CWRR1 cells treated with Enzalutamide, a potent AR antagonist. We assessed the functional impact of SOX2 depletion by measuring proliferative capacity and cell survival of CWRR1 cells infected with virus containing CRISPR-Cas9 constructs siliencing SOX2 expression. We used a tissue microarray (N = 499) to assess SOX2 protein expression in FFPE prostate cancer tissues, evaulating associations with biochemical recurrence and metastatic disease. Results: ChIP-Seq revealed SOX2 binding of prostate-specific, prostate-cancer specific, and both stem cell and non-stem cell gene targets in prostate cancer for SOX2. Functional analyses of these cells show significantly decreased proliferative capacity and survival in SOX2-silenced cells under treatment with Enzalutamide, as compared to cells infected with a non-silencing control. SOX2 protein expression in FFPE tissues not associated with time to biochemical recurrence, but was associated with lymph node metastases ($p = 0.006$). Conclusions: Our data identify non-stem, and cancer-specific functions for SOX2 in the development of CRPC. In addition to its role in CRPC, our data suggest a role for SOX2 in mediating resistance to AR-targeted therapies in CRPC. The SOX2 target genes and their pathways identified herein represent potentially novel therapeutic targets to better manage advanced prostate cancer and CRPC.

PS2-04 Tumor-derived exosomes regulate Enzalutamide resistance of prostate cancer

Y Tai[1], C Lee[2], M Lee[2], AC Gao[3], T Shen[2], J Hsieh

[1]UT Southwestern Medical Center; [2]National Taiwan University; [3]UC Davis, School of Medicine

Introduction & Objective: Although new generation of anti-androgen agents such as Abiraterone and Enzalutamide can prolong overall survival of castration-resistant prostate cancer (CRPC) patients, CRPC eventually progresses drug resistance often associated with loss of AR. Therefore, unveiling the regulatory mechanism(s) of anti-androgen resistance has emerged as an urgent problem. Exosomes, cell-secreted nanosized vesicles, has been highlighted as carriers for bioactive molecules and waste products, indicating a critical role of tumor-derived exosomes (TDEs) in leading the treatment-induced resistance by exporting of drug or drug targets. However, the role of PCa-derived exosomes in Enzalutamide resistance of CRPC remains unclear. Methods: Both LNCaP and C4-2B cells were treated with incremental concentrations of Enzalutamide to generate the resistant cell. We isolated exosomes from Enzalutamide resistance or sensitive PCa cells by differential ultracentrifugation. Characterization of TDEs was analyzed by dynamic light scattering technique, exosome quantitation kit and Western blot. Additionally, the effect of exosome inhibitor and Enzalutamide were analyzed by MTT assay. Results: Based on characterization of TDEs, particle number, protein content and size exhibited no significant difference between exosomes derived from Enzalutamide resistant or sensitive cells, suggesting no significant impacts of Ezalutamide on exosome biogenesis. Our studies indicated that Enzalutamide-induced resistance in PCa cells exhibited the decreased AR level. Surprisingly, significant amount of AR protein was detected in exosomes derived from Enzalutamide resistant PCa compared to that from Enzalutamide sensitive PCa. In addition, targeting ESCRT (endosomal sorting complex required for transport)-independent exosome biogenesis by GW4869 ablated AR loading into exosomes. Functionally, the resistant CRPC cells were re-sensitized to Enzalutamide in the presence of exosome inhibitor, suggesting that targeting exosome packing can delay the onset of resistance. Conclusions: This study unveils that TDEs can facilitate Enzalutamide-induced resistance of PCa. Understanding the mechanism of exosome-mediated anti-androgen resistance of PCa will not only provide valuable information on the treatment-modulated exosome biogenesis/cargo selectivity but also offer a new therapeutic strategy in Enzalutamide resistant PCa. The emerging lineage transdifferentiation in recurrent PCa represents a major clinical challenge for PCa therapy. This study will certainly contribute to delay this process in reducing the mortality of PCa.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-05 Significance of Prostate Tumor Phenotypic Reprogramming in Resistance to Cabazitaxel Chemotherapy

D Begemann[1], H Pu, N Kyprianou[2]

[1]University of Kentucky; [2]Department of Urology, Toxicology and Cancer Biology, Molecular and Cellular Biochemistry

Introduction & Objective: The plasticity of prostate tumors contributes to the heterogeneity in response and acquisition of therapeutic resistance in advanced castration-resistant prostate cancer (CRPC). Disruption of the phenotypic landscape via epithelial-mesenchymal transition (EMT) enables prostate tumors to invade and metastasize. Our previous studies demonstrated that cabazitaxel (2nd generation taxane chemotherapy that causes mitotic catastrophe and FDA-approved for treatment of CRPC) causes reversal of EMT to mesenchymal-epithelial transition (MET). This study investigated the impact of cabazitaxel chemotherapy-mediated MET on tumor phenotypic reprogramming towards overcoming resistance in models of advanced prostate cancer. Methods: A cabazitaxel resistant cell line (PC3-CR) was developed in our lab from androgen-independent PC3 cells and were comparatively assessed by cell viability, cell migration and matrigel invasion assays. PC3-CR cells have significantly increased survival in response to cabazitaxel. RNA-seq analysis was performed to compare PC3-CR transcriptome gene expression to PC3 cells. *In vivo* therapeutic targeting by cabazitaxel in combination with castration-induced-androgen deprivation therapy (ADT) was investigated using the androgen sensitive LNCaP and CRPC 22Rv1 xenografts. Tumor-bearing male nude mice were treated with cabazitaxel (3 mg/kg) alone, before or after ADT for 14-days. Endpoints of evaluation were EMT profiling, apoptosis, cell proliferation, and differentiation status. Results: RNA-sequencing analysis identified that PC3-CR cells acquire a mesenchymal phenotype via transforming-growth factor-β (TGF-β) signaling. PC3-CR cells exhibited enhanced invasive properties *in vitro* compared to PC3 cells. *In vivo* cabazitaxel treatment induced MET in both LNCaP and 22Rv1 prostate xenografts as shown by increased E-cadherin and decreased N-cadherin levels. Our results also revealed increased apoptosis and decreased cell proliferation among prostate tumor cells in response to cabazitaxel treatment that was enhanced by ADT. Conclusions: These studies provide new insights into re-programming prostate tumor cell subpopulations into an epithelial phenotype by TGF-β driven EMT to MET interconversion contributing to taxane resistance. Our findings are of high translational impact in treatment sequencing, by re-sensitizing tumor cells (after cabazitaxel chemotherapy) to ADT, towards an improved therapeutic response in patients with advanced CRPC.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-06 AR activity patterns in treatment-naïve prostate cancer correlate with drug response signatures and cancer subtypes

S Ben-Salem[1], M Takhar, M Alshalalfa, Q Hu, J Billaud, S Liu, E Davicioni[2], H Heemers[3]

[1]Lerner Research Institute Cleveland Clinic Foundation; [2]Genome Dx; [3]Cleveland Clinic

Introduction & Objective: Androgen receptor (AR) action drives prostate cancer (CaP) progression and is the target for first-line treatment of non-organ-confined CaP. Responses to androgen deprivation therapy (ADT) and radiation therapy (RT), which impact on AR action, however, vary widely among patients. Differential AR coregulation controls gene sets with distinct roles in CaP progression, and CaP genomic heterogeneity is well-recognized, yet AR activity in clinical CaP remains poorly understood and understudied. Methods: Expression of 452 well-characterized direct AR target genes was analyzed in transcriptomes of 6,532 treatment-naïve localized low-to-high risk CaPs. AR target gene expression was aligned with molecular subtypes (ERG+, ETV+, SPINK+, triple-), basal or luminal subtype, PAM50 classifier score, response to ADT or RT, docetaxel or dasatinib sensitivity signatures, genomic instability, and clinical parameters (serum PSA, Gleason Grade, TNM stage). Pathway and Cistrome analyses were done on AR target gene sets and their genomic AR binding peaks. Results: Unsupervised clustering showed that expression patterns of 8 AR target gene sets gave rise to 5 major CaP clusters, consisting of 11 subclusters. CaPs clustered based on cancer subtypes and treatment responses, but not clinical parameters. One major "basal" cluster showed lower response to ADT, higher response to RT and lower docetaxel sensitivity signatures. Considerable variability between 4 luminal clusters included 3 separate ERG+ subclusters that differed in PAM50 classifier score, ADT response and response to docetaxel. Pathway analysis on reconstituted AR target expression patterns from 11 CaP subclusters confirmed differences in associated canonical pathways, upstream regulators and biofunctions. Diversity was seen also in the composition of AR binding sites in the 8 target gene sets. Genes highly expressed in the basal cluster were enriched in SOX transcription factor binding sites, whereas those with pronounced expression in luminal clusters contained motifs recognized by ETS, GATA and Oct family members. Conclusions: Novel insights in heterogeneity in AR action, its molecular determinants and resulting biology in primary CaP were obtained that can be used to optimize CaP treatment plans.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-07 PRMT5 is a master epigenetic regulator of the DNA damage response and is a novel therapeutic target for prostate cancer radiosensitization

JL Owens[1], E Beketova[1], S Tinsley[1], A Asberry[1], S Liu[2], X Deng[1], C Li[3], J Wan[2], C Hu[1]

[1]Purdue University; [2]Indiana University School of Medicine; [3]University of Florida

Introduction & Objective: Radiation therapy (RT) is one of two curative treatments for prostate cancer and kills cells by inducing double-strand breaks (DSBs) in DNA. Following RT, tumors regrow for about 60% of patients with high-risk disease within 5 years. The only clinical approach to enhance RT is androgen deprivation therapy (ADT), which targets androgen receptor (AR) signaling. The use of ADT is limited due to side effects; thus, identification of novel therapeutic targets to enhance RT will save lives of prostate cancer patients. We recently reported that PRMT5 epigenetically activates AR expression and our finding led us to investigate if targeting PRMT5 may enhance RT. Methods: To evaluate if targeting PRMT5 may sensitize prostate cancer cells to radiation, we performed a clonogenic assay of irradiated cells. To determine if PRMT5 is required for repair of radiation-induced DSBs, we performed foci analysis via immunocytochemistry. We then used RNA-seq, qPCR, western blot, and ChIP to evaluate a potential epigenetic role of PRMT5 in activating the expression of genes critical to DSB repair. To extend our findings, we analyzed clinical data from around 18,000 of cancer patients encompassing 43 cancer types to assess if PRMT5 expression correlates with the expression of its putative target genes. Results: Targeting PRMT5 sensitizes prostate cancer cells to radiation independently of AR status. RNA-seq analysis revealed putative PRMT5 target genes including several involved in DSB repair and G_2 arrest. Mechanistically, PRMT5 functions as an epigenetic activator of these genes: upon radiation, PRMT5 is quickly upregulated and recruited to their promoters and methylates histones to activate gene expression, including BRCA1, BRCA2, and RAD51. Targeting PRMT5 decreases expression of these proteins and hinders repair of radiation-induced DSBs in multiple cancer and non-cancer cell types. Clinically, PRMT5 expression positively correlates with the expression of putative target genes involved in DSB repair across all 43 cancer types analyzed. Conclusions: PRMT5 acts as a master epigenetic activator of genes involved in DDR and is critical for cells to survive radiation treatment. Given that PRMT5 is often overexpressed in cancer and its expression correlates with its target genes involved in DSB repair, our findings suggest that PRMT5 is a therapeutic target in cancer to improve radiation therapy.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-08 SRD5A2 promoter methylation predicts the sensitivity to androgen deprivation therapy in Castration-Resistant Prostate Cancer

T Deng[1], X Lin[1], S Wu[1], Z Zhang[2], C Wu[1], M Taplin[2], Z Wang[1], A Olumi[1]

[1]Massachusetts General Hospital, Harvard Medical School; [2]Dana-Farber Cancer Institute, Harvard Medical School

Introduction & Objective: Steroid 5-alpha reductase (SRD5A2) is a critical enzyme for prostatic development and growth. We have found that epigenetic modifications suppress expression of *SRD5A2* in one-third of adult prostates, a condition associated with an androgenic to estrogenic switch in adult prostate tissues accounting for changes in hormonal milieu. Our objective is to demonstrate, in a well-defined subset of PCa patients, whether the *SRD5A2* promoter methylation is associated with cancer progression during androgen deprivation therapy in castration-resistant prostate cancer (CRPC). Methods: Fifty-eight CRPC samples were used for testing: 42 CRPC prostate biopsies were collected from Massachusetts General Hospital (MGH), and 16 baseline and progression bone biopsies from patients treated with abiraterone and dutasteride (NCT01393730). As controls, twenty-three benign prostatic specimens were collected from patients with benign prostatic hyperplasia (BPH). The methylation status of CpG site(s) at *SRD5A2* promoter regions was tested, and the methylation level was calculated. Data was retrieved on primary and secondary androgen deprivation therapy (ADT) treatment response. Overall survival (OS) was calculated from time of diagnosis to time of death. Results: Compared with benign prostatic tissue, CRPC samples demonstrated higher *SRD5A2* methylation in the whole promoter region (MGH samples: $P<0.0001$; samples from NCT01393730: $P = 0.002$). Higher ratio of methylation was correlated with better OS ($R^2 = 0.11$, $P = 0.032$). Hypermethylation of specific regions (nucleotides -434 to -4 (CpG#: -39 to CpG#: -2)) was associated with a better OS (11.3±5.8 vs 6.4±4.4 years, $P = 0.001$) and progression-free survival (PFS, 8.4±5.4 vs 4.5±3.9 years, $P = 0.005$) with cutoff value of 37.9%. Multivariate analysis showed that *SRD5A2* methylation was associated with OS independently (whole promoter region: $P = 0.035$; specific region: $P = 0.02$). Conclusions: Our study demonstrates that *SRD5A2* hypermethylation in specific promoter regions of *SRD5A2*, a condition that favors estrogenic as opposed to an androgenic milieu in the prostate, is significantly associated with better survival in CRPC patients. We show that a well-defined subset of prostate cancers with *SRD5A2* methylation might predict the sensitivity to ADT. Recognition of epigenetic modifications of *SRD5A2*, which affects the prostatic hormonal environment, may affect the choices and sequence of available therapies for management of CRPC.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-09 Therapeutic targeting of eukaryotic translation initiation factor 4 gamma 1 (EIF4G1) in Castrate Resistant Prostate Cancer

PK Jaiswal[1], S Koul[2], R Shi, HK Koul[3]

[1]Louisiana State University Health Sciences Center-Shreveport; [2]M.Sc.; [3]LSU Health-Shreveport

Introduction & Objective: Cap-dependent mRNA translation, the rate limiting step of initiation of protein synthesis, is being recognized as an essential process for translation of key oncogenic proteins at optimal levels, and as such offers a novel target for therapeutic intervention. An interaction between EIF4G1-EIF4E is crucial for the formation of the EIF4F complex and initiation of cap-dependent translation. Our exciting recent observations demonstrate that EIF4G1 is amplified and/or overexpressed in a majority (59%) of patients in CRPC/NEPC data set, and plays an important role in PCa cell growth and metastasis. In the present study we observed differential expression of EIF4G1 in low grade vs high grade PCa and evaluated the effects of modulating EIF4G1 in PCa and CRPC/ENZ resistant PCa. Methods: Enzalutamide (ENZ) sensitive LNCaP, C4-2B cells and ENZ resistant C4-2B (C4-2B ENZR) cells were used in present study. To evaluate function of EIF4G1, EIF4G1 was either knocked down using ShRNA or activity of EIF4G1 was inhibited using small molecule inhibitors (CC-123 or 4EGI-1). The levels of EIF4G1 were measured by Immunoblotting and RT-PCR. Cap-binding activity was evaluated using m^7-GTP Sepharose binding assay. Association of EIF4G1 with cap-complex was determined by Western blot assays. *In-vitro* functional assays were performed in the cells following EIF4G1 knockdown or EIF4G1 inhibition. Prostasphere assays were performed using single cell suspensions (5×10^3 cells/well) cultured in ultralow attachment surface dishes, and photographed using Cytation 5 imager. Results: We discovered that EIF4G1 is amplified and/or upregulated in only 11% patients in TCGA data set as compared to over 59% patients in CRPC/NEPC data set. In complementary laboratory studies we observed increased level of EIF4G1 protein in C4-2B ENZR (ENZ resistant) cells as compared to parental C4-2B (ENZ sensitive) cells. Cap-binding assay by m^7-GTP Sepharose revealed that treatment with EIF4G1 inhibitors resulted a decreased in EIF4G1 associated with cap complex. Treatment of C4-2B ENZR cells with EIF4G1 inhibitors (4EGI-1 and CC-123) resulted a decrease in EIF4G1 protein levels, dose and time dependent fashion. Moreover, addition of EIF4G1 inhibitors or knock down of EIF4G1 using shRNA inhibited colony formation and 3D-prostasphere formation, suggesting a critical role of EIF4G1 in CRPC. Conclusions: EIF4G1 expression is elevated in CRPC, and plays a critical role in oncogenic properties. Small molecule inhibitors of EIF4G1 decrease protein levels of EIF4G1 and inhibit Clonogenic activity. This is the first study suggesting a critical role for EIF4G1 in CRPC and ENZ resistance.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-10 RORγ inhibitors resensitize docetaxel and cabazitaxel cross-resistant CRPC to taxanes

Y Wang[1], Z Huang[1], C Liu[1], AC Gao[2], F Zhou[3], H Chen[1]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine; [3]Sun Yat-sen University Cancer Center

Introduction & Objective: Docetaxel (DTX) and cabazitaxel (CTX) are widely used as chemotherapy drugs for mCRPC patients treatment. Drug resistance often occurred. ATP binding cassette subfamily B member 1 (ABCB1), also known as multidrug resistance protein 1 (MDR1), pumps its small molecular substrates out of the cells and causes resistance to drugs, including DTX and CTX. Our previous study found that RORγ, a nuclear receptor family member, is a potential therapeutic target for CRPC. RORγ functions by directly control of gene expression. In this study, we wish to examine whether inhibition of RORγ can sensitizes DTX and CTX cross-resistant CRPC to taxanes. Methods: Cell viability and growth assay and colony formation were performed to examine the synergistic cell-growth inhibition of CRPC cell lines. qRT-PCR and Western blotting were performed to measure MDR1 mRNA and protein level changes. Rhodamine 123 is a MDR1 substrate. Rhodamine efflux assay was used to measure the MDR1 drug pumping activity. SCID mice bearing C4-2B-TaxR tumors were treated with RORγ small-molecule inhibitor and DTX alone or in combination. Results: We show that the RORγ inhibitors inhibit growth of CTX and DTX cross-resistant CRPC cells. When combined with DTX or CTX, the inhibitors display strong synergistic growth inhibition of C4-2B-TaxR and DU145-TaxR, but not regular C4-2B and DU145 cells. In a C4-2B-TaxR tumor model, a low does of RORγ inhibitor resensitized the tumor to DTX. Our mechanistic studies demonstrate that RORγ inhibitors alone can strongly decrease the MDR1 mRNA and protein expression of DTX and CTX cross-resistant CRPC cells. Results from our rhodamine efflux assay show that inhibition of RORγ strongly decrease the drug pumping activity of MDR1. Conclusions: When combined with DTX or CTX, RORγ inhibitors can have potent synergistic growth inhibition of DTX and CTX cross-resistant CRPC tumors through suppressing MDR1 expression.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-12 Combating lineage plasticity to suppress therapeutic resistance in advanced prostate cancer

