

Poster Session 3: Basic Science

June 27, 2016 0800-0930

MP-03.01

The interaction of urinary components with biomaterials in the urinary tract: Ureteral stent discolouration

Chew, Ben H.¹; Chan, Justin¹; Hirayama, Takahiro²; Iwamura, Masatsugu²; Branda, Neil³; Lange, Dirk¹

¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada;

²Urology, Kitasato University, Kitasato, Minami, Sagami-hara, Kanagawa, Japan; ³Chemistry, Simon Fraser University, Burnaby, BC, Canada

Introduction and Objectives: Ureteral stents are fraught with complications, including infection, encrustation, and significant patient discomfort. Recent studies from Japan suggested that the discolouration of indwelling stents changes their surface characteristics in a way that promotes stent-associated complications. Occasional stent discolouration has been observed, however, not much is known about how it happens and whether it changes the surface characteristic of stents. This work studies mechanisms that lead to stent discoloration and characterizes changes in stent surface characteristics.

Methods: 20 indwelling Polaris Ultra and Percuflex Plus stents (made of the same polymer material) with varying degrees of discolouration were collected from patients in both Japan and Canada. Surface characterization was conducted using scanning electron microscopy, Fourier transmission infrared spectroscopy, and secondary ion mass spectrometry. Conditioning film components from each stent were analyzed using quadrupole time of flight mass spectrometry. Degrees of encrustation of stents removed from patients and artificially discoloured stent pieces were investigated using an established stent encrustation model.

Results: Stent discolouration was caused by a reaction between sulfur-containing compounds in urine and the bismuth subcarbonate in the stents used to make them radiopaque. It was determined that a reducing agent is also required, making this a reaction involving multiple urinary compounds. Furthermore, no significant changes in the bulk material of discoloured stents were observed compared to non-discoloured stents. No evidence of unusual crystal deposition on the surface of discoloured stents was found.

Conclusions: Ureteral stent discolouration is triggered by a reaction between bismuth subcarbonate within the stent material and sulfur containing urinary components and does not result in significant changes in the bulk material of indwelling stents.

MP-03.02

Pre-clinical rationale for combination PI3K and epigenetic pathway inhibition in advanced prostate cancer

Toren, Paul J.^{1,2}; Allman, Jared¹; Kim, Soojin¹; Zoubeidi, Amina¹

¹Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Department of Surgery, Université Laval, Quebec, QC, Canada

Introduction and Objectives: The PI3K/Akt pathway is frequently activated in aggressive and resistant prostate cancer. Here, we detail our pre-clinical evaluation of AZD8186, a novel β and δ selective PI3K small-molecule inhibitor. Further, we investigate how increased transcription of the Myc oncogene may represent a targetable mechanism of resistance to monotherapy PI3K/Akt inhibition through targeting of the epigenetic reader BET proteins with JQ1.

Methods: Human prostate cancer cell lines LNCaP and 22RV1 were tested for sensitivity to AZD8186 in vitro. Caspase-3 activity and flow cytometry were used to assess apoptosis. Western blotting and RT-qPCR were used to measure AR and myc pathway genes and protein expression. Castrate

resistant LNCaP xenografts were treated with increasing doses of AZD8186.

Results: In vitro, LNCaP cells were sensitive to AZD8186, with decreases in cell proliferation and increases in apoptosis. In vivo, there was a dose-dependent decrease in tumour growth velocity with AZD8186 with on-target decreases in pAkt observed in xenograft tumours. Further, increases in Myc protein and mRNA levels were seen in AZD8186-treated xenografts compared to control. Downstream increases in EGFR and IGF-IR mRNA transcripts induced by AZD8186 were also seen in vitro and in vivo. Addition of the BET inhibitor JQ1 decreased the increase of Myc induced by AZD8186 and also partially abrogated the increases in EGFR and IGF-IR. Greater suppression of PSA expression was seen with the combination of AZD8186 and JQ1 compared to AZD8186 and enzalutamide. Similar results were seen in the 22RV1 cell line.

Conclusions: Inhibition of PI3K with AZD8186 inhibits growth of PTEN-negative LNCaP cells. However, feedback activation of Myc and AR pathways occur. Our results suggest that combined BET inhibition and PI3K inhibition is a rational combination strategy and further studies are warranted for prostate cancers with an activated PI3K/Akt pathway.

MP-03.03

Targeting Gli1 and Gli2 in the sonic hedgehog pathway inhibits bladder cancer progression

Raven, Peter A.^{1,2}; Frees, Sebastian²; Zhou, Betty²; Chavez-Munoz, Claudia²; Cox, Michael²; So, Alan I.^{1,2}

¹Urology, University of British Columbia, Vancouver, BC, Canada;

²Vancouver Prostate Centre, Vancouver, BC, Canada

Introduction and Objectives: The sonic hedgehog (SHH) signaling pathway regulates a variety of embryonic developmental processes and abnormal activation of the SHH pathway may lead to an increase in cell survival and metastasis in different cancers, including bladder cancer (BC). Glioma-associated proteins (Gli), a family of transcription factors, have been inter-related with the expression levels of the SHH pathway, suggesting Gli activity is a direct indicator of SHH pathway activity. Here we hypothesize that blockage of SHH pathway may be an efficacious way of stopping BC progression.

Methods: SHH pathway protein expression was assessed by immunohistochemical staining in our human BC tissue array of superficial, invasive, and lymph node-metastasized transitional cell carcinoma. In addition, a panel of BC cell lines was assessed for SHH pathway activation, function, and responsiveness. Proliferation and cell death were measured in response to small-molecule pathway inhibitors and antisense oligonucleotides (ASO) targeted to Gli 1 and 2. These results were confirmed in a novel in-vivo murine non-muscle invasive BC model.

Results: An array of human BC tissues showed SHH pathway activation through a significant upregulation of the SHH pathway proteins PTCH, SMO, and Gli1, and Gli2 in invasive BC when compared to non-invasive cancer and normal bladder tissue. Similarly, in a panel of urothelial carcinoma cell lines, Gli1 and Gli2 proteins were highly expressed in more aggressive cell lines (UM-UC3, T24) compared to less aggressive ones (RT4, SV-HUC). Inhibition of Gli1 and Gli2 signalling with ASO reduced tumour cell proliferation, migration, and invasion in vitro in the more aggressive cell lines. In vivo, Gli2 ASO significantly abrogated tumour growth when compared to control tumours.

Conclusions: Gli