A Davies[1], S Nouruzi[1], D Thaper, S Kim, CE Bostock[2], S Kumar[1], L Puca[3], M Ahmed, J Bishop, L Fazli, H Huang, DW Goodrich[4], H He, H Beltran[3], A Zoubeidi

[1]Vancouver Prostate Centre, University of British Columbia; [2]Queen's University; [3]Weill Cornell Medicine; [4]Roswell Park Comprehensive Cancer Center

Introduction & Objective: Potent targeting of the androgen receptor (AR) in castration-resistant prostate cancer has altered the archetypal course of the disease, fueling the emergence of aggressive and incurable neuroendocrine prostate cancer (NEPC). These tumors can arise from non-neuroendocrine cells in response to AR pathway inhibitors (ARPIs), such as enzalutamide (ENZ), an observation consistent with lineage plasticity. Recent evidence suggests that evolution toward a NEPC phenotype is aligned with dynamic epigenetic reprogramming, but the molecular basis underlying this phenomenon remains poorly understood. Methods: We developed an *in vivo* model of acquired ENZ resistance to (a) identify reprogramming factors that facilitate lineage plasticity, and (b) determine how to best capitalize on therapeutic strategies aimed at blocking or reversing lineage transformation. Cell lines derived from ENZ-resistant tumors were profiled by RNA-seq and ChIP-seq, and functionally assessed for stem cell-associated properties. Our findings were validated across NEPC cell lines (NCI-H660), genetically engineered mouse models (PBCre4:*Pten*$^{f/f}$:*Rb1*$^{f/f}$), and patient tumors and organoids. CRISPR/Cas9-mediated genomic editing allowed us to assess the effect of knocking out reprogramming factors on therapy-induced neuroendocrine transdifferentiation. Results: Using a multicolor genetic lineage tracing approach, we demonstrate that prostate cancer cells convert to a stem cell-like state permissive of lineage plasticity and, in turn, transition to a neuroendocrine phenotype under the pressure of ARPIs. This plasticity was found to be driven by EZH2; in particular, we identified EZH2 to be phosphorylated at threonine-350 (pEZH2-T350) in NEPC cell lines, mouse models, and patient tumors. Notably, RB1 loss was sufficient to enhance pEZH2-T350 via CDK1 activation, which facilitated rapid NEPC transdifferentiation. This transition was associated with a marked redistribution of the EZH2 cistrome, specifically to a core set of genes governing lineage identity. AR colocalized at the reprogrammed EZH2 binding sites, and was found to be part of the same complex with EZH2. Treating AR-indifferent/NEPC cell lines with clinically relevant EZH2 inhibitors reversed the lineage switch and mitigated ENZ resistance. Conclusions: This research establishes the centrality of epigenetic reprogramming in driving the insurgence of a neuroendocrine phenotype in response to ARPIs, and posits that drugging the epigenome via EZH2 inhibition may reverse or delay lineage transformation to extend the durability of clinically beneficial ARPIs.

PS2-14 PTH1R-mediated down-regulation of TGFBR2 in osteoblasts contributes to enzalutamide resistance in prostate cancer bone metastasis

J Cao[1], X Meng[1], A VanderArk[1], E Woodford[1], X Zhang[2], Z Madaj[1], E Xu[1], X Li[1]

[1]Van Andel Research Institute; [2]Mayo Clinic

Introduction & Objective: Second-line hormonal therapies, such as enzalutamide (Enz), only improve overall patient survival by a few months in about 50% of prostate cancer (PCa) patients, and almost all patients develop drug resistance. Due to the heterogeneity and plasticity of cancer cells, the mechanisms of and approaches to overcome Enz resistance that focus on PCa cell autonomous effects are not adequate and effective. On the other hand, the tumor microenvironment was found to contribute drug resistance. Thus, determining the cancer cell non-autonomous mechanism of Enz resistance might provide novel targets for PCa bone metastasis. Methods: The efficacies of Enz treatments were compared among orthotopic, subcutaneous, and intratibial PCa xenograft models. Immunohistochemical analysis of the TGF-β type II receptor (TGFBR2) was performed in a PCa bone metastasis tissue microarray. The staining was blindly scored, and an unbiased survey of correlations was conducted among cell-specific expression of TGFBR2 and clinical parameters. To dissect the role of Enz on the bone microenvironment, western blots were used to detect protein changes in osteoblasts and osteoclasts. Results: Enz is a small-molecule inhibitor of the androgen receptor. We showed that although Enz inhibited the tumor growth of C4-2B cells in orthotopic or subcutaneous xenografts, it had no effect on the growth of C4-2B tumors in the bone or on the development of bone lesions. This data suggested a crucial role of the microenvironment in Enz resistance in PCa bone metastasis. We found that Enz significantly and specifically reduced the TGFBR2 protein in osteoblasts. This observation was confirmed in PCa bone metastatic tissue microarray, in which we found a significant decrease of TGFBR2 expression specifically in osteoblasts from patients who had undergone treatment with second-line hormonal therapies (either Enz or abiraterone) and that the decrease of TGFBR2 in osteoblasts was a causative effect from the hormonal therapies. To determine the role of TGFBR2 in the osteoblasts in bone metastasis, we used a mouse model (*Tgfbr2*^{Col1CreERT} KO) with inducible *Tgfbr2* knockout specifically in the osteoblasts. The loss of TGFBR2 in osteoblasts significantly induced C4-2B tumor establishment in bones. We therefore studied how Enz downregulated TGFBR2 in osteoblasts. We found that Enz up-regulated PTH1R and blocking PTH1R rescued the expression of TGFBR2 by Enz. Conclusions: Our data showed that a reduction of TGFBR2 in osteoblasts by Enz causes resistance to the drug and that blocking PTH1R might be able to overcome the Enz resistance in PCa bone metastasis.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-15 Circulating tumor DNA profiling reveals a deficiency of biomarkers predictive of docetaxel response in patients with metastatic castration-resistant prostate cancer

C Herberts[1], D Khalaf, G Vandekerkhove[2], M Annala, E Ritch, K Beja, BJ Eigl[3], C Kollmannsberger, L Nappi[3], J Vergidis[4], K Chi, A Wyatt

[1]University of British Columbia; [2]Vancouver Prostate Centre, University of British Columbia; [3]BC Cancer-Victoria; BC [4]Cancer-Victoria

Introduction & Objective: Docetaxel chemotherapy is a standard of care for men with metastatic castration-resistant prostate cancer (mCRPC). However, therapeutic benefit is eclipsed by the inevitability of resistance-45% of patients do not respond, and all will eventually relapse. There are currently no established markers to predict whether patients will respond to docetaxel. Herein lies an urgent clinical need for biomarkers predictive of docetaxel response, thereby allowing patients earlier access to potentially effective therapies while avoiding the unnecessary toxicity of taxane treatment. Methods: Our objective was to identify molecular biomarkers in plasma cell-free DNA (cfDNA) that are associated with docetaxel therapy response. We enrolled a prospective cohort of 58 mCRPC patients who had progressed on androgen receptor (AR) targeted agents but had not received prior taxane therapy. A blood sample for cfDNA analysis was collected before initiation of docetaxel, and targeted sequencing of 73 prostate cancer-relevant genes was performed on both leukocyte (germline) and plasma cfDNA. Patient records were reviewed for baseline clinical characteristics, PSA response (≥50% decline from baseline), and time to PSA progression (TTPP) (PCWG3 criteria) on docetaxel. Clinical outcomes were correlated with circulating tumour DNA (ctDNA) fraction and the genomic status of key prostate cancer genes. Results: We identified frequent disruption to *TP53* (20/58, 35%), *PTEN* (14/58, 24%), and *RB1* (11/58, 19%), as well as recurrent *AR* copy number amplification (24/58, 41%). The majority of the cohort had >2% ctDNA in their plasma sample (39/58, 67%). Of the 52 patients for which clinical outcomes were assessed, the median TTPP was 3.93 months, and 38.4% (20/52) experienced a PSA response. No significant relationships with either PSA response or TTPP were identified among genomic factors previously implicated in response to AR-targeted drugs. Many of the same genomic variables, however, display a clear association with patient overall survival, consistent with previous reports of their prognostic utility in mCRPC. Conclusions: These findings point to a lack of relationship between common prostate cancer molecular subtypes and taxane therapy response in mCRPC. However, this analysis does not address the role of regulatory or epigenetic mechanisms, nor rarer somatic alterations not covered by our gene panel. Nevertheless, since genomic factors such as *TP53* mutations and *AR* amplifications are linked to lack of mCRPC response to abiraterone and enzalutamide, our study suggests that patients that are unlikely to respond to AR-targeted therapy may instead benefit from docetaxel.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-16 Re-activation of the Androgen Receptor Pathway Accompanies Failure of Medical Therapy in Patients with Benign Prostatic Hyperplasia (BPH) and Lower Urinary Tract Symptoms (LUTS)

R Jin[1], T Case[1], I Ikwuanusi[2], J Wilton[3], J Cates[1], N Miller[1], DW Strand[4], J Mohler[3], R Matusik[1]

[1]Vanderbilt University Medical Center; [2]Meharry Medical College; [3]Roswell Park Comprehensive Cancer Center; [4]UT Southwestern Medical Center

Introduction & Objective: BPH is the most common urologic disease in men over the age of fifty that results in lower urinary tract symptoms (LUTS). Prostate enlargement is regulated by the androgen receptor (AR) pathway. There are two major medical approaches to relieve the symptoms of BPH/LUTS: alpha-adrenergic receptor antagonist (α-blockers) that relax the prostate to provide relief and steroid 5α-reductase (SRD5A2) inhibitors (5ARIs) that block the conversion of Testosterone (T) to Dihydrotestosterone (DHT) resulting in prostate shrinkage. Most patients initially respond to medical therapy; however, failure occurs often resulting in a need for surgery. Although 5ARI inhibits conversion of T to DHT in the prostate, the mechanism by which failure happens is unclear. AR splice variants (AR-Vs), which lack the ligand-binding domain (LBD), can constitutively activate the AR pathway. Our HYPOTHESIS is that *AR-V7 expression increases and the backdoor pathway for DHT synthesis by prostatic tissue occurs to contribute to the failure of medical therapy for BPH/LUTS*. Methods: In this study, we evaluated AR-V7 expression in human BPH specimens and we measure the levels of androgens and cholesterol within human BPH tissue by tandem mass spectrometry (LC/MS/MS). Results: In BPH patients that require an operation to relieve LUTS, we see an increase in dehydroepiandrosterone (DHEA) and a decrease in cholesterol levels in the tissue. Further, we find detectable AR-V7 and detectable levels of prostatic DHT that can activate the AR full length (AR-FL). The increase in enzymes in the BPH samples that are responsible for the synthesized of DHT by the non-conventional “backdoor” pathway suggest that the need for T is bypassed. Conclusions: Together, these data suggest that failure to medical therapy is due, in part, to renewed activation of the AR pathway by ligand independent expression of AR-V7 and by prostatic synthesis of DHT that can activate the ligand dependent AR-FL (Supported by 5R01 DK111554).

PS2-17 Transcription Factor Network Drives Prostate Cancer Drug Resistance

PM Schnepp, A Ahmed, G Shelley, E Keller

University of Michigan

Introduction & Objective: Drug resistance is a critical hindrance to the success of chemotherapy. Transcriptome modulation plays a role in chemotherapeutic drug resistance. In the past, bulk tissues have been analyzed to determine how the transcriptome confers a resistant phenotype. However, it is possible that subpopulations, which would not be identified in bulk analysis, contribute to the development of drug resistance. Accordingly, we evaluated for drug resistance transcriptional networks at the single cell level to provide a deeper understanding of the development of drug resistance. Methods: To understand the role of gene expression modulation in drug resistance, we conducted single cell mRNA-seq of docetaxel-resistant PC-3 and Du145 prostate cancer (PCa) cell lines. We utilized a method that uses an integrative approach to model information flow in transcription factor-gene regulatory networks (Passing Attributes between Networks for Data Assimilation or PANDA) to construct transcription factor (TF) networks from both docetaxel-sensitive and resistant cells and identified differences between the network models. Results: Ten TFs were identified to be similarly dysregulated in both PC-3 and Du145 resistant cell lines compared to their sensitive variants. Additionally, there were 210 signature genes between the sensitive and resistant networks in both cells with 118 of them similarly dysregulated. To identify which TF drive resistance in our networks, we constructed a shared TF network using the statistically significant TF/gene nodes and edges. When compared to docetaxel sensitive cells, the TFs CUX1, ATF4, and FOSL2 were connected to a higher number of downregulated genes; whereas, NFYB, E2F5, and GABPA were connected to a higher number of upregulated genes in the resistant cells. To identify potential therapeutics to modulate the combined network, we used the connectivity map analysis method (CMAP) to evaluate the impact of various signaling inhibitors *in silico* using a large-scale perturbation database (database constructed by microarray analysis of various drug induced gene expression changes in multiple cell lines). GW-8510, vorinostat, kaempferol, and trichostatin A were predicted to restore the sensitive phenotype. Conclusions: Our data indicate TF activity plays a role in the development of docetaxel resistance in PCa. Our model reveals candidate TFs that drive the resistant phenotype. Furthermore, we can identify potential novel drugs to reverse the resistance and improve patient outcomes after a diagnosis of chemotherapeutic resistance.

PS2-18 Identifying the lethal ancestral clones of metastatic disease in primary prostate cancer

E Warner, A Nurminen, C Herberts, W Struss, L Fazli, M Annala, K Chi, A Wyatt

University of British Columbia

Introduction & Objective: Localized prostate cancer (PCa) is managed with surgery or other local interventions, but ~10% of men relapse and develop metastatic PCa (mPCa). Although primary prostate cancer is often multifocal with multiple genetically distinct clones, it is unclear how disease progresses from this stage to mPCa which is typically more homogeneous with a major dominant tumor clone. Methods: We obtained metastatic ctDNA and matched DNA from archival prostate tumor tissue collected at time of radical prostatectomy from 13 patients who developed lethal mPCa after initial localized diagnoses. All distinct tumor foci within each patient's primary tumor were identified pathologically, and a combination of deep-targeted and whole exome sequencing was used to profile all DNA specimens. Results: Across the 13 patients the average PSA at diagnosis was 10.23, and time between diagnosis and initiation of androgen-deprivation therapy (ADT) for metastatic disease was 3.8 years (95% confidence interval [CI] 2.06-5.60). Time from ADT initiation to development of castration-resistance was 3.58 years (95% CI 1.70-5.46). 88% of patients (with sufficient levels of ctDNA for analysis) had a clear clonal relationship between subsequent metastatic disease and at least one tumor foci within the archival primary tissue. In all instances where a *TP53* mutation was detected in the mPCa setting via ctDNA, the identical *TP53* mutation was present in the radical prostatectomy specimen. Of note, we observed one example of a *BRCA2* truncation mutation confined to a single primary tumor foci that was completely absent from ctDNA collected at mPCa progression. Conclusions: The ancestral somatic clones of metastatic disease were detected in the primary tumors of patients with initial localized diagnoses. Primary prostate cancer is highly heterogeneous, and multi-region sampling was necessary to identify ancestral clones as they were often confined to a lone tumor foci. Our data suggests that the driver mutations of mPCA can arise early in disease progression and could be used to identify primary tumors with high metastatic potential.

PS2-19 Post-transcriptional Regulation of Androgen Receptor by DDX3 in Prostate Cancer Progression and CRPC

JE Vellky, W Ricke

University of Wisconsin-Madison

Introduction & Objective: Prostate cancer (CaP) driven by androgen receptor (AR) can be targeted therapeutically by androgen deprivation therapy (ADT); however, in 10-20% of cases, ADT fails, allowing disease recurrence. Mechanisms for recurrence include dysregulation of AR at the gene, RNA, and protein level. Recently, AR negative cell growth has been implicated as a mechanism of resistance. This study aims to identify mechanisms of drug resistance in castration-resistant prostate cancer (CRPC) that can be exploited therapeutically to reduce disease recurrence. Methods: Using the BCaP model, we can study the progression of CaP from benign to metastasis. Additionally, using the LNCaP-C4 series we can study progression to CRPC. To determine the RNA binding capacity, we utilized 1) RIP-qPRC for identification of mRNA targets and 2) RNAscope for visual colocalization. Changes in RNA and protein expression were determined using qPCR, Western blot, and IF. Results: DDX3 is an ATP-dependent RNA helicase that can aid or prevent translation of target mRNAs. While DDX3 is implicated in several cancers, its role as a translational regulator in CaP remains unstudied. In BCaP and LNCaP-C4, DDX3 protein expression increases through progression, concurrent with localization to cytoplasmic puncta. RIP-qPCR identified AR as an mRNA target of DDX3 in the metastatic and CRPC cell lines. Because of the dual role of DDX3 in translational control, AR expression was investigated; while AR mRNA expression increased through progression, AR protein expression decreased. LOF experiments using siRNA or small molecule inhibitor RK33 1) reduced DDX3 punctate localization, 2) restored the protein expression of AR, and 3) upregulated PSA. Similarly, with overexpression of DDX3, we saw DDX3 localized to puncta, reduction in AR protein, and reduction in PSA. These data suggest DDX3 acts as a translational repressor of AR in metastasis and CRPC. Because DDX3 is sufficient to reduce AR protein expression in metastases and CRPC, co-treatment of DDX3 inhibitors with anti-androgen therapy may prevent AR negative cell growth underlying recurrence. *In vitro* co-treatments with RK33 and bicalutamide, an AR antagonist, show decreased proliferation compared to either treatment alone, suggesting increased efficacy of bicalutamide with DDX3 inhibition. Conclusions: DDX3 as a repressor of AR translation could have clinical implications as a mechanism of resistance to ADT. Based on preliminary data, DDX3 could be contributing to the regulation of AR post-transcriptionally, and targeting DDX3 could reduce resistance and disease recurrence by sensitizing CRPC growth to ADT.

PS2-20 The mitotic kinase Citron Kinase drives prostate cancer growth

S Ben-Salem[1], S Bachour[2], V Venkadakrishnan, Y Su, E Cortes Gomez, Q Hu, E Klein, M Nicolas, C Magi-Galluzzi, S Liu, H Heemers[3]

[1]Lerner Research Institute Cleveland Clinic Foundation; [2]Cleveland Clinic, Lerner College of Medicine; [3]Cleveland Clinic

Introduction & Objective: Ligand-activated androgen receptor (AR) controls prostate cancer (CaP) growth. Androgen deprivation therapy (ADT), the first-line treatment for metastatic CaP, initially blocks CaP growth but eventually fails because of AR re-activation. CaP recurrence directly contributes to ~29,000 CaP deaths annually in the US. Novel actionable targets downstream of AR that control lethal CaP progression are needed to prolong CaP survival. Here, we identify citron kinase (CIT), a regulator of cell division, as a target of AR action and mediator of CaP cell proliferation. Methods: Dose response curves and time courses were done using AR agonists and antagonists, and siRNAs, inducible shRNAs, and site-directed mutagenesis assays were performed to define CIT expression and activity before and after ADT. Cell viability, cell proliferation, cell cycle analysis assays and CaP xenograft studies were done. RNA-Seq, MSigDB, GSEA, cBIO and TCGA analyses were performed. CIT was evaluated using immunoblotting and immunohistochemistry in archival CaP tissue from radical prostatectomy specimens. Results: Low doses of androgens selectively induced CIT expression while high doses, silencing of AR or enzalutamide treatment decreased CIT expression, indicating AR-dependence of CIT. Loss of CIT decreased cell proliferation, reduced cell viability, and delayed cell division in ADT-naïve and -recurrent CaP cell lines and reduced CaP volumes in xenograft models. Transient or stable CIT overexpression promoted cell proliferation, which depended entirely on CIT's kinase moiety. CIT- and androgen-dependent genes controlled cell cycle progression. Mechanistically, regulation of CIT depended on activity of the E2F family of cell cycle regulators, and E2F2 was the major determinant. Analysis of clinical CaP datasets demonstrated increased CIT mRNA expression during CaP progression, which correlated with shorter disease-free and overall survival. Higher CIT protein levels were found in primary CaP compared to adjacent non-neoplastic prostate tissue; higher CIT protein expression correlated with increasing Gleason scores, an indicator of CaP aggressiveness. Conclusions: Our studies identify the cell cycle regulator CIT as a novel druggable target downstream of AR that plays an important role in CaP cell proliferation and clinical progression.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-21 AKR1C3 promotes AR-V7 protein stabilization and confers anti-androgen resistance in advanced prostate cancer

C Liu[1], JC Yang[1], W Lou[1], L Liu, CP Evans[1], AC Gao[2]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine

Introduction & Objective: The resistant mechanisms of next generation anti-androgens in castration resistant prostate cancer are incompletely understand. Numerous studies found constitutively active AR signaling or full-length AR bypassing mechanisms contributes to the resistance. Our previous work demonstrated that AKR1C3 and AR-V7 play important roles in enzalutamide and abiraterone resistance, and that targeting AKR1C3 or AR-V7 could be valuable strategies to overcome this resistance. In this study, we investigated the interaction between AKR1C3 and AR-V7 and their coordinated roles in resistance. Methods: Global gene expression analysis was analyzed by microarray. Steroid profile including androgens in tumor tissues was analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). The effects of AKR1C3 expression and activation were examined by knock down of AKR1C3 expression by lenti-shRNA or inhibition of AKR1C3 enzymatic activity by indomethacin. AKR1C3 and AR-V7 interaction were determined by co-immunoprecipitation and dual immunofluorescence. The effects of AKR1C3 activation on anti-androgen sensitivity were examined *in vitro* and *in vivo*. Results: AKR1C3 formed a complex with AR-V7 and induced AR-V7 overexpression through increasing its protein stabilization. Targeting AKR1C3 by indomethacin significantly decreased AR/AR-V7 protein expression through the ubiquitin mediated proteasome pathway activation. AKR1C3 activated steroid hormone biosynthesis pathway and reprogramed AR signaling in enzalutamide resistant prostate cancer. Additionally, bioinformatic analysis of indomethacin treated resistant cells discovered that indomethacin significantly activated unfolded protein response pathway and suppressed Myc, cell cycle and AR/AR-V7 signaling pathways. Finally, we revealed that enzalutamide and abiraterone prostate cancer cells were cross resistant to apalutamide and darolutamide, possibly through overexpression of AKR1C3 and AR-V7 pathways. Targeting ARK1C3 re-sensitized resistant cells to apalutamide and darolutamide treatment through the AR-V7 inhibition. Conclusions: AKR1C3 induces AR-V7 overexpression and stabilizes AR-V7 protein in resistant cells through the ubiquitin proteasome system alteration. Apalutamide and darolutamide are cross resistant to enzalutamide and abiraterone via AKR1C3/AR-V7 complex regulation.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-22 HSP70/STUB1 complex regulates androgen receptor variants through proteostasis and confers enzalutamide and abiraterone resistance in lethal prostate cancer

C Liu[1], W Lou[1], JC Yang[1], L Liu, CM Armstrong[1], AP Lombard[1], H Chen[1], CP Evans[1], AC Gao[2]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine

Introduction & Objective: Proteostasis is a complementary process by which cells control the protein biosynthesis, folding, trafficking and degradation. Evidences suggested proteomic instability, such as protein misfolding and aggregation play pivotal roles in cancer cell survival and progression. AR-V7 lacking the ligand binding domain confers resistance to enzalutamide and abirateorne, and targeting AR-V7 is an ideal strategy to overcome the resistance. The mechanisms of AR-V7 proteostasis haven't been fully studied so far. Methods: Expression of HSP70 and STUB1 were determined by qRT-PCR and western blot. Expression of HSP70 and STUB1 was downregulated using specific siRNA. HSP70/STUB1 and AR-V7 interaction was determined by co-immunoprecipitation and dual immunofluorescence. The gene regulating mechanisms underlying the HSP70 inhibition in drug resistant prostate cancer cells was determined by RNA sequencing analyses. The effects of HSP70 inhibition on enzalutamide sensitivity were examined *in vitro* and *in vivo*. The correlation between HSP70 and AR-V7 in high Gleason score prostate tumors was determined by qRT-PCR. Results: In the present study, we analyzed enzalutamide and abiraterone resistant prostate cancer cells and found ubiquitin mediated proteolysis pathway was suppressed, and E3 ubiquitin ligases STUB1 was downregulated in the resistant cells. STUB1 bound to AR-V7, degraded AR-V7 expression and suppressed its activity. HSP70, the STUB1 binding protein, also bound to AR-V7 and enhanced AR-V7 transcriptional activity. STUB1 disassociated HSP70 from AR-V7 binding and increased AR-V7 degradation. Targeting HSP70 by siRNA or small molecular inhibitors (Apotozole and Ver155008) significantly suppressed prostate cancer growth and improved enzalutamide and abiraterone treatment through AR-V7 inhibition *in vitro* and *in vivo*. Additionally, HSP70 was upregulated in mCRPC patients and correlated with AR-V7 level in high Gleason score and metastatic prostate tumor specimens. Conclusions: Enzalutamide and abiraterone treatment induces the imbalance of AR-V7 proteostasis through the ubiquitin-proteolysis alteration. STUB1/HSP70 complex controls AR and AR variants proteostasis. Targeting HSP70 could be a valuable strategy to overcome the next generation anti-androgen resistance and improve their therapy.

PS2-23 Identifying DNA biomarkers of Bacillus Calmette-Guerin (BCG) resistance in non-muscle-invasive bladder cancer

JV Bacon[1], G Vandekerkhove[2], M Annala, D Mueller, C Rentsch, A Wyatt

[1]University of British Columbia; [2]Vancouver Prostate Centre, University of British Columbia

Introduction & Objective: Bacillus Calmette-Guerin (BCG) therapy is the gold standard of treatment for non-muscle-invasive bladder cancer (NMIBC). However, clinicopathological factors predicting response to BCG remain limited in scope and utility. Despite high initial efficacy, 60% of cases experience local recurrence or progress to muscle-invasive bladder cancer (MIBC). Establishing genetic biomarkers for predicting treatment response will be vital to improving NMIBC patient outcomes. Methods: Archived formalin-fixed, paraffin-embedded tissue sections were obtained from 12 relapsed NMIBC patients and 8 BCG responsive patients. Of the 12 relapsed patients, six had an NMIBC recurrence and six showed MIBC progression. We performed targeted DNA sequencing of 50 relevant bladder cancer genes on all samples. Relapsing patients each had samples from both pre- and post-BCG initiation, while responding patients only had pre-BCG samples available. Results: FGFR3 and KMT2D mutations were enriched in the pre-BCG cohort relative to the post-BCG cohort (21% vs 0% and 32% vs 8%) while TP53 mutations were enriched post-BCG (57% vs 75%). We identified two BCG responders with somatic hypermutation, each featuring over 30 mutations per MB. Lastly, 5/12 relapsed patients had a major somatic clonal switch over the course of BCG treatment indicative of strong selective pressure by BCG. Conclusions: Resistance to BCG and progression of NMIBC to MIBC may be linked to loss of tumour suppressor genes and baseline clonal heterogeneity. Temporal changes at relapse or progression are suggestive of dramatic clonal shifts in response to therapy, despite uniform presence of a common ancestral clone. Further accrual and sequencing are ongoing.

PS2-24 Elucidating the Mechanism of PIM-Mediated Resistance to Anti-Angiogenic Agents

AL Casillas, NA Warfel

University of Arizona

Introduction & Objective: Our group identified PIM kinases as proteins that drive resistance to anti-angiogenic agents in mouse models of prostate cancer. Notably, combined inhibition of PIM kinases and vascular endothelial growth factor (VEGF) displayed synergistic anti-vascular effect, as well as reduced tumor volume and metastasis. To facilitate the clinical translation of this therapeutic strategy, we sought to elucidate the mechanism by which PIM induces angiogenesis. The main hypothesis of this research is that PIM kinases promote angiogenesis and resistance to anti-VEGF therapies through sustained activation of HIF signaling. Methods: *In vitro* endothelial cell (EC) assays were performed using conditioned media from cancer cells with and without PIM overexpression. Vascularity *in vivo* was measured using DCE-MRI, an imaging technique that utilizes a paramagnetic contrast agent to measure perfusion into tissue. *In vitro* and *in vivo* kinase assays followed by mass spectrometry analysis were performed with and without PIM inhibitor to identify direct PIM phosphorylation sites on HIF-1α. Results: In normoxic conditions, induction of PIM1 is sufficient to stabilize HIF-1/2α protein, and treatment with AZD1208 blocks this effect. Importantly, PIM1 overexpression was sufficient to induce the expression of HIF-target genes. EC assays and DCE-MRI data indicate PIM increases vascularity *in vitro* and *in vivo*. *In vitro* kinase assays and phospho-proteomic analysis identified that PIM1 directly phosphorylates HIF-1α at T455, a novel post-translational modification. Notably, phosphorylation at T455 was detected in live cells using mass spectrometry, and no phosphorylation was detected with AZD1208. Moreover, T455 is located within the oxygen dependent degradation domain (ODDD), a key region controlling HIF-1 stability, and the Ser/Thr residue at T455 is conserved between HIF-1/2α. Finally, overexpression of T455D is sufficient to stabilize HIF in normoxia, and cycloheximide chase assays show an increase in HIF-T455D half-life in hypoxia. Conclusions: Overall, the data indicate that PIM controls the protein stability of HIF-1/2α in both normoxic and hypoxic conditions, and a novel role for PIM in facilitating tumor angiogensis was identified. Future studies will explore the effect of HIF-T455D in a mouse model of prostate cancer in order to determine whether concomitant targeting of VEGF and PIM kinase is still sufficient to stop angiogenesis. In general, this research yields important information regarding a viable treatment of prostate cancer.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-25 FGF5 Increases Androgen Receptor Expression and Signaling in Prostate Cancer

D McLean

University of Wisconsin-Madison

Introduction & Objective (Background): Prostate cancer (PRCA) is the 3rd most prevalent type of cancer behind breast and lung cancer, with over 220,000 new cases arising per year in the United States. The 5-year survival rate for prostate cancer is over 98% (SEER 2017), but patients that have undergone androgen deprivation therapy (ADT) to treat advanced prostate cancer will develop castration resistant prostate cancer (CRPC) in 10-20% of cases, with survival rates dropping to 29% (Kirby et al, 2011). The purpose of this study was to investigate the role of increasing FGF5 expression in the progression of PRCA and emergence of CRPC. Methods: Utilizing the BCaP cell line model of prostate cancer progression, we assessed FGF5 expression and function *in vitro* and *in vivo*. Overexpression plasmids and recombinant FGF5 (rFGF5) protein were used for gain of function studies, while siRNA and natural inhibitors were used for loss of function. 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: Gain of function experiments found that FGF5 significantly increases the expression of androgen receptor (AR) mRNA and protein. In addition, FGF5 was found to induce nuclear localization and activation of AR in the absence of ligand, suggesting ligand-independent activation of AR by FGF5. FGF5 overexpression also led to a significant increase in proliferation and colony formation of various cell lines. When androgen-dependent LNCaP cells are subjected to an androgen-depleted environment, FGF5 protein and RNA significantly increased within 24 hrs. Additionally, when AR is stimulated using androgen, FGF5 protein and RNA levels significantly decreased. These effects are not observed in cell lines that do not express AR or are AR-independent. These results suggest a negative feedback loop exists between FGF5 and AR. Finally, there was a significant increase in the both RNA and protein expression of FGF5 in tumorigenic cell lines compared to nontumorigenic in our model. In TMAs that were analyzed, there was a significant increase of FGF5-positive cells across progression of prostate cancer from benign to metastatic. Conclusions: These results suggest that the increase in FGF5 expression in PRCA progression could lead to an increase in AR overtime, and that this increase in FGF5 could be induced during androgen deprivation therapy. The mechanism of FGF5 increasing AR will be further investigated as this could be implicated in the emergence of CRPC.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-26 Cross-talk between the bone microenvironment and metastatic prostate cancer

T Case[1], J Sterling[2], R Jin[1]

[1]Vanderbilt University Medical Center; [2]Department of Medicine and Division of Clinical Pharmacology

Introduction & Objective: Men with metastatic castrate-resistant prostate cancer (mCRPC) have incurable disease with poor survival despite intensive therapy. Importantly, unlike other types of cancer, initial metastasis of PC is almost strictly limited to bone. Previously, we and others have reported that activation of NF-κB signaling plays a critical role in the cancer progression to bone mCRPC and to resistant to antitumor agents. However, how NF-κB signaling is activated in advanced PC and the detailed mechanism by which NF-κB signaling contributes osseous metastasis of PC remains unclear. Methods: In this study, we developed bone-growing anti-androgen resistant PC cells by long-term treatment of androgen-dependent non-bone-growing PC cells and investigated the mechanism by which PC progress to a bone metastatic CRPC. Results: Our studies show that long-term treatment with anti-androgens induces PC cell transdifferentiation to neuroendocrine prostate cancer (NEPC) and reprograms PC cells to change their characteristics enabling them to interact with bone marrow-derived macrophages and to grow in the bone microenvironment. We demonstrated that the antiandrogen-resistant or therapy (t) induced tNEPC cells can stimulate bone marrow-derived macrophages (BMM) result in expression of proinflammatory cytokine IL-1α. Further, we demonstrated IL-1α feed-forward loop activates NF-κB signaling and increases osteotropism gene expression in PC cells which benefit cancer cell survival, colonization and growth in the bone microenvironment. Also, it has been well described that IL-1α can play an important role to induce immunosuppression. Conclusions: These findings indicate although ADT initially inhibits PC tumor growth and PSA expression, long-term ADT can reprogram PC cells to change their characteristics enabling them to i) interact with BMM, ii) to escape from immune surveillance, and iii) to grow in the bone microenvironment. Therefore, blocking the interacting loop between cancer cells and the bone microenvironment by targeting IL-1α signaling may be a novel sufficient approach to prevent/treat bone metastatic CRPC.

PS2-27 Protocadherin 7 is overexpressed in CRPC and Promotes Aberrant MEK and AKT Signaling

G Shishodia, S Koul[1], R Shi, HK Koul[2]

[1]M.Sc.; [2]LSU Health-Shreveport

Introduction & Objective: Most of prostate cancer (PCa) deaths are a result of distant metastasis and due to emergence of castrate resistant PCa (CRPC). Aberrant ERK and AKT signaling has been implicated in subsets of CRPC patients, but mechanisms are not completely understood. Protocadherins (PCDHs) are members of the cadherin superfamily that regulate cell adhesion. PCDH7, a cadherin superfamily transmembrane protein, is involved in cell-cell recognition and adhesion. Recent reports suggest overexpression of PCDH7 promotes metastasis in lung and breast cancer. We evaluated expression and function of PCDH7 in Prostate cancer. Methods: PCa cells-LNCaP, C4-2B, DU145, PC3, 22Rv1 and RWPE1 were used in this study. Protein expression was measured by Western blotting in cell lines and immunohistochemistry in FFPE TRAMP and human prostate tissue sections. Gene expression was monitored by quantitative real time PCR. PCDH7 expression in clinical samples was extracted from publically available data sets (CRPC/NEPC data set-Trento *et al* 2016 and TCGA data set-TCGA, Cell 2015; cBioPortal). PCDH7 was knocked down by shRNA using lentiviral expression. Cell migration and invasion were performed 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. Co-IP studies were performed to check interactions of PCDH7 with SET and PP2A. Results: PCDH7 mRNA and protein is overexpressed in CRPC (C4-2B, 22Rv1, DU145 and PC3) cells as compared to castrations sensitive (LNCaP) cells and normal prostate (RWPE1) cells. We also observed increased expression of PCDH7 in prostate tissues of TRAMP mice during PCa progression as well as human PCa as compared to normal tissues. Our results also show that PCDH7 mRNA is overexpressed in 43% patients in CRPC as compared to 4% of patients in TCGA data set. We observed that knocking down PCDH7 reduced ERK and AKT activities, decreased cell migration, reduced cell invasion, and decreased colony formation. Co-IP studies revealed that PCDH7 interacts with SET and PP2A suggesting that PCDH7 could be promoting aberrant ERK and AKT activities upon interaction with SET and PP2A. Collectively, these data suggest PCDH7 is overexpressed during PCa progression and promotes aberrant activation of ERK/AKT signaling in CRPC. Conclusions: Taken together, our results show overexpression of PCDH7 promotes aberrant activation of ERK and AKT signaling in CRPC, as such PCDH7 may be an attractive target for therapeutic intervention in subsets of CRPC patients.

PS2-28 Identification of genes that drive therapeutic resistance to enzalutamide resistance in castrate resistant prostate cancer cells

W Awadallah[1], R Phillips[2], R Jin[3], X Yu[4], T Case[3], P Clark[4], R Matusik[3], Y Yi[3], P Anderson[2], M Grabowska[5]

[1]Case Western Reserve University; [2]Salisbury University; [3]Vanderbilt University Medical Center; [4]LSU Health-Shreveport; [5]Case Western Reserve University School of Medicine

Introduction & Objective: The majority of prostate cancers are adenocarcinomas and express androgen receptor (AR), making anti-androgens a standard therapy for advanced prostate cancer. Patients initially respond to androgen deprivation therapy, but eventually progress to castrate resistant prostate cancer. Castrate resistant prostate cancer patients are treated with next-generation androgen deprivation therapies, like the potent anti-androgen enzalutamide. However, eventually patients progress to enzalutamide-resistant castrate resistant prostate cancer. One of the mechanisms by which castrate resistant prostate cancer can become resistant to anti-androgens is through the over-expression of androgen receptor or induction of androgen receptor splice variants. The goal of this study was to define novel therapeutic targets for re-sensitizing castrate resistant prostate cancer to enzalutamide. Methods: One third of a commercially available bar-coded lentiviral short hairpin (sh)RNA library (DECIPHER from Cellecta) was transduced into castrate resistant C4-2B cells, targeting approximately 5,000 genes. Cells were split into three groups. Cells in the first group were collected immediately and represent the initial population of shRNA quantity. The cells in the remaining groups were treated with vehicle control (DMSO) or enzalutamide for six days. Genomic DNA was isolated and shRNA quantities were quantified by sequencing. The ratio of the abundance of each shRNA in the enzalutamide group versus both control groups was calculated. A given shRNA was considered a “hit”; if it showed at a least 2-fold abundance decrease relative to both vehicle control and initial sample shRNA. We verified our candidate genes in C4-2B and 22RV1 castrate resistant prostate cancer cells using duplicate short interfering (si)RNA constructs. Results: Our shRNA screen identified 11 genes that when knocked down, resulted in sensitivity to enzalutamide. These genes include transcription factors, kinases, and enzymes involved in cell metabolism. Importantly, androgen receptor was also identified as a gene driving resistance to enzalutamide, consistent with the literature. In subsequent validation studies, most genes sensitized both C4-2B and 22RV1 cells to enzalutamide. Conclusions: Our studies have identified 11 genes that drive resistance to enzalutamide in prostate cancer cells in vitro. Ongoing studies are working to elucidate how these genes are driving resistance and whether these genes drive resistance in vivo.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-29 Nuclear factor I/B regulates invasion and proliferation in prostate cancer cells

JS Nanda[1], W Awadallah[1], M Grabowska[2]

[1]Case Western Reserve University; [2]Case Western Reserve University School of Medicine

Introduction & Objective: Androgen receptor (AR) is the major therapeutic target in prostate cancer, and therapies target the ligand binding domain of AR through competition with androgens or inhibition of androgen synthesis. These strategies are not curative and prostate cancer cells become resistant by expressing AR splice variants (AR-Vs), which are constitutively active. Our studies have focused on identifying AR cofactors that regulate full length AR (AR-FL) and AR-Vs to identify therapeutic targets for both AR-FL and AR-Vs. Previous studies described nuclear factor I/B (NFIB) as frequently associated with genomic AR and FOXA1 binding sites in an androgen dependent prostate cancer cell line (LNCaP). Our studies focused on determining the role of NFIB in prostate cancer. Methods: In order to determine if NFIB can interact with AR-FL or AR-Vs, we performed co-immunoprecipitation studies in JEG-3 cells, which do not express AR-FL, AR-V7, FOXA1, or NFIB. We also generated NFIB over-expressing androgen-dependent (LNCaP) and castrate resistant (C4-2B, 22RV1) prostate cancer cell lines and assessed changes in cell proliferation, invasion, and gene expression in response to dihydrotestosterone (DHT) and the anti-androgens enzalutamide and bicalutamide Results: Co-immunoprecipitations from transiently transfected JEG-3 cells show that NFIB can interact with AR-FL and AR-V7. Although NFIB over-expressing LNCaP cells (LNCaP-NFIB) were less proliferative than their vector control (LNCaP-vector) counterparts, this proliferation defect could be over-come with DHT treatment. Indeed, AR-target gene expression was higher in the DHT treated LNCaP-NFIB versus LNCaP-vector cells. The presence of NFIB also resulted in increased sensitivity to bicalutamide and enzalutamide in proliferation assays. However, LNCaP-NFIB cells were more invasive. Conversely, while NFIB over-expressing C4-2B cells were more proliferative in the presence of enzalutamide and bicalutamide and maintained higher levels of AR-target gene expression versus C4-2B-vector cells, they were also less invasive than vector control cells. Like LNCaP cells, 22RV1-NFIB cells were more invasive than their 22RV1-vector control counterparts. Conclusions: Our studies show that NFIB can interact with both AR-FL and AR-Vs, independently of FOXA1. This suggests that NFIB may be able to regulate both AR-FL and AR-V genes. Our studies have also demonstrated a cell-dependent role for NFIB in regulating cell proliferation and invasion. These studies support a pro-tumorigenic role for NFIB in prostate cancer *in vitro*. Our current studies are working to determine how NFIB promotes tumorigenesis and castrate resistance *in vivo.*

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-30 Targeting PRMT5 as a novel approach for the treatment of castration-resistant prostate cancer

E Beketova, X Deng, C Hu

Purdue University

Introduction & Objective: Metastatic prostate cancer is commonly treated with the first-line treatment option androgen deprivation therapy (ADT). Following ADT, the disease inevitably progresses within 18-24 months to a lethal stage called castration resistant prostate cancer (CRPC). In general, androgen receptor (AR) reactivation is the major cause of CRPC, and it occurs through multiple mechanisms such as AR gene amplification, expression of androgen-independent mutants or ligand-independent splice variants, and intratumoral androgen synthesis. Current CRPC therapies that target androgen synthesis or full-length AR are not curative and only prolong survival by 4-5 months. Thus, development of novel therapeutic approaches for CRPC treatment is in urgent need. Recently it was demonstrated that protein arginine methyltransferase 5 (PRMT5), an emerging oncogene in various cancers that symmetrically dimethylates arginine residues of numerous substrates, regulates hormone-naïve prostate cancer (HNPC) growth in AR-dependent manner. Mechanistically, it was demonstrated that PRMT5 epigenetically activates AR transcription. Considering the role of AR signaling in CRPC and that PRMT5 regulates AR in HNPC, we aimed to determine whether PRMT5 also regulates expression of AR in CRPC. Methods: Short hairpin RNA (shRNA) against PRMT5 and small molecule inhibitor BLL3.3 were used to target PRMT5 in CRPC cell lines C4-2 (AR overexpression), 22Rv1 (AR-V7 expression) and VCaP (AR gene amplification). AR and AR target genes expression were analyzed using Western Blot and/or RT-qPCR. Cell proliferation was measured using MTT assay. Chromatin immunoprecipitation was used to analyze presence of PRMT5 and PRMT5-mediated methylation marks at the proximal AR promoter. 22Rv1 lines with doxycycline-inducible expression of PRMT5-targeting shRNA and control shRNA were established for use in xenograft studies. Results: PRMT5 targeting reduced cell proliferation and downregulated the expression of both AR full length and V7 at the protein and mRNA levels in all CRPC cell lines tested. Consistently, expression of AR target genes regulated by either full length AR or AR-V7 was decreased. PRMT5 and H4R3me2s were present at the AR proximal promoter and decreased upon PRMT5 knockdown. PRMT5 knock down significantly reduced growth of 22Rv1 xenografts in castrated NRG male mice. Conclusions: Taken together, these results suggest that PRMT5 acts as epigenetic activator for both full length and spliced variants of AR in CRPC cells. Basing on these findings, we propose that targeting PRMT5 may present a novel treatment approach for CRPC via eliminating the expression of AR and its splice variants.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-31 Differential chromatin binding and cofactor requirements contribute to the androgen receptor splice variant AR-V7 specific gene signature

B Paul[1], M Robertson[1], B Piyarathna, W Bingman, H Nagandla[1], Z Xia[2], C Coarfa[1], N Weigel[1]

[1]Baylor College of Medicine; [2]Oregon Health and Science University

Introduction & Objective: Expression of constitutively active AR splice variants including AR-V7 is important in reactivated AR signaling in CRPC. We are seeking to identify an AR-V7 mediated gene signature and to understand the molecular basis for AR-V7 specific activities. Methods: LNCaP and VCaP cell lines were engineered to express AR-V7 in response to doxycycline to probe isoform specific transcriptomes and cistromes. Bioinformatics, qPCR, siRNA, RNA-Seq and ChIP-exo approaches were used to test the mechanisms for differential regulation. Results: Although more than a thousand genes were regulated by both isoforms, a large sub-set of genes were differentially regulated by AR or AR-V7. An integrated analysis was performed to generate an AR-V7 specific gene signature from LNCaP and VCaP which revealed 299 AR specific genes and 100 AR-V7 specific genes to be used as a gene signature predictive of AR-V7 action. Potential mechanisms for differential transcriptional regulation include differential DNA binding, differential recruitment of factors or a combination of these two. We found that AR specific genes frequently have differential AR binding sites (>5 fold difference in binding) distal to the promoter whereas AR-V7 target genes have differential AR-V7 binding sites in the regulatory regions characterized by promoter elements and histone marks of transcriptional regulation. Motif analysis of the specific binding sites revealed different frequencies of motifs with AR sites enriched for FOXA1 motifs. Further depletion studies revealed that requirements for preferential induction were complex. While many AR specific genes required FOXA1 for AR dependent induction there were a number of other classes of genes as well. For example, although induction of SGK1 is AR specific, depletion of FOXA1 further increased induction by AR, but AR-V7 remained unable to induce SGK1. HOXB13 has been suggested as an important factor for AR-V7 dependent activities. However, depletion studies revealed that although there is a requirement for HOXB13 for induction of ORM-1 and NKX3.1, several AR-V7 dependent genes exhibited higher expression when HOXB13 was depleted and others were not affected. Studies of AR revealed that several also required HOXB13 for optimal induction. Conclusions: We conclude that AR-V7 has both common and unique actions relative to AR; the unique gene targets may be useful as an AR-V7 specific gene signature. Many of AR-V7's unique actions are regulated by differential binding to chromatin. Although FOXA1 contributes to differential regulation, HOXB13 plays a role in regulating sub-sets of both AR and AR-V7 regulated genes.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-32 Isoform Specific Activities of Androgen Receptor Splice Variants in Prostate Cancer Cells

H Nagandla, M Robertson, B Piyarathna, S Donepudi, N Putluri, C Coarfa, N Weigel

Baylor College of Medicine

Introduction & Objective: Expression of constitutively active androgen receptor (AR) splice variants such as ARV7 and ARv567, which lack the ligand binding domain of AR is enriched in castration resistant prostate cancer (CRPC) and is a proposed mechanism for escape from androgen deprivation therapy (ADT). One unresolved question is whether these AR splice variants simply mediate a subset of full length AR activities or have unique activities of their own, which may also be variant isoform specific. We had previously shown that the variant ARV7 exhibits differences in gene expression and metabolism relative to AR. The variant ARv567 lacks exons 5, 6 and 7 of full length AR. ARv567 differs from ARV7 in that it contains the hinge region of AR, which was previously shown to mediate interactions with the pioneering factor FOXA1. The objective of our study was to test if the activities mediated by ARv567 are unique compared to AR or ARV7. Methods: We developed an LNCaP prostate cancer cell line with dox inducible expression of ARv567. To evaluate the transcriptomic and metabolomic activities of ARv567, we performed RNA Seq. and steady state metabolomics in LNCaP cells expressing ARv567. We compared the resulting data from ARv567 expression with our previous data sets for AR and ARV7. Mechanistic studies were performed to test the role of FOXA1 in AR isoform specific gene transcription. Results: Comparison of RNA-Seq. data showed that the majority of the gene expression changes mediated by AR and the two variants were similar although the magnitude of regulation differed. We discovered that a number of gene expression changes were unique to only the two variant receptors or to AR and ARv567, indicating that ARv567 is an intermediate between AR and ARV7 in terms of regulation of target gene expression. Steady state metabolomics revealed that the pattern of metabolites resulting from Dox induced expression of ARv567 partially resembled the patterns resulting from the action of AR or ARV7 but also induced novel changes. For example, we found that ARv567 also led to a decrease in citrate levels similar to ARV7, but the increased levels of cytosine were unique to ARv567. Global β-oxidation analysis revealed that both variants induce oxidation of lipids, though not as well as AR. Moreover although ARv567 can bind FOXA1, this difference does not explain variant specific differences in gene expression. Conclusions: In summary, our data suggests that the pattern of ARv567 activities differs not only from that of full length AR but also from that of ARV7. Thus, the response of tumors may depend on the specific splice variants expressed.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-33 Targeting RET Kinase in Neuroendocrine Prostate Cancer

H Van Deusen[1], J Ramroop[2], KL Morel[3], L Cheng, Z Li, L Ellis[3], JM Drake[1]

[1]University of Minnesota; [2]Ohio State University; [3]Dana-Farber Cancer Institute, Harvard Medical School

Introduction & Objective: The advent of second generation androgen deprivation therapies have provided much needed life-extending treatments for metastatic castration resistant prostate cancer (mCRPC) patients. However, the implementation of these novel therapies has created a shift in the molecular characteristics of recurrent tumors, with a greater incidence of tumors that have lost androgen receptor (AR) signaling (collectively termed aggressive variant prostate cancers or AVPC). AVPC tumors may also gain neuroendocrine markers creating a distinct subclass of AVPC known as neuroendocrine prostate cancer (NEPC). Methods: To understand how these AR negative AVPCs differ from their AR positive counterparts, we analyzed and compared the phosphoproteome of adenocarcinomas (AdCa) and AVPC cell lines to identify altered kinase activity between the two groups. Kinase substrate enrichment analysis identified RET kinase as an activated kinase in the NEPC cell lines. We then analyzed mRNA transcript data from multiple studies of patients with AVPC to determine if RET kinase was upregulated in NEPC patient tumors. Finally, we used AD80 to pharmacologically inhibit RET kinase in NEPC tumor models including ex-vivo cultured *PTEN/RB1* deleted mouse organoids and NCI-H660 xenograft tumors to determine if this upregulated kinase contributes to the survival and aggressive phenotype of NEPC. Results: Our results revealed distinct phospho-serine/threonine and tyrosine phosphorylation patterns between AdCa and AVPC and predicted RET kinase activity to be elevated in the AVPC cell lines, despite the absence of RET activating mutations. Large primary tumor transcript data sets revealed that NEPC patients had higher levels of neuroendocrine markers like SYP and CHGA, and also had increased levels of RET kinase mRNA. We found that the RET pathway inhibitor, AD80, dramatically increased cell death in cultured mouse organoids and reduced xenograft tumor growth. Interestingly, in the organoid tumor model, treatment with both enzalutamide and AD80 further increased cell death beyond AD80 treatment alone. Conclusions: These results implicate RET as an important kinase for NEPC tumor survival and progression and suggests that targeting RET kinase may be a treatment option in patients with AVPC containing NE features.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-34 Characterizing the metabolic effect of enzalutamide on prostate cancer

EB Blatt[1], R Sonavane, GH Henry[2], DN Ekoue[1], DW Strand[2], GV Raj

[1]University of Texas Southwestern Medical Center; [2]UT Southwestern Medical Center

Introduction & Objective: Enzalutamide is an FDA approved androgen receptor (AR) antagonist widely used for the treatment of metastatic prostate cancer. The effect of enzalutamide on prostate cancer metabolism is poorly understood. Given that AR's main role in prostate cancer is as a pro-proliferative transcription factor, we hypothesized that inhibiting AR activity with enzalutamide would have substantial metabolic implications. Methods: To test the impact of enzalutamide on prostate cancer metabolism, we have used a combination of metabolomic and transcriptomic analyses in prostate cancer cell lines and primary tumor explants in the presence and absence of enzalutamide. Results: With these unbiased approaches, we have found that acute enzalutamide treatment of prostate cancer cell lines significantly alters their metabolic prolife. Notable among these changes were alterations in nucleotide and polyamine metabolism. Importantly, we have validated these findings in primary tumor explants cultured *ex vivo* with enzalutamide. Transcriptomic analysis of enzalutamide sensitive prostate cancer cell lines shows that enzalutamide affects the transcription of genes that regulate nucleotide and polyamine synthesis. Similar analyses of enzalutamide resistant cell lines reveal changes consistent with those seen in enzalutamide sensitive models and suggest potential mechanisms of enzalutamide resistance. Validation of these data in enzalutamide sensitive and resistant models is ongoing. Conclusions: These results suggest that enzalutamide treatment can modulate nucleotide and polyamine metabolism. Given that these changes are also noted in enzalutamide resistant prostate cancers, alterations in these pathways may represent potential mechanisms of resistance to enzalutamide.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-35 Overcoming prostate cancer therapeutic resistance by modulating mitochondria-mediated cell death signaling

J Lyu[1], GC Chu[2], Y Zhang, S You[2], S Mrdenovic[3], HE Zhau, LW Chung[2]

[1]Cedars Sinai Medical Center; [2]Cedars-Sinai Medical Center; [3]University Hospital Osijek

Introduction & Objective: Advanced prostate cancer (PC) initially responds to androgen-deprivation and taxane-based therapies, but progresses to castration-resistant and fails to respond to additional therapy. A novel PC targeting drug, DZ, chemically conjugated to a cholesterol-lowering statin, simvastatin (SIM), was tested using enzalutamide (MDVR)-, abiraterone (AbiR)- or docetaxel (TaxR)-resistant PC cell and tumor models. Methods: C4-2B, MDVR, AbiR, TaxR and 22RV1 cells were used for cell proliferation, apoptosis, and mechanistic studies. DZ-SIM in cells was tracked by Mito- or Lyso-tracker. PC metabolism and oxygen consumption were assessed by Seahorse XF24 for extracellular acidification rate (ECAR), oxygen consumption rate (OCR), status of oxidative phosphorylation, glycolysis, and fatty acid oxidation (FAO). Flow cytometry was used to determine mitochondria membrane integrity and apoptosis. RNA-seq data were subjected to computational analyses for shared common genes in MDVR/AbiR/TaxR cells, and results validated by qRT-PCR. Results: DZ-SIM accumulates in mitochondria and lysosome fractions, inhibits growth and induces apoptosis in MDVR/AbiR/TaxR C4-2B cells (Figures 1 and 2). DZ-SIM disrupts mitochondrial membrane integrity through reduced cholesterol levels, depresses OCR, and FAO, but stimulates ECAR in MDVR C4-2B, and induces apoptosis and mitochondrial fission (Figure 2). Mitochondrial fission, depressed by Mdivi1, rescues PC cell death. RNA-seq revealed the expression of 72 genes shared in MDVR/AbiR/TaxR C4-2B cells; among them, CAMK2N1, HMGCS2, NR2F1 and CDH3, are identified and confirmed to be depressed by DZ-SIM in PC cells. Conclusions: DZ-SIM can be developed as a novel agent to overcome MDV/Abi/Tax resistance in PC. DZ-SIM provokes PC cell death likely through a coordinated function of enhanced mitochondrial fission, selectively depressed gene transcription, FAO and cell junctional protein function.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-36 HSD3B1 Utilizes Pregnenolone to Promote Resistance to Anti-androgens in Prostate Cancer

CM Armstrong[1], C Liu[1], W Lou[1], AC Gao[2]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine

Introduction & Objective: Most prostate cancer (PCa) patients receiving enzalutamide (Enza) or abiraterone (Abi) develop drug resistance within 24 months of exposure. This creates a need to better understand the underlying causes of anti-androgen resistance so that improved treatment methods can be developed. Previous studies demonstrate that uncontrolled intraprostatic androgen synthesis promotes Enza and Abi resistance. HSD3B1 is a key enzyme contributing to androgen synthesis and its expression is associated with PCa progression. The aim of this study is to determine the contribution of HSD3B1 to Enza and Abi resistance in PCa. Methods: Enza resistant (C4-2B MDVR) and Abi resistant (C4-2B AbiR) C4-2B PCa cells were generated by chronically exposing parental C4-2B cells to increasing Enza or Abi concentrations for >12 months and maintained in 20 μM Enza or 10 μM Abi. Differences in gene expression between parental and anti-androgen resistant cells were determined by microarray, RNA-seq, and rtPCR. HSD3B1 expression was knocked down in C4-2B MDVR and C4-2B AbiR cells and cell number was determined in media containing FBS, charcoal dextran stripped FBS (CDFBS), or CDFBS supplemented with 100 nM pregnenolone (P5), 100 nM DHEA, or 10 nM DHT in the presence and absence of 20 μM Enza. PSA secretion was determined by ELISA and PSA-luciferase activity was measured by reporter assay. Results: HSD3B1 expression is higher in C4-2B MDVR and C4-2B AbiR cells compared to parental C4-2B cells. This correlates to increased intracrine androgens in C4-2B MDVR cells. Knockdown of HSD3B1 in C4-2B MDVR resensitized cells to Enza in FBS, CDFBS+DHT and CDFBS+P5 conditions as determined by a reduction in cell number and PSA secretion and/or PSA-luciferase activity in response to Enza. In the C4-2B AbiR cells, inhibition of HSD3B1 re-sensitized cells to treatment with Abi in FBS, CDFBS, and CDFBS+P5 conditions with the greatest effects seen in the FBS and CDFBS+P5 containing media. Supplementation with P5, but not DHEA, was able to induce PSA-luciferase activity and cell growth in C4-2B AbiR cells and this could be blocked by knockdown of HSD3B1. Conclusions: HSD3B1 overexpression in C4-2B MDVR and C4-2B AbiR cells contributes to Enza and Abi resistance and modulation of this enzyme could be a viable strategy to improve anti-androgen response in PCa cells. HSD3B1 activity appears to be reliant on select androgen precursors indicating preference towards a specific androgen synthesis pathway by HSD3B1 in mediating anti-androgen resistance in these resistant model systems.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-37 Mechanisms of anti-androgen resistance in a 3D patient-derived organoid model of bone metastatic prostate cancer

D Burner[1], T Mendoza, M Muldong[1], S Lee[2], C Arreola[3], O Miakicheva-Greenburg, WY Zhu[2], C Wu[4], N Cacalano, C Jamieson, C Kane, A Kulidjian, C Jamieson[2]

[1]University of California San Diego Moores Cancer Center; [2]University of California, San Diego; [3]Surgery-Urology, University of California, San Diego, Moores Cancer Center; [4]University of California, San Diego, Moores Cancer Center

Introduction & Objective: Over 80% of advanced prostate cancer (PCa) patients develop bone metastases for which there is no cure. PCa lines have been used for the development of new therapies, however they do not fully represent PCa heterogeneity nor the bone metastatic disease. Thus, we established new patient-derived models for bone metastatic prostate cancer. We generated three-dimensional (3D) organoids from our patient-derived xenograft (PDX) model and characterized their response to either dihydrotestosterone (DHT) or the anti-androgen, Enzalutamide. Methods: Intra-femoral PDX tumor cells were used to establish 3D/organoid cultures according to previously described protocols for prostate cancer. Culture medium was supplemented with 10% v/v fetal bovine serum (FBS) and ±1 nM DHT or ±10 μM Enzalutamide in 0.1% v/v dimethyl sulfoxide (DMSO) for four weeks. Overall viability was quantified using a luciferase-based viability assay. Changes in various multi-cellular masses were quantified as changes in cyst number, cyst lumen diameter and spheroid area using the Keyence microscope and image analysis software. Statistical significance was determined using a student's t-test. Quantitative RT-PCR was performed for Prostate-Specific Antigen (PSA) and Prostate-Specific Membrane Antigen (PSMA). PSA immunohistochemistry (IHC) and immunofluorescence cytochemistry (IFC) for androgen receptor (AR), and cytokeratins 5 and 8 (CK5, CK8) were performed. Results: Enzalutamide treatment did not reduce overall viability of our PDX-derived 3D/organoid cultures (PDO). Intriguingly, the PDO cultures consisted of heterogeneous cell populations that formed hollow cysts or spheroids. Treatment with DHT significantly increased the average cyst lumen diameter, cyst count as well as the spheroid area ($p<0.05$). Conversely, treatment with Enzalutamide significantly decreased the average cyst lumen diameter, cyst count and spheroid area ($p<0.05$). Analogous structures were observed in the PDX tumors. Quantitative RT-PCR and PSA IHC showed that Enzalutamide reduced PSA expression but did not change PSMA expression. Conclusions: The overall resistance of the PDX-derived 3D/organoids to enzalutamide treatment mirrored the in vivo response of our bone metastatic prostate cancer PDX model. The heterogeneous populations of hollow cysts and spheroid clusters exhibited unique responses to Enzalutamide and may require distinct therapeutic interventions.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-38 NCOR2/SMRT Re-Wires The Epigenome And Transcriptome To Drive Androgen Deprivation Therapy Resistant Prostate Cancer

MD Long[1], DJ Smiraglia[1], MJ Campbell[2]

[1]Roswell Park Comprehensive Cancer Center; [2]The Ohio State University

Introduction & Objective: NCOR2/SMRT is altered in advanced prostate cancer (PCa), and supports a cancer-driver role. It interacts with multiple transcription factors but the key targets and functions remain unknown. Therefore we dissected NCOR2 genomic and epigenomic functions during PCa progression. Methods: NCOR2 dependent ChIP-Seq, RNA-Seq and DNA methylation arrays were undertaken in androgen sensitive (LNCaP), isogenic androgen deprivation therapy (ADT) resistant (LNCaP-C42) cells, and the CWR22 xenograft model. Cells were either stimulated with DHT and/or stable NCOR2 knockdown. Endpoints were validated in TCGA data sets. Data integration was undertaken using the R language for statistical computing. Results: NCOR2 genomic binding differed significantly by distribution and enriched motif; in LNCaP-C42 cells, NCOR2 bound to ~1700 sites that were significantly more distal, associated with HIF and TEAD motifs and overlapped FOXA1 cistrome. The NCOR2-dependent transcriptomes were also largest in LNCaP-C42 (~7000 genes) and were significantly enriched for DHT regulated genes. NCOR2 knockdown significantly increased DNA hypermethylation, notably at poised enhancer regions. Using a 700-case TMA and TCGA PCa data, we demonstrated elevated NCOR2 significantly associated with the risk of biochemical progression in local PCa. However, it was not elevated in advanced disease. Instead, stable NCOR2 knockdown in the CWR22 model induced ADT resistance ($p = 0.0044$). RNA-Seq revealed that NCOR2 knockdown only impacted the transcriptome during ADT resistance, and significantly skewed ~1800 genes towards activation ($p = 0.00012$), of which, about 35% were direct NCOR2 targets such as the pro-metastatic AGR2, which is bound in a distal intergenic region. Surprisingly, many activated gene targets gained significant hypermethylation. For example, NCOR2 knockdown in ADT resistant tumors significantly upregulated HOXA3, which also gained significant DNA hypermethylation. Conclusions: In localized PCa, elevated NCOR2 associates with disease progression but not in advanced disease. Rather we stably reduced NCOR2 expression and demonstrated significant changes in the epigenome and transcriptome. *In vivo* we used the CWR22 model of PCa progression to demonstrate that NCOR2 knockdown led to ADT resistance in advanced PCa. In the absence of exogenous androgens, the resistance is driven by genomic, epigenomic and transcriptomic re-wring of NCOR2-dependent gene networks to phenocopy androgen actions.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-39 Enzalutamide Treatment Results in Anti-Androgen Resistance in Patient Derived Bone Metastatic Prostate Cancer Models

M Muldong[1], S Lee[2], D Burner[1], T Mendoza, C Wu[3], N Cacalano, C Jamieson, C Kane, A Kulidjian, C Jamieson[2]

[1]University of California San Diego Moores Cancer Center; [2]University of California, San Diego; [3]University of California, San Diego, Moores Cancer Center

Introduction & Objective: Castration-resistant prostate cancer (CRPC) is an advanced form of prostate cancer associated with poor survival rates. Upon diagnosis of CRPC, 84% of patients present with metastases and the survival rate is 28% despite improvements in treatment options. Our group has previously shown that a well characterized patient derived xenograft known as PCSD1, is resistant to the anti-androgen drug, Bicalutamide. To further understand the mechanisms of androgen deprivation resistance, we have treated PCSD1 and PCSD13, a novel xenograft model with the next generation anti-androgen, Enzalutamide. Methods: PCSD1 cells derived from a human prostate cancer right femoral metastasis were serially transplanted into male Rag2-/-; γc-/- mice intra-femorally (IF) or subcutaneously (SC). 4 weeks post SC or IF injection, mice were given oral gavage treatments of Enzalutamide 10 mg/kg/day or Vehicle control for 35 days (SC) or 30 days (IF). Tumor growth was measured bi-weekly with calipers and in vivo bioluminescence (IVIS). PCSD13 cells were derived from a human prostate cancer femoral metastasis and serially transplanted into 6-8 week old male Rag2-/-; γc-/- mice intra-femorally. 8 weeks post IF injection, mice were given oral gavage treatments of Enzalutamide 10 mg/kg/day or Vehicle control for 50 days. Tumor growth was measured bi-weekly with calipers. PSA was measured using qPCR and immunohistochemistry. Results: In PCSD1 intra-femorally injected mice, Enzalutamide treatment had a partial effect in reducing tumor burden. However, in PCSD1 SC injected mice, tumors were sensitive to treatment and regressed in tumor size. In PCSD13 intra-femorally injected mice, Enzalutamide had a small tumor inhibitory effect. Conclusions: Our patient derived xenograft models demonstrate that the bone niche promotes tumor growth even with treatment of anti-androgen. Tumor growth is inhibited when injected subcutaneously, resembling the effectiveness of androgen deprivation therapy toward localized prostate cancer. This elucidates the importance of utilizing in vivo patient derived models to gain a better understanding of why the bone niche supports tumor growth.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-41 High-resolution survey of the AR variant expression landscape in metastatic castration-resistant prostate cancer

Y Zhu[1], P Zhao[2], AM DeMarzo, E Antonarakis[1], J Luo[1]

[1]Johns Hopkins University; [2]Johns Hopkins University

Introduction & Objective: Detection of constitutively active AR splice variants (AR-Vs) in patients with metastatic castration-resistant prostate cancer (mCRPC) indicates emergence of therapeutic resistance to agents targeting the androgen receptor signaling pathway. Among the AR-Vs, androgen receptor variant-7 (AR-V7) is the most well-studied AR-Vs with prognostic values in the settings of resistance to novel hormonal therapies. The detection of other AR-Vs has also been reported. However, no systemic study of the overall burden of AR-Vs has been conducted due to lack of validated assays. We have previously developed a novel junction-specific RNA in situ hybridization (RISH) assay that enabled highly specific detection of cytoplasmic AR-FL and AR-V7 mRNA. In this study, we conducted a survey of the AR-V expression landscape using this novel method in clinical mCRPC specimens. Methods: We designed RISH probes targeting distinct splice junctions of total AR, AR-FL, AR-V7, AR-V9, AR-V3, AR^{v567es} and AR exon 3 duplication (as detected in CWR22Rv1 cells). All the probes were validated using prostate cancer cell lines with known AR-V profiles prior to testing in 23 metastatic biopsies collected from both castration-sensitive prostate cancer (CSPC, n = 2) and mCRPC (n = 21) patients. Quantitative RISH scores for each AR-V were analyzed. Results: All biopsies had positive (greater than zero) AR-FL RISH score (median 1.68, range 0.09-15.31). AR-V7 and AR-V9 are the most highly expressed AR-Vs analyzed. AR-V7 was detected in 15/23 (median 0.14, range 0.04-0.47) samples. AR-V9 was detected in 13/23 (median 0.12, range 0.02-0.43) samples, all of which also co-expressed AR-V7 and AR-FL. The AR exon 3 duplication, AR-V3, and AR^{v567es} were detected in 3/23, 6/23, and 4/23 samples, respectively, with all RISH scores below 0.10, indicating lower expression frequency and intensity compared to AR-V7 and AR-V9. The overall AR-V burden is mainly driven by AR-V7 and AR-V9. Conclusions: Using a specific and quantifiable RISH method, we confirm the relative importance of AR-V7 and AR-V9. The high-resolution AR-V expression landscape also confirms the co-existence of AR-FL and various AR-Vs, and lack of sub clones that may express a predominant AR-V mRNA other than AR-V7 and the often co-existing AR-V9. Given that AR-V7 and AR-V9 are the most abundant AR-Vs in CRPC biopsies, and that AR-V7 is associated with AR-V9 in most cases, it is unlikely that detection of AR-Vs other than AR-V7 would add additional value in clinical biomarker development. Further characterization of the clinical significance of additional AR-Vs derived from AR gene rearrangements may be evaluated with this novel method.

PS2-42 Elucidating Cross-Resistance between Taxanes and Next-Generation Anti-Androgens in Castration Resistant Prostate Cancer

AP Lombard[1], C Liu[1], CM Armstrong[1], W Lou[1], CP Evans[1], AC Gao[2]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine

Introduction & Objective: Recent advances have added effective therapies for castration-resistant prostate cancer (CRPC) including next-generation anti-androgen therapies (NGATs), enzalutamide and abiraterone, and taxanes, docetaxel and cabazitaxel. Despite improvements in outcomes using these two drug classes, patients still succumb to the disease due to resistance. Further complicating this is lack of a well-defined treatment sequence and potential for cross-resistance. We have developed several models representing therapeutic resistant CRPC. Here we utilize these models to assess putative cross-resistance between treatments. Methods: Enzalutamide resistant C4-2B-MDVR (MDVR), abiraterone resistant C4-2B-AbiR (AbiR), and docetaxel resistant C4-2B-TaxR (TaxR) cells have been previously described and shown to be robustly resistant to respective therapies versus parental C4-2B cells. Cell growth and colony formation assays were used to test drug response. Western blot and qPCR were used to test for markers of resistance. Overexpression vectors and siRNAs were used to manipulate levels of AR-v7 via transfection with lipofectamine 2000 or lipofectamine RNAiMAX respectively. Results: We find that resistance to enzalutamide induces resistance to abiraterone and vice versa but resistance to neither alters sensitivity to taxanes. Acquired resistance to docetaxel induces cross-resistance to cabazitaxel but not to enzalutamide or abiraterone. Correlating responses with known mechanisms of resistance indicates that androgen receptor (AR) variants correlate with resistance to NGATs while ABCB1 correlates with taxane resistance. Mechanistic studies show that AR-v7 is involved in resistance to NGATs but not taxanes. Conclusions: Our findings suggest the existence of intra cross-resistance within a drug class (i.e., within NGATs or within taxanes), while inter cross-resistance between drug classes does not develop in CRPC. Furthermore, our data suggests resistance mechanisms differ between drug classes. We conclude that treatments of one class can be sequenced with those of another, but caution should be taken when sequencing drugs of a similar class. Also, the development of additional resistance biomarkers, such as ABCB1, will improve treatment decisions.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-43 Overcoming ABCB1-Mediated Olaparib Resistance in Advanced Prostate Cancer

AP Lombard[1], C Liu[1], CM Armstrong[1], W Lou[1], CP Evans[1], AC Gao[2]

[1]University of California Davis, School of Medicine; [2]UC Davis, School of Medicine

Introduction & Objective: Despite great progress in the management of advanced, castration-resistant prostate cancer (CRPC), it is estimated that over 29,000 men will lose their lives to this disease in 2018. The recent phase II TOPARP-A study highlighted the potential role of using PARP inhibitors for the treatment of a subset of heavily pre-treated prostate cancer patients harboring DNA-repair defects in their tumors. Although these new findings are exciting, many questions remain regarding the optimal use of these therapies including; 1) whether there is cross-resistance with other approved drugs, 2) where to place PARP inhibitors in the treatment paradigm, and 3) what combinations of currently approved drugs may produce the best effects? The aim of our study was to utilize our labs models of therapeutic resistant CRPC to explore putative cross-resistance with olaparib. Methods: C4-2B based enzalutamide resistant MDVR, abiraterone resistant AbiR, and docetaxel resistant TaxR cells as well as docetaxel resistant DU145 based DU145-DTXR cells were previously described. Cell growth and colony formation assays were used to test response to olaparib and putative combination treatments. Western blots were used to assess expression of PARP, PAR, and ABCB1. Cell compartment fractionation was used to assess chromatin bound PARP. Results: Our data demonstrate that docetaxel resistant cells confer cross-resistance to olaparib, while enzalutamide and abiraterone resistant cells remain sensitive to olaparib, albeit less sensitive compared to the parental cells. We focused our study on docetaxel mediated cross-resistance as it was the most robust. Our previous studies showed that ABCB1 mediates taxane resistance. We hypothesized it may also mediate olaparib resistance. shRNA-knockdown of ABCB1 re-sensitizes docetaxel resistant cells to olaparib treatment. Small molecule inhibition of ABCB1 using either elacridar or enzalutamide also re-sensitizes docetaxel resistant cells to treatment. Conclusions: Our findings highlight the potential for cross-resistance with currently used therapeutics and suggest that olaparib combination therapies with ABCB1 inhibitors such as elacridar or enzalutamide may yield the highest efficacy.

PS2-44 Stromal endoglin signaling regulates prostate cancer glutamine metabolism and contributes to neuroendocrine differentiation

B Smith, R Mishra, A Madhav, K Rohena-Rivera, V Placencio-Hickok, N Bhowmick

Cedars-Sinai Medical Center

Introduction & Objective: Targeting tumors using androgen receptor signaling inhibitors (ARSI) remains a major approach to treat prostate cancer (PCa). However, this strategy is not curative due to cell-autonomous mechanisms associated with tumor progression. We have previously reported that endoglin (CD105), a TGF-β co-receptor, is upregulated in PCa epithelia and cancer associated fibroblasts (CAF) by ARSI, and its expression is closely associated with castration-resistant PCa. We also identified that ARSI induce epigenetic imprinting on prostatic CAF that result in metabolic reprogramming, and a more neuroendocrine profile of the cancer epithelia. We hypothesize that stromal CD105 signaling mediates PCa metabolic reprogramming and castrate resistance through a novel stromal-epithelial crosstalk. Methods: We employed co-culture *in vitro* models with CRISPR/Cas9 knockout of CD105 and species-specific neutralizing antibodies to CD105 to demonstrate the impact of this signaling axis on the epigenetic regulation ARSI can impart on CAF. Further mouse models were used to identify the relevance of glutamine metabolism on PCa epithelial neuroendocrine differentiation. Results: In response to ARSI, bone morphogenic protein (BMP) signaling downstream of CD105 was found to cause epigenetic silencing of RASAL3 expression by way of DNMT3b regulation. This resulted in elevated Ras signaling and glutamine secretion by the CAF in response to ARSI. Glutamine metabolism to glutamate by GLS (glutaminase) was critical for both the generation of ATP and neuroendocrine differentiation of PCa epithelia. Conclusions: We discovered that blocking either CD105 or GLS in the context of ARSI could limit the expansion of castrate resistant PCa models. These findings corroborate our previous demonstration that men with poor responsiveness to ARSI have elevated circulating blood glutamine.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-45 Particle Encapsulation of a Prostate-Targeted Biologic for the Treatment of Liver Metastases in a Preclinical Model of Castration-Resistant Prostate Cancer

O Rogers[1], L Antony, O Levy, N Joshi, B Simons, S Dalrymple[2], D Rosen, A Pickering, HH Lan[3], H Kuang, S Ranganath, L Zheng[4], JM Karp[5], S Howard, S Denmeade, J Isaacs, W Brennen[4]

[1]Revolve Biotechnologies; [2]Johns Hopkins University; [3]University of Toronto; [4]Johns Hopkins University School of Medicine; [5]Brigham and Women's Hospital

Introduction & Objective: The liver is a common site of prostate cancer (PCa) metastasis, particularly in advanced castration-resistant disease. This is associated with a worse median overall survival compared to men with disseminated disease in other tissue sites. These facts document that secondary liver metastasis is a significant health burden in need of novel targeted therapeutic approaches. Pharmacokinetics and biodistribution of systemically-infused particles are greatly influenced by their chemical and physical properties. Indeed, certain types of particles have been shown to selectively accumulate in the liver due to increased phagocytic uptake. This suggests that poly-lactic-co-glycolic acid (PLGA) particles may be ideal vectors for targeting liver disease. We have previously engineered a highly potent mutant bacterial pore-forming protoxin for selective activation by prostate specific antigen (PSA). Though currently being developed clinically as a local therapy for benign prostatic hyperplasia and localized PCa, this protoxin cannot be administered systemically as a treatment for metastatic disease due to its mechanism of action, which leads to poor accumulation within the tumor microenvironment. To overcome this limitation, PLGA particles encapsulating the protoxin were developed. Methods: A highly sensitive sandwich ELISA to quantify protoxin release was developed. Hemolysis and MTT assays performed to demonstrate functional pore formation and PSA-dependent toxicity of the released protoxin *in vitro*. *In vivo* efficacy demonstrated using a model of multifocal castration-resistant liver disease. Results: Protoxin release from different particle formulations was quantified over 10 days. Hemolysis assays documented PSA-dependent pore formation and lytic potential of the released protoxin. Conditioned supernatant from protoxin-loaded but not blank (i.e. unloaded) PLGA particles was highly cytotoxic to PC3 and DU145 in the presence of exogenous PSA. Particle encapsulation increased the therapeutic index of the protoxin *in vivo*, and anti-tumor efficacy was demonstrated following a *single IV dose* of protoxin-loaded particles in a preclinical model of PCa liver metastasis with no obvious toxicity. Conclusions: These results document robust methods to accurately quantify the release and function of a mutant PSA-activated protoxin from PLGA particles and *in vivo* efficacy in a clinically-relevant preclinical model of metastatic PCa, which are essential for future studies aimed at optimizing a systemic delivery strategy for this protoxin.

PS2-46 Near-infrared fluorescence heptamethine carbocyanine dyes mediate imaging and targeted delivery of gemcitabine for treatment of human prostate cancer

S Liu, R Wang, HE Zhau, LW Chung, GC Chu

Cedars-Sinai Medical Center

Introduction & Objective: Advanced prostate cancer (PC) has poor prognosis and patients with progressing disease to castration resistant PC (CRPC) develop deadly bone metastases with limited therapeutic options. We previously identified and synthesized a unique NIRF heptamethine carbocyanine dye, DZ, as a dual imaging and targeting agent, which shows preferential uptake by the tumor cells but not normal cells, via activated organic anion-transporters OATPs in the cancer cells, as demonstrated in various cancer cell lines and tumor xenografts. Taking the unique characteristics of DZ, we recently developed DZ-GEM, a conjugate between DZ and chemotherapy drug gemcitabine (GEM) with the goal to effectively target and treat PC and overcome castration resistance in CRPC patients. Methods: Human PC 22Rv1, PC3, and enzalutamide-resistant C4-2B (MDVR) cells were treated with GEM and DZ-GEM at various concentrations to determine the half maximum inhibitory concentration (IC50) *in vitro*. Seahorse Analysis was performed to measure the mitochondrial function and basal respiration of MDVR cells treated with DZ, GEM, and DZ-GEM. Mice bearing subcutaneous PC3 tumors were treated with DZ (7.5 mg/kg), GEM (2.5 mg/kg), and DZ-GEM (10 mg/kg) for 13 weeks and subjected to fluorescence and bioluminescence imaging and tumor measurement weekly to determine the tumor growth and efficacy of the treatment. Accumulation of DZ-GEM in the mitochondria and lysosomes was assessed by using Mito and Lyso Tracker and examined by fluorescence microscopy to determine the co-localization of DZ-GEM in the suborganelle compartments. Results: We demonstrated that DZ-GEM effectively inhibited the cell proliferation of 22Rv1, PC3, and MDVR cells and provoked their death more completely *in vitro* at significant lower IC50 compared to GEM. We also found that DZ-GEM targeted specifically the PC tumors in mice and the uptake of DZ-GEM by the tumors corresponded with the tumor size. Importantly, DZ-GEM more effectively prevented and attenuated PC3 tumor growth and prolonged the survival of the mice *in vivo*. Mechanistically, DZ-GEM induced cell death is mediated by the accumulation of DZ-GEM in the mitochondria and lysosomes of PC cells, causing mitochondrial membrane depolarization and inducing defects in mitochondrial and lysosomal functions. Conclusions: Our results strongly demonstrated that in comparison to GEM, DZ-GEM more effectively inhibited PC cell and tumor growth *in vitro* and *in vivo* by specific targeting and delivery of DZ-GEM to the tumors and subsequently inducing mitochondria/lysosome-mediated cell killing. DZ-GEM therefore offers a poteintial therapeutic strategy for treatment of PC progression and metastasis.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-47 Exploiting FcRn-mediated Antibody Internalization for Targeted Imaging and Directed Therapy

D Ulmert

University of California, Los Angeles

Introduction & Objective: Understanding the mechanisms of this resistance in prostate cancer is confounded by a lack of non-invasive means to assess the down-stream androgen-receptor (AR) activity in vivo. Here, we exploit the neonatal fc-receptor (FcRn) mediated immunological mechanism that allows cells to internalize antibodies that have formed complexes with antigens. We applied this immunobiological mechanism for in vivo targeting of hK2; an AR-governed and prostate specific antigen. Specifically, we investigated the applicability of humanized high affinity hK2-antibody (hu11B6) against to guide and deliver therapy, and to monitor response in advanced and novel models of prostate cancer that recapitulate critical phases of the disease in man. Methods: A novel anti-free hK2 antibody, 11B6, was produced and humanized. Zirconium-89 (for immunoPET) and alpha emitting Actinium-225 (for molecular radiotherapy) conjugates were generated using clinical labeling protocols and characterized. Imaging and therapy studies were evaluated in vivo using models of human disease (VCaP, LNCaP, 22RV-1 and PC3) and in a genetically engineered mouse model (GEMM) of prostate-specific hK2 expression. Fluorescently labeled (Cy5.5) 11B6 was evaluated in vitro (using validated human PCa cell lines) in order to evaluate cellular interaction using laser scanning confocal microscopy. Results: Internalization of the antigen-antibody complex was confirmed by confocal microscopy and the relation to FcRn mechanism was confirmed through a missense mutation of the hu11B6's fc-region. Uptake reflects AR pathway activity (validated by chemical and surgical inhibition) in advanced models of cancer development and treatment. Noninvasive immunoPET imaging provides high contrast quantitative delineation of disease, while targeted alpha emitting therapies cures or significantly inhibit disease progression. Humanized 11B6 has undergone toxicologic tests in non-human primates, with no noted toxicity. Conclusions: Fluorescent and radio-conjugates of an anti-free hK2 antibody, hu11B6, are internalized and non-invasively report AR pathway activity in human cells as well as advanced GEMM of disease. The role of neonatal Fc-receptor is still being explored, but paves the way to achieve sustained uptake for imaging and therapy of other secreted antigens. Humanized 11B6 has significant potential to improve patient management in these cancers.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-49 ONECUT2 is a Targetable Master Regulator of Lethal Prostate Cancer that Suppresses the Androgen Axis

M Rotinen[1], S You[1], J Yang[2], S Coetzee[1], M Reis-Sobreiro[1], W Huang[3], A Yáñez[1], D Hazelett[1], GC Chu[1], K Steadman[1], C Morrissey[4], P Nelson[5], E Corey[4], LW Chung[1], S Freedland[1], D Di Vizio[1], I Garraway[6], R Murali[1], B Knudsen[1], M R Freeman[1]

[1]Cedars-Sinai Medical Center; [2]City of Hope; [3]China Medical University; [4]University of Washington School of Medicine; [5]Fred Hutchinson Cancer Research Center; [6]University of California, Los Angeles

Introduction & Objective: Treatment of prostate cancer by hormone suppression leads to the appearance of aggressive variants of metastatic castration-resistant prostate cancer (mCRPC) with variable or no dependence on the androgen receptor (AR). Here we identify the transcription factor ONECUT2 as a negative regulator of the AR axis, that emerges in aggressive PC variants to control transcriptional networks linked to CRPC and neuroendocrine (NE) differentiation. We further demonstrate that ONECUT2 can be targeted with a small molecule that inhibits metastasis in mice. Methods: ONECUT2 was confirmed as an mCRPC-relevant protein and to be targetable by computational modeling, enforced expression, silencing, microarray, ChIP-seq, immunohistochemistry, functional assays, in vivo experiments and surface plasmon resonance. Results: We have performed a master regulator analysis using 260 mCRPC transcriptome profiles and developed a model transcription factor network that associates ONECUT2 activity 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 aggressive PC signatures, and a negative correlation with AR activation pathways. ONECUT2 is a negative regulator of AR expression and a repressor of its transcriptional program through direct binding to AR target genes, and is estimated to regulate expression of over 500 transcription factor genes by direct binding to the gene promoters. ONECUT2 is significantly increased in high grade PC, PC with mixed adeno-NEPC features, and NEPC, and it activates a neural differentiation program in CRPC through direct down-regulation of the NEPC inhibitor FOXA1 and direct up-regulation of the NEPC driver PEG10. Finally, we show that ONECUT2 is required for cell growth and survival in CRPC models and can be targeted with a small-molecule that, by binding to the ONECUT2 HOX domain, inhibits metastasis in mice. Conclusions: ONECUT2 is a targetable master regulator of lethal mCRPC variants that drives AR-dependent adenocarcinoma toward NEPC differentiation by blocking AR/FOXA1-activity, inducing PEG10 and activating an oncogenic program associated with metastasis. Patients with OC2-active tumors may benefit from ONECUT2 inhibitor therapy.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-50 Metformin regulates PSMA expression in prostate cancer

JM Gutierrez[1], D Bacich[1], D O'Keefe[2]

[1]University of Texas Health Science Center San Antonio; [2]UT Health San Antonio

Introduction & Objective: Prostate cancer is the second most diagnosed cancer among men and second leading cause of male cancer death in the U.S. As prostate cancer progresses into late-stage, the effectiveness of current strategies begins to diminish. Establishing new, less toxic therapies that are effective at early stage through late stage prostate cancer is crucial for improving patient quality of life. Recently, metformin has been shown to be effective at decreasing proliferation, disrupting cell cycle progression, and increasing cell death in prostate cancer cell lines and mouse models. Metformin use has also been associated with a decrease in prostate cancer risk in epidemiologic studies, but the effective treatment of existing prostate cancer has yet to be established. The low toxicity and ease of dose administration makes metformin an ideal candidate for a new first-line prostate cancer therapy. Prostate-specific membrane antigen (PSMA), also known as folate hydrolase 1 (FOLH1), is known to aid in supplying the folate source needed for one-carbon metabolism by cleaving C-terminal glutamates, allowing for enteral absorption of folate in human cells. As malignant tumors increase and become more aggressive, increased PSMA expression is observed, with studies done *in vitro* resulting in increased cellular folate content conferring a proliferative advantage in PSMA expressing cells. Epidemiologic studies show high PSMA protein expression is correlated with increased tumor angiogenesis and increased risk of prostate cancer progression, and higher expression of PSMA is observed when comparing high grade prostate cancer to low grade prostate cancer. Methods: Two human prostate cancer cell lines (LNCaP and C4-2) were examined for their gene and protein expression of PSMA, and intracellular accumulation of the folate analog, methotrexate, following treatment with metformin at different concentrations and time points, using quantitative PCR, Western blot, and scintillation counting. Results: Metformin treatment resulted in the downregulation of gene expression of the folate transporters, reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT), and a decrease in gene and protein expression of PSMA. Treatment with metformin also led to a decrease in intracellular accumulation of the folate analog, methotrexate. Conclusions: Metformin potentially targets PSMA, a chief component of one-carbon metabolism, elucidating a novel mechanism by which metformin's anti-tumorigenic effect may be enacted.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-51 PROTUX Clinical trial: An open label, single institution, pilot study of rituximab neoadjuvant therapy in patients with high risk prostate cancer scheduled to undergo radical prostatectomy

S Ryan, M Liss, A Shabaik[1], M Muldong[2], E Pittman[1], D Burner[2], J Zhang, S Shalapour, M Karin, K Messer, J Woo, S Howell, C Kane, C Jamieson[1]

[1]University of California, San Diego; [2]University of California San Diego Moores Cancer Center

Introduction & Objective: A novel, immunosuppressive B cell subpopulation which accelerated the emergence of castrate resistant prostate cancer (PCa) was discovered in mouse models and PCa patients. Ablation of B lymphocytes with anti-CD20 antibody in the mouse models delayed regrowth of PCa. Our objective was to determine whether neoadjuvant treatment of high risk PCa patients with the anti-CD20 immunotherapy, Rituximab, could reduce B cell infiltration of prostate tumors. We report B cell density in tumor and adjacent tissue for 8 patients with high risk PCa who received neoadjuvant rituximab when compared to 11 historical controls. Methods: An open label, non-randomized, single arm clinical trial for high risk PCa prior to prostatectomy ("PROTUX" NCT01804712) was performed. Subjects were candidates for prostatectomy with curative intent. Enrolled men received one cycle of rituximab (375 mg/m^2 IV once weekly for 28 days), followed in 2 weeks by prostatectomy. Controls were selected from a pathologic biobank with similar patient characteristics and stained concurrently for CD20. Tumor regions were marked by a blinded pathologist and a computer algorithm quantified the immunofluorescence in tumor and adjacent tissue regions. Mean immunohistochemical (IHC) staining area of CD20+ B-cells within the tumor was compared against historical controls. Results: Mean CD20 IHC stained area in the tumor region of the untreated and treated groups was 0.044 (95% CI 0.028-0.062) and 0.027 (95% CI 0.021-0.033) p = 0.02, respectively. Mean within patient difference (CD20 IHC stained area in tumor-adjacent tissue) was compared against controls, both utilizing unequal variances *t* test. Mean within patient difference of CD20 IHC stained area was 0.009 (95% CI -0.004-0.023) and -0.005 (95% CI -0.028-0.017) (p = 0.11) in the untreated and treated groups, respectively. Conclusions: Neoadjuvant rituximab treatment significantly decreased B cell density within tumors compared to historical controls (p = 0.02, relative to controls) and appeared to reduce the density of tumor-resident B cells to levels comparable to adjacent normal tissue, (p = 0.11 relative to controls). These results provide evidence that rituximab can modify the immune environment of the tumor.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-53 Detecting prostate cancer using a targeted dual imaging nanoparticle platform

D Martin, J Lee, G Galiana, P Humphrey, R Weiss, T Fahmy

Yale University

Introduction & Objective: There is a need to design more effective diagnostic tools for metastatic prostate cancer. We developed a nanoparticle (NP) platform functionalized with the carboxyl-terminal domain (290-319) of the *Clostridium perfringens enterotoxin* (cCPE) peptide that is selective in targeting a subset of aggressive prostate cancers that overexpress claudin-3 (Cldn3) and claudin-4 (Cldn4) tight junction proteins. As magnetic resonance imaging (MRI) is already being used in standard diagnostic protocols, the encapsulation of iron oxide and a fluorophore, for real-time surgery, may provide an opportunity to improve detection of metastatic disease. Methods: NPs were prepared by dissolving poly (lactide-co-glycolide), rhodamine B, and superparamagnetic iron oxide in ethyl acetate or chloroform and then the mixture was added to a 5% polyvinyl alcohol solution and avidin-palmitate conjugate, resulting in an emulsion of NPs: 150 nm (RS1) and 400 nm (RS4). For peptide conjugation, NPs were incubated with biotinylated cCPE. A prostate cancer xenograft flank tumor model was developed by subcutaneously injecting 5 million PC3 cells into the flank of severe combined immune deficiency mice. For MRI studies, mice were anesthetized and respiratory gating was used. T2*-weighted images were acquired using a 4T Bruker spectrometer magnetic. Tumor volume and NP uptake (ie tumor contrast) were quantified using BioImage Suite 3.01. For biodistribution studies, prostate tumor-bearing mice were injected intravenously with and without cCPE-NPs, and fluorescence was quantified using ImageJ. Results: Human metastatic prostate cancer PC3 cells, with high Cldn expression, had a greater uptake of cCPE-NPs than normal prostate RWPE1 cells, with low Cldn expression. Also, there was a cCPE surface density dependent effect. MRI was performed on prostate tumor bearing mice that expressed Cldn3 and Cldn4 receptors. We demonstrated a 4-fold increase in NP tumor coverage with cCPE-RS1 than RS1. Fluorescent biodistribution studies performed on mice showed that functionalized NPs were localized mainly to the tumor with little off target effects. Also, NP size played a role in tumor coverage as cCPE-RS1 had a 3-fold greater tumor uptake compared to cCPE-RS4 for both imaging methods. In addition to *in vivo* detection, we exposed our NPs to human *ex vivo* prostate biopsies and found that the NPs localized to a greater degree to the cancerous region compared to the non-cancerous region. Conclusions: We demonstrate that our CPE functionalized NPs have the ability to enhance prostate tumor detection using a combination of fluorescence and MR imaging.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-54 Extracellular Vesicles of Prostate Origin Contain Prognostic microRNAs in Prostate Cancer Patients

M Zenner, L Nonn, M Abern, R Sauer, K Valyi-Nagy

University of Illinois at Chicago

Introduction & Objective: Prostate cancer is the most common cancer diagnosis and the second leading cause of cancer-related deaths in men. A common treatment is radical prostatectomy (RP) surgery, but frequent side effects of this surgery are incontinence and impotence. Many men with indolent disease unnecessarily undergo RP surgery due to the possibility that they harbor aggressive disease missed on the biopsy. In order to improve identification of patients who should undergo RP treatment, we previously discovered a pre-surgical serum microRNA signature that predicts prostate cancer aggressiveness. Herein we describe our work to validate the microRNA signature, determine the origin of the serum microRNAs and determine if they have biological significance. The hypothesis is that the serum microRNAs in the signature originate from the prostate via secreted exosomes. Methods: We are also in the process of collecting serum prospectively to validate the prognostic microRNA signature. To identify prostate-derived microRNAs, we have run small RNA sequencing on exosomes collected from serum from 20 patients before and after RP surgery and exosomes from prostate tissue explants. microRNAs secreted in exosomes from primary prostate cells were also examined. Results: We show that some of the microRNAs from the signature are present in serum exosomes from men with prostate cancer. The microRNAs are also present in exosomes from primary prostate cells and human prostate tissue slices. Conclusions: In conclusion, we have found that microRNAs can be quantified from serum exosomes and may house prognostic markers for prostate cancer. Both epithelial and stromal prostate cells release exosomes which may contribute to the serum exosome population and facilitate cross-talk in the prostate microenvironment. Ongoing experiments include examining the effects of stromal cell exosomes on epithelial cell culture and vice versa to determine the effects of exosomes on cellular cross-talk.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-55 Orally effective antagonists of ROR-γ inhibit CRPC tumor growth and aggressive-tumor progression programs

Z Huang[1], J Wang[1], C Zhang[1], JC Yang[1], J Li[1], E Corey[2], CP Evans[1], A Yu[1], H Chen[1]

[1]University of California Davis, School of Medicine; [2]University of Washington School of Medicine

Introduction & Objective: Patients with locally advanced prostate cancer (PCa) undergoing androgen-deprivation therapy have high chances to progress to metastatic castration-resistant prostate cancer (mCRPC). We previously discovered that retinoic acid receptor-related orphan receptor γ (ROR-γ) drives the overexpression of androgen receptor (AR) in CRPC and that ROR-γ antagonists potently block tumor growth and metastasis. Here we aimed to investigate whether ROR-γ small-molecule antagonists are orally bioavailable and effective in inhibition of patient-derived xenograft (PDX) tumors and to identify the potential therapeutic mechanism of the ROR-γ inhibitors in CRPC. Methods: Mice with LuCaP-35CR PDX tumors were orally administrated with different doses of small-molecule antagonists of ROR-γ or vehicle for 40 days. Tumor volume and body weight was monitored. Tumors were subjected to Immunohistochemistry (IHC) staining of Ki-67 and cleaved caspase-3. Additionally, organoids developed from PDX tumors were also treated with the antagonists. Cell viability was measured thereafter. To address the oral bioavailability of ROR-γ antagonists, a pharmacokinetics (PK) study was performed with CD-1 mice treated, p.o. or i.v., with the ROR-γ inhibitors. To further explore the mechanism of action (MOA) of the antagonists, RNA-seq gene expression profiling and gene set enrichment analyses (GSEA) was performed. Results: The three ROR-γ antagonists exhibited strong potency in inhibition of LuCaP-35CR tumor growth and an attractive PK profile when orally administered. The antagonists also significantly inhibited the proliferation and survival of tumor and organoid cells. Mechanistically, they strongly suppressed the aggressive progression pathways or programs such as those of AR-variants (AR-V) and NEPC as well as the PCa-subtyping PCS1 signature genes. Conclusions: Our study using PDX and organoid models demonstrates the oral bioavailability and anti-CRPC potency of small-molecule antagonists of ROR-γ. ROR-γ may promote tumor cell proliferation and survival by stimulating gene programs such as the ones that are indicative of aggressive PCa subtypes (PCS1 and NEPC).

PS2-56 Reactivation of tissue inhibitor of matrix metalloproteinase-3 in prostate cancer by green tea polyphenols

G Deb[1], E Shankar[2], VS Thakur[3], L Ponsky, D Bodner, P Fu[2], S Gupta[2]

[1]City of Hope; [2]Case Western Reserve University; [3]University of Miami

Introduction & Objective: Diet and lifestyle factors contribute to cancer development by inducing both epigenetic and genetic changes that, in combination with genetic make-up, result in the disruption of key cellular processes leading to neoplastic progression. Green tea polyphenols (GTP) and its major constituent, epigallocatechin-3-gallate (EGCG) have been reported to demonstrate many remarkable biological activities, including induction of epigenetic changes and cancer prevention. Recent studies in prostate cancer provide strong evidence that epigenetic mechanisms are major players in the regulation of the MMP-2 and MMP-9 and their binding partners Tissue Inhibitor of Matrix metalloproteinases viz. TIMP-2 and TIMP-3 involved in prostate cancer progression. Methods: We demonstrate that GTP and EGCG mediate epigenetic reactivation of TIMP-3 levels and play a key role in suppressing invasiveness and gelatinolytic activity of MMP-2 and MMP-9 in prostate cancer cells. Results: Treatment of human prostate cancer DU-Pro and LNCaP cells with 20 μM EGCG and 10 μg/mL GTP for 72 h significantly induces TIMP-3 mRNA and protein levels, reduced MMP-2/9 gelatinolytic activity and abrogated invasive and migration capabilities in cancer cells. Interestingly, investigations into the molecular mechanism revealed that TIMP-3 repression in prostate cancer cells is mediated by epigenetic silencing mechanism(s) involving increased activity of the enhancer of zeste homolog 2 (EZH2) and class I histone deacetylases (HDACs), independent of promoter DNA hypermethylation. Treatment of cancer cells with GTP/EGCG significantly reduced EZH2 and class I HDAC protein levels. Transcriptional activation of TIMP-3 was found to be associated with decreased EZH2 localization and H3K27 trimethylation enrichment at the TIMP-3 promoter with a concomitant increase in histone H3K9/18 acetylation. Furthermore, clinical trial performed at the University Hospitals on patients undergoing radical prostatectomy consuming 800 mg EGCG (Polyphenon E) up to 8 weeks and the grade-matched controls demonstrate increase in plasma TIMP-3 levels, compared to controls. A marked decrease in HDAC activity; decrease in the protein expression of class I HDACs and EZH2; trimethylation of H3K27 were noted in the prostate tissue from GTP supplemented group. Conclusions: Our findings highlight TIMP-3 induction as a key epigenetic event modulated by green tea in restoring the MMP: TIMP balance to suppress prostate cancer progression.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-57 PARP inhibitors as potential therapeutic agents against bladder cancer

M Sullivan, S Bhattacharjee, R Wynn, N Nadiminty

University of Toledo Health Science Campus

Introduction & Objective: Bladder cancer (BLCA) is the sixth most common cancer in the US. BLCA management has not changed significantly in the last 3 decades, with platinum-based chemotherapy (MVAC) being the systemic therapy of choice since 1985. MVAC can reduce recurrence after tumor resection, but only 50% of patients are either eligible or respond, and it is associated with several life threatening toxicities. Breakthrough immunotherapy with checkpoint inhibitors produces responses in only 20-30% of patients with metastatic BLCA. Hence, the need for more therapeutic options is paramount. Cells deficient in homologous recombination (HR) via loss of BRCA proteins and in SSB repair via inactivation of PARP are subject to synthetic lethality when treated with PARP inhibitors (PARPi). BLCA exhibit somatic loss of function mutations in several HR genes, which can amplify the effects of DNA damage caused by ionizing radiation or platinum drugs. Hence, we tested the sensitivity of BLCA cells to commercially available PARPi either singly or in combination with cisplatin. Methods: BLCA cell lines and xenografts in SCID mice were treated with different concentrations of PARPi (niraparib, olaparib, veliparib, rucaparib, or talazoparib) as single agents or in combination with cisplatin. Cell survival, apoptosis, and clonogenic ability were measured in vitro. Tumor growth of xenografts was measured in vivo. The levels of proliferation and apoptosis in the xenograft tumors were assessed using immunohistochemistry. Expression of PARP enzymes in the BLCA cells and xenograft tumors was examined using Western blotting. Results: BLCA cells responded differently to each PARPi. Niraparib and talazoparib were the most effective at reducing cell survival of BLCA cells compared with normal urothelial cells. Veliparib was not effective in reducing cell survival even at high concentrations. Combinations of PARPi with cisplatin exhibited strongly additive effects on cell survival and apoptosis in vitro. In vivo, niraparib, rucaparib, and talazoparib were the most effective at reducing tumor growth of BLCA xenografts. The combination of olaparib with cisplatin exhibited strongly additive effects in reducing tumor growth. Conclusions: We provide preliminary evidence that PARPi can be effective therapeutic options against BLCA cells. Given that the anti-tumor activity of PARPi is via the inhibition of PARP catalytic activity and/or PARP trapping, our results suggest that the higher PARP trapping activity of niraparib, olaparib, rucaparib, and talazoparib plays the major role in their activity against BLCA cells compared with veliparib, which is a primarily catalytic inhibitor.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-59 LAT1 promote bladder cancer progression through IGFBP-5

M Maihulan[1], S Shinichi[1], T Nobuyoshi, S Masahiro, F Tomomi, K Atsushi, I Yuzuru[1], K Yoshikatsu[2], A Naohiko, I Tomohiko

[1]Chiba University; [2]Osaka University Graduate School of Medicine

Introduction & Objective: Cancer cells require massive amounts of amino acids for survival. LAT1 is a major route for the transport of large neutral essential amino acids through the plasma membrane. L-type amino acid transporter 1 (LAT1) that transports essential amino acids is highly expressed in several tumors, however no previous study investigated in bladder cancer. Here, we examined the role of LAT1 in bladder cancer. Methods: Gene expression was compared by Real-Time PCR and expression of related proteins was examined by Western blotting. SiRNA was used for knocking down the target gene. A functional experiment of bladder cancer cell line was performed using LAT1 inhibitor JPH203. Genes related to LAT1 were analyzed using RNA-Seq. 68 total cystectomy patient's data was used to study the clinical relevance. Results: LAT1 was highly expressed in 5637 cells and T24 cells. LAT1 knockdown and JPH203 (10 μM and 20 μM) suppressed cell proliferative, invasive and migratory ability. JPH203 inhibited phosphorylation of MAPK/Erk, AKT, and p70S6K. IGFBP-5 was identified as a gene that significantly changed (0.35 times) by SiLAT1 by RNA-Seq analysis. SiIGFBP-5 suppressed the cell proliferation in two bladder cancer cell lines. The LAT1 expression is the only independent predictor of OS in bladder cancer patients who received total cystectomy on multivariate analysis (HR27.3 p = 0.0114). Patients with high expression of LAT1 plus IGFBP5 showed the highest mortality rate by Kaplan-Meier analysis (p = 0.0045). Conclusions: The study demonstrated that LAT1 potentially contributed to the progression of bladder cancer. Since JPH 203 underwent the phase I clinical trial in solid cancer patients, the clinical benefit of JPH203 is awaited to be investigated.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-60 Eliminating aberrant OXPHOS activities by simultaneously targeting multiple Krebs cycle enzymes in prostate cancer cells

H Xu, B Li

Kansas University Medical Center

Introduction & Objective: Aberrant metabolism is one of the hallmarks in human cancers. Especially in prostate cancer, metabolic transformation has been recognized as “citrate-producing” in normal or benign prostate tissue to “citrate oxidizing” through Krebs cycle OXPHOS activities in malignant prostate tissue. Disrupting citrate OXPHOS activities represents a novel therapeutic approach in prostate cancers. We recently demonstrated that the natural compound Alternol exerts a cancer-specific killing effect with very limited effect on benign prostate cells through an oxidative stress-dependent mechanism. Methods: Prostate cancer PC-3, C4-2 and 22RV1 and benign BPH1 cell lines were used in the experiments. A comprehensive strategy was utilized to identify protein targets and functional analysis in vitro and in vivo. Results: In attempt to elucidating the mechanism underlying Alternol-induced oxidative stress and apoptosis in prostate cancer cells, we identified four Krebs cycle enzymes as Alternol-interacting protein targets. These enzymes are dihydrolipoamide acetyltransferase (DLAT) as a E2 enzyme in pyruvate dehydrogenase complex (PDHC), dihydrolipoamide S-succinyltransferase (DLST) as a E2 enzyme in a-ketoglutarate dehydrogenase complex (KGDHC), fumarate hydratase (FH) and malate dehydrogenase-2 (MDH2). Our data revealed that at the basal level, PDHC and KGDHC activities were significantly higher in prostate cancer cells compared to benign BPH1 cells. Alternol treatment reduced their activities to the levels similar to BPH1 cells. Although FH and MDH2 activities were comparable among multiple malignant and benign cells, Alternol enhanced their activities only in malignant cells. Metabolomic analysis revealed that Alternol treatment remarkably reduced Krebs cycle intermediates including isocitric acid, fumaric acid and malic acid, as well as mitochondrial respiration rate. Alternol treatment also drastically reduced ATP contents in PC-3 cells *in vitro* and in PC-3 cell-derived xenograft tissues but not in BPH1 cells or in mouse liver tissues. Conclusions: These results suggest that Alternol interacts with and attenuates the functions of multiple Krebs cycle enzymes, disrupts mitochondrial OXPHOS activities, resulting in reduced mitochondrial respiration and energy production in malignant cells and xenograft tissues. Therefore, Alternol possesses a great potential to be developed as a successful clinical therapy. As the authors aware, Alternol is the first natural compound reported targeting multiple Krebs cycle enzymes in cancer cells.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-61 Direct Targeting of the FKBP52 Cochaperone for the Treatment of Castration Resistant Prostate Cancer

AN Payan, NC Guy, MB Cox

University of Texas at El Paso

Introduction & Objective: The folding, activation and nuclear translocation of steroid hormone receptors involves no less than twelve proteins and at least four distinct complexes. At least one of these proteins, the FKBP52 cochaperone, is a highly promising therapeutic target for the disruption of a number of mechanisms important in prostate cancer (PCa). FKBP52 is a positive regulator of androgen (AR), glucocorticoid (GR) and progesterone receptor (PR) hormone binding, nuclear translocation, and transcriptional activity. FKBP52 regulates multiple, distinct steps within the AR signaling pathway, some of which are independent of Hsp90. The direct targeting of FKBP52 will inhibit beta-catenin, FKBP51, and FKBP52-dependent potentiation of AR activity in addition to inhibiting GR and PR activity. Our data suggest that the proline-rich loop surface that overhangs the FKBP52 PPIase pocket is important and likely represents an AR interaction surface. For that reason, our strategy aims to identify specific PPIase binding molecules that, when docked in the pocket, reorient the proline-rich loop conformation leading to the disruption of FKBP52 interactions within the steroid hormone receptor heterocomplex. Thus, small molecules targeting FKBP52 would simultaneously hit multiple pathways known to have, or suspected of having, a role in PCa. Methods: We used *in silico* structure-based drug design to identify molecules predicted to bind the FKBP52 PPIase pocket with high affinity and assessed hit molecules for effects on steroid hormone receptor signaling, hormone-dependent gene expression, prostate cancer cell proliferation and prostate tumor growth in xenografts. Results: A primary screen of the predicted hits identified GMC1, a molecule that specifically inhibits FKBP52-mediated AR, GR and PR activity in reporter assays, AR-dependent gene expression in prostate cancer cells, AR-dependent proliferation of prostate cancer cells, and tumor growth in mouse xenograft models. We are currently performing hit-to-lead optimization by screening rationally designed GMC1 modifications and assessing them for activity in AR-mediated luciferase assays. Conclusions: We have identified and characterized GMC1, a hit molecule that inhibits AR, GR and PR signaling, PCa cell proliferation and prostate tumor formation likely by targeting the FKBP52 PPIase pocket. While we have not yet verified the drug target site, the data strongly suggest FKBP52 as the target and also suggest that disruption of FKBP52 proline-rich loop interactions is possible through targeting the PPIase pocket. Future studies will be aimed at hit-to-lead optimization and characterization of the drug(s) mechanism of action.

PS2-62 Trop2 is a Novel Driver of Metastatic Castration Resistant Prostate Cancer with Neuroendocrine Phenotype

E Hsu[1], MA Rice[1], A Bermudez[1], F Garcia-Marques[1], M Aslan[1], A Ghoochani[2], C Zhang[1], Y Chen[1], A Zlitni, F Habte[1], S Kumar[3], R Nolley, D Peehl[4], A Zoubeidi, S Gambhir[5], CA Kunder[1], S Pitteri[1], J Brooks, T Stoyanova

[1]Stanford University; [2]Stanford University; [3]Vancouver Prostate Centre, University of British Columbia; [4]University of California San Francisco; [5]Stanford University/Department of Radiology

Introduction & Objective: Prostate cancer is the most common non-cutaneous cancer and second leading cause of cancer related deaths in men in the United States. The first line of treatment for men with advanced prostate cancer is androgen deprivation therapy. Although initial responses are observed, prostate cancer commonly relapses in its lethal metastatic form referred to as castration resistant prostate cancer (CRPC) with 1-2 years mean survival time. Neuroendocrine prostate cancer (NEPC) is highly aggressive, AR independent and usually emerges post castration resistance. Thus, there is an urgent need to identify novel targets and therapeutic strategies for advanced CRPC/NEPC. Methods: Tissue microarrays were used to evaluate correlation of Trop2 level with clinical outcome and association with CRPC and NEPC.CRISPR/Cas9 technology was used to generate Trop2 knock-out clone and lentiviral infection was used to generate Trop2 overexpressed stable cells. *In vitro* functional assays were performed including colony formation, tumorsphere formation, and cell migration assays. *In vivo* tumor growth was assessed by subcutaneous xenograft tumor model. *In vivo* metastasis was addressed by intracardiac injection of cancer cells with stable luciferase expression. *In vivo* tumor growth in castrated mice were utilized to evaluate androgen independence of prostate cancer cells. Whole proteomic profiling was performed to identify Trop2 down-stream targets. Results: Our results demonstrate that Trop2 is strongly elevated in CRPC, metastasis and correlates with unfavorable clinical outcome. Additionally, we demonstrate that overexpression of Trop2 promotes cancer cell proliferation, migration, invasion and drives androgen independent growth *in vitro*. We further show that overexpression of Trop2 drives bone metastasis, liver metastasis, and castration resistance *in vivo*. Trop2-driven CRPC exhibit AR loss andneuroendocrine markers. Whole proteomic profiling identified PARP1 as the top up-regulated protein in Trop2 driven CRPC/NEPC. Trop2 driven CRPC/NEPC cells are sensitive to PARP1 inhibitor *in vitro*. Moreover, treatment with PARP1 inhibitor suppresses Trop2 driven tumor growth, and reverses Trop2 up-regulated neuroendocrine markers *in vivo*. Conclusions: In our study, we identified Trop2 as a novel driver of metastatic CRPC with neuroendocrine features through up-regulation of PARP1. Our study revealed that high level of Trop2 is associated with prostate cancer recurrence, CRPC, NEPC, and metastasis. Thus, targeting Trop2 driven aggressive prostate cancer with PARP inhibitors may represent a novel therapeutic strategy for metastatic CRPC with neuroendocrine features.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-63 Molecular Dissection of Magnetic Resonance Imaging Visible and Invisible Prostate Cancer

SS Salami[1], JB Kaplan[1], S Nallandhighal[1], M Lee[1], J Yoon, DH Hovelson[2], K Plouffe[1], AK George, M Davenport[3], S You[4], SA Tomlins[1], NE Curci[1], H Kim[5], DE Spratt, AM Udager[1], GS Palapattu

[1]University of Michigan; [2]Strata Oncology; [3]Michigan Medicine; [4]Cedars-Sinai Medical Center; [5]Cedars Sinai Medical Center

Introduction & Objective: Up to 20% of patients with negative multiparametric magnetic resonance imaging (mpMRI) harbor high grade prostate cancer. In this study, we sought to characterize and compare the molecular profiles of multiparametric magnetic resonance imaging (mpMRI) visible and invisible prostate cancer to elucidate the molecular basis of cancer visibility on mpMRI. Methods: Patients who underwent mpMRI prior to radical prostatectomy were identified for this IRB-approved study. mpMRI for each patient was re-reviewed by a radiologist with expertise in prostate mpMRI and histopathology was re-reviewed by a genitourinary pathologist. Whole-mount histopathology was co-registered with axial mpMRI images. DNA and RNA were co-isolated from all tumor foci pre-identified on formalin-fixed paraffin-embedded specimens. High depth, targeted DNA and RNA next generation sequencing was performed to characterize the molecular profile of each tumor focus using the Oncomine Comprehensive Panel (DNA) and a custom targeted RNAseq panel assessing prostate cancer relevant genes. A multigene RNAseq model was developed and validated to predict MRI visible prostate cancer. Results: A total of 26 primary tumor foci from 10 patients were analyzed. The median number of prostate cancer foci was 3. Of the 14 (54%) invisible lesions on mpMRI, 5 (36%) were Gleason 3+4 = 7. We detected high-confidence prioritized genetic mutations in 54% (14/26) of tumor foci, 43% (6/14) of which were in mpMRI-invisible lesions. Additionally, 64% (9/14) of lesions exhibiting prioritized mutations were Gleason 7. Notable point mutations were in *APC, AR, ARID1B, ATM, ATRX, BRCA2, FAT1, MAP3K1, NF1, SPEN, SPOP, TP53,* and a frameshift mutation was detected in *SOX2.* A multiplex model, composed of 9 genes (Figure 1), majority of which are involved in cellular organization and structure, was developed to predict MRI visible tumor with an AUC of 0.89. Validation of this model in an independent data set (n = 16) yielded an AUC of 0.88. Conclusions: Prostate cancer lesions visible on mpMRI exhibited differential expression in cellular organization and structural genes. More work is needed to discern the significance of this model and mpMRI to predict prostate cancer oncological outcomes.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-64 Myofibroblasts Promote Prostate Cancer Progression by Causing Aberrant Prostate Epithelial Differentiation

S Tahsin[1], L Jiang[1], F Ivich[1], Y Zohar[1], C Miranti[2]

[1]University of Arizona; [2]University of Arizona College of Medicine

Introduction & Objective: Differentiation of the prostate ducts into basal and luminal cell types is driven by the normal underlying stroma. The stroma surrounding the tumor is predominantly made up of myofibroblasts. However, what generates the myofibroblasts and how they are involved tumor development is not well understood. Recently it was shown that the PIM1 serine-threonine protein kinase is elevated in patient tumor-associated fibroblasts. The ability of the normal prostate stroma to support normal basal and luminal cell differentiation is dependent on androgen and AR activation in the stroma. Interestingly, the loss of stromal-expressed AR is directly proportional to the decrease in tumor differentiation (i.e. Gleason grade) and increased progression of prostate cancer. We hypothesize that myofibroblasts generated by PIM1 overexpression promotes prostate cancer progression by disrupting normal basal-luminal differentiation through downregulation of AR signaling in the stroma. Methods: Using a microfluidics model, BHPrS1 prostate stromal cells, co-cultured with iPrEC prostate epithelial cells, were stimulated with androgen, to induce luminal cell differentiation. BHPrS1 cells with or without PIM1 kinase were co-cultured for 16-21 days with iPrEC or tumorigenic EMP-iPrEC respectively to measure the generation of myofibroblasts. Differentiation state of basal and luminal cells and generation of myofibroblasts was monitored by immunostaining of lineage markers. Stromal AR target gene expression (mRNA) was monitored by qRT-PCR. Results: we successfully differentiated basal epithelial cells into luminal cells through direct stimulation of stromal cells with androgen. We observed nuclear localization of AR and AR induced gene expression in the stroma. We successfully generated myofibroblasts, with increased expression of α-SMA and Collagen A1, with PIM1 overexpressing BHPrs1 stroma. Normal BHPrsS1 developed into myofibroblasts when co-cultured with tumorigenic EMP-iPrEC. Conclusions: Studies using this unique bioengineered prostate-on-chip model will ultimately determine how myofibroblasts suppress prostate epithelial differentiation and lead to aggressive prostate cancer. This approach will potentially assist in the subcategorization of prostate tumors based on their differentiation status to provide patients with better prognostic information.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-65 Gene signature involved in genomic instability underlies the effect of EZH2 inhibitors in castration resistant prostate cancer

K Xu[1], C Chen[2], SX Liu, M Brown

[1]University of Texas Health Science Center San Antonio; [2]Harvard University

Introduction & Objective: Pharmacological inhibition of the methyltransferase EZH2 has been proposed as a promising anticancer strategy. Selective inhibitors that disrupt EZH2 enzymatic activity preferentially suppress the growth of lymphoma cells with activating mutations in EZH2 gene. Although substantial evidence indicates a vital role of EZH2 in driving the aggressive features of cancer cells, it is unknown whether these EZH2-targeting compounds have inhibitory effects in solid tumors that generally do not carry somatic mutations of the methyltransferase. Our previous study demonstrated that EZH2 functions as a coactivator of AR-mediated gene expression in castration resistant prostate cancer (CRPC) cells. Genes co-regulated by both proteins are generally overexpressed in metastatic, hormone-refractory prostate tumors, and show strong prognostic correlation with aggressive forms of the disease. EZH2 facilitates AR recruitment to particular regulatory elements of target genes, and the methyltransferase activity of EZH2 is required for its cooperation with AR signaling. Based on that work, it is appealing to posit that the selective EZH2 inhibition may be a new initiative against AR activation in advanced prostate cancer. Methods: We performed CRISPR knockout screens in parental, androgen-dependent LNCaP cells and its derived hormone-refractory abl cells. We also did CRISPR screens in prostate cancer cells with or without EZH2 inhibitors. We tested two of EZH2 selective inhibitors, GSK126 and EPZ-6438, in a panel of human prostate cell lines, including two benign prostate epithelial cells, two AR-null prostate cancer cells, and eight AR signaling competent prostate cancer cells. We then characterized EZH2 inhibitor-induced gene expression profiling using RNA-Seq and epigenomic alterations using quantitative ChIP-Seq, and compared between sensitive and irresponsive prostate cancer cells. Results: We found that prostate cancer cells with competent androgen receptor (AR), especially castration resistant ones, were sensitive to EZH2 inhibitors (EZH2i). EZH2i treatment impaired AR recruitment to target chromatin loci and disrupted the interaction between EZH2 and AR. A group of EZH2-activated genes are essential for the inhibitory effects of EZH2i in prostate and other types of cancer. These signature genes are functionally involved in regulation of genomic integrity, cannot be transcriptionally controlled by EZH2 mutations conferring EZH2i resistance and may predict cellular responses to EZH2i. Conclusions: Our work revealed a novel mechanism of action of EZH2i in cancer and challenged prevalent rationale behind the design of EZH2-targeting drugs.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-66 ROLE OF ID4 (Inhibitor Of Differentiation 4) IN FKBP52-AR PATHWAY

SK Komaragiri

Clark Atlanta University

Introduction & Objective: Inhibitor of DNA binding/differentiation protein 4 (ID4), acts as a dominant negative regulator of basic helix loop helix (bHLH) family of transcription factors. In normal prostate epithelial cells, ID4 collaborates with androgen receptor (AR) and p53 to exert its tumor suppressor activity. Previous studies have shown that ID4 promotes tumor suppressive function of AR whereas loss of ID4 results in tumor promoter activity of AR through selective interaction with FKBP52. Pharmacological inhibition of FKBP52, by MJC13, attenuated the tumor growth, weight, and volume of LNCaP cells lacking ID4(L(-)ID4) xenografts. The L(-)ID4 cells also gains CRPC phenotype through FKBP52-mediated AR signaling. In this study we investigated the role of ID4 in pharmacological inhibition of FKBP52 with GMC1 (Initial hit molecule by in-silico screening with the most potent inhibition of FKBP52) in regulating the AR pathway Methods: We determined ID4 protein expression levels in prostate cancer cell lines (LNCaP, LNCaP(-)ID4, DU145, DU145(+)ID4). GMC1 drug treatments in LNCaP-ID4 generated xenograft tumors which were further analyzed for tumor volume and tumor weight. We also performed Immunohistochemistry on GMC1 treated xenografts to determine AR, PSA, FKBP51 and FKBP52 protein expression. Results: Our in vivo studies with GMC1 drug treatments in castrated male SCID mice injected with LNCaP-ID4 cells showed decreased tumor size and volume when compared to untreated mice tumors. IHC results on GMC1 treated LNCaP-ID4 generated xenograft tumors showed no change in ID4 expression with decrease expression of AR, PSA, FKBP51, FKBP52 and KI67 when compared to untreated xenograft tumors. These results suggest that inhibition of FKBP52-regulated AR activity (via GMC1) demonstrated a significant reduction in the tumor volume of subcutaneous xenografts in vivo Conclusions: ID4 appears as a potential tumor suppressor gene that selectively regulates AR activity through direct interaction with FKBP52, and its loss, promotes CRPC through FKBP52-mediated AR signaling.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-67 Nucleolin Functions as a Negative Regulator of Androgen Receptor Expression

E Reyes-Reyes[1], S Moore[1], K Ramos[1], L Hurley[1], C Miranti[2]

[1]University of Arizona; [2]University of Arizona College of Medicine

Introduction & Objective: Androgen receptor (AR) drives the development and progression of prostate cancer (PCa). Men who develop regionally advanced or metastatic prostate cancer often have long-term cancer control when treated with androgen-deprivation therapies (*ADT*), but their disease inevitably becomes resistant to ADT and progresses to castration-resistant prostate cancer (*CRPC*). ADT involves the use of potent competitive AR antagonists and androgen synthesis inhibitors. Resistance to these treatments often emerges through maintenance of AR signaling via *ligand*-independent *activation* mechanisms. There is a need to identify the molecular mechanism that regulate AR expression to develop novel anti-AR therapies that enhance the efficacy of existing systemic therapies for CRPC patients. Here, we present evidence that implicates nucleolin (NCL) as a negative regulator of AR expression, through its ability to stabilize a G-quadruplex structure (G4) in the AR promoter. Methods: We genetically manipulated NCL expression in PCa cell lines expressing AR and analyzed changes in AR expression. We evaluated NCL binding to the *AR* promoter to the G4 area by chromatin immunoprecipitation assays. We treated PCa cell lines with G4-binding drugs to analyze *AR* expression and NCL binding to the *AR* promoter. The dependence on the G4 structure for nucleolin-mediated AR suppression was evaluated using dual reporter assays. Results: Genetic knockdown of NCL increases the levels of both AR mRNA and protein in PCa cells, while NCL overexpression suppresses AR. The ability of NCL to modulate AR expression was independent of AR activation. We found that NCL binds to the G4 region within the *AR* promoter. A dual reporter assay showed that genetic knockdown of NCL increases the transcription activity of AR promoter only when the AR promoter G4 sequence is present. Moreover, compounds that stabilize G4 structures and increase NCL association with the G4 of the *AR* promoter decrease AR expression. Conclusions: These results indicate that NCL functions as a transcriptional repressor of the *AR* gene, and raises the important possibility that G4-stabilizing drugs can increase NCL transcriptional repressor activity to block AR expression. These findings contribute to a clearer understanding of the mechanisms that control the expression of AR and may be of significance for the development of alternative therapeutic options for men with CRPC.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-68 Novel Methoxychalcone Inhibits Prostate Cancer Growth and Synergizes with Anti-androgen Therapy

MA Rice[1], V Kumar, D Taylor, M Aslan[1], F Garcia-Marques[1], A Bermudez[1], A Su[1], A Ghoochani[2], CC Going, S Pitteri[1], SV Malhotra[3], T Stoyanova

[1]Stanford University; [2]Stanford University; [3]Stanford University School of Medicine

Introduction & Objective: Prostate cancer remains the most commonly diagnosed non-cutaneous tumor in men in the United States. Among these cases, about 10% will progress to aggressive, potentially metastatic disease. These patients are treated aggressively with anti-androgen therapy with great short term success. However, patients ultimately relapse to incurable hormone refractory, or castration resistant prostate cancer (CRPC). Standard of care therapies include second-generation anti-androgens or chemotherapeutics which only modestly increase patient lifespan. We sought herein to generate new therapeutic strategies to improve patient survival with CRPC. Methods: We generated a compound library of chalcones, derivatives of common natural products, flavonoids. SSMDL-0086 was selected based on a viability screen of normal prostate and prostate cancer (PC) cells exhibiting high potency with minimal toxicity. In vitro clonogenic assays were performed with SSMDL-0086 on 22RV1, C4-2 and DU145 androgen-independent aggressive PC cells. These cell lines were used to implant subcutaneous xenografts into NSG animals. Mice were treated i.p. with 50 mg/kg SSMDL-0086 daily. To identify the target pathways of SSMDL-0086, quantitative liquid chromatography/mass spectrometry (LC/MSMS) was performed on each cell line following SSMDL-0086 treatment. Targets identified by LC/MSMS were validated with western blot and immunohistochemistry of treated xenografts. In vitro colony formation, and in vivo xenografts were performed combining SSMDL-0086 and second-generation anti-androgens for CRPC treatment, Enzalutamide (Enz) or Abiraterone (Abi). Results: We identified a novel compound, SSMDL-0086, a methoxychalcone derivative with structural similarity to Enz. SSMDL-0086 was determined to be a potent inhibitor of aggressive PC cells in vitro. In vivo, SSMDL-0086 decreased tumor growth in three models of aggressive, androgen-independent PC. Quantitative mass spectrometry identified several down-regulated targets of SSMDL-0086 indicating impairment of glycolysis. Further, SSMDL-0086 synergized with Enz or Abi at submicromolar concentration to abrogate colony formation, and significantly impaired tumor growth in either combination compared to independent treatment of SSMDL-0086, Enz, or Abi. Conclusions: SSMDL-0086 is a novel methoxychalcone inhibitor of aggressive prostate cancer. SSMDL-0086 possesses great potential for treatment of aggressive prostate cancer as a single agent and its effects synergize in combination with current standard of care treatments for CRPC, Abiraterone and Enzalutamide, making it a candidate for improvement of CRPC therapy.

Am J Clin Exp Urol 2018;6(Supplementary Issue 1):1-138
www.ajceu.us /ISSN:2330-1910/2018 SBUR Annual Meeting

PS2-69 Peroxisome Proliferator-Activated Receptor Gamma Inhibition as a Novel Therapeutic and Chemoprevention Strategy in Prostate Cancer

J Jones[1], C Elix[1], M Salgia[1], M Otto-Duessel[1], C Yoo[1], B Copeland[1], B Tew[2], D Ann[1]

[1]City of Hope; [2]University of Southern California

Introduction & Objective: Prostate Cancer (PC) remains a leading cause of cancer mortality and the most successful chemopreventative and treatment strategies for PC come from targeting the androgen receptor (AR). Although AR plays a key role, it is likely that other molecular pathways also contribute to PC, making it essential to identify and develop drugs against novel targets for PC treatment. New studies have identified PPARγ, a nuclear receptor that regulates fatty acid metabolism, as a novel target in PC, and suggest that inhibitors of PPARγ could be used to treat existing disease and to prevent PC development. We hypothesize that PPARγ acts through AR-dependent and -independent mechanisms to control PC development and growth and that PPARγ inhibition is a viable PC treatment strategy. Methods: Growth curves, MTT assays, immunofluorescence, Western blot, and flow cytometry were used to determine the effects of PPARG antagonists and siRNA on PC cell viability, while luciferase and RT-qPCR assays were used to determine the effects on AR and PPARG expression and activity. Nude mice were used to determine the effect of PPARG antagonists on human PC xenograft growth and a syngeneic B6 mouse model is being used to determine if PPARG antagonism can increase response to checkpoint inhibitors and control the growth of RM9 PC tumors. Results: We found that small molecule inhibition of PPARγ decreases the growth of AR+ and AR-PC cells in vitro and that T0070907, a potent PPARγ antagonist, significantly decreased the growth of human PC xenografts in nude mice. We found that PPARγ antagonists or siRNA do not affect mitochondrial activity nor do they cause apoptosis; instead, they arrest the cell cycle. In AR+PC cells, antagonists and siRNAs reduce AR transcript and protein levels, which could also contribute to growth inhibition. AR-independent effects on growth appear to be mediated by effects on fatty acid metabolism as the specific FASN inhibitor Fasnall inhibited PC cell growth but did not have an additive effect when combined with PPARγ antagonists. Having elucidated the direct cancer cell effects of PPARγ inhibition, we next are looking at potential effects on immune response to cancers. We have shown that PPARγ inhibition increases inflammatory cytokine expression in PC cells in vitro and in vivo. Conclusions: Taken altogether, we postulate that treatment with PPARγ antagonists affects immune cell recruitment to PCs and can potentially boost the effectiveness of immunotherapies when used in adjuvant. Our studies will help determine the role of PPARγ in PC progression, and whether PPARγ inhibition is an effective strategy for PC treatment.

PS2-70 Can Urinary Biomarkers from Epigenetic Alterations in Non-Tumor Prostate Cells Detect the Presence of Prostate Cancer?

TA Khemees, A Schultz, GO Allen, J Gawdzik, KA Richards, TM Downs, E Abel, B Yang, DF Jarrard

University of Wisconsin-Madison

Introduction & Objective: Prostate cancer (PCa) development and progression are driven by the interplay of genetic and epigenetic changes that include DNA methylation. Detection of PCa cells in urine has been hindered by their infrequent shedding. Nontumor prostate cells are found more frequently (14-20%) in the urine, and contain DNA methylation alterations associated with a cancer field defect. In the current study, we analyzed a series of DNA methylation markers to determine if they could predict the presence of PCa using urine samples of patients undergoing biopsy for PCa screening. Methods: Following IRB approval, urine samples were collected after a prostate biopsy procedure done for patients who presented with an elevated PSA from 2012 to 2016. Ninety urine samples were collected from patients with biopsy proven PCa, and 77 urine samples were collected from patients without PCa. We purified genomic DNA using a kit from IBI Scientific (Valley Park, MO). Methylated DNA was analyzed across several regions using bisulfite treatment and pyrosequencing. EVX1, CAV1 have previously been demonstrated as representing a tissue methylation defect. PLA2G16 was evaluated in nontumor tissues from patients with and without PCa. Results: The mean patient age was 64 yo and mean PSA was 13 ng/ml. Methylation changes in urine cell pellets showed significantly increased methylation at CpG shores associated with EVX1, CAV1 and PLA2G16 genes from patients who had PCa compared to those without PCa. EXV1 methylation to detect PCa revealed a AUC of 0.75 (OR 1.09; 95% CI 0.94-1.25), CAV1 an AUC of 0.75 (OR 1.07; 95% CI 0.96-1.2) and PLA2G16 0.75 (OR 1.19; 95% CI 1.02-1.38). PSA AUC was 0.61. The combined three-marker assay performed better than PSA with AUC of 0.76 vs PSA AUC of 0.61 ($P = 0.01$) (Figure 1). PLA2G16 gene showed significant hypermethylation in the histologically normal prostate tissues/biopsies from patients with PCa compared to those without ($p<0.05$). Decreased PLA2G16 gene expression was detected in normal prostate tissues associated with PCa, and it negatively correlated to methylation. Conclusions: The methylation and gene status of PLA2G16, EVX1 and CAV1 distinguishes between tumor associated and non-tumor associated prostate tissues marking a field of susceptibility associated with the development of PCa. Genes methylated in a field defect in normal prostate cells can be detected in urine and may be utilized as a novel biomarker approach to detect PCa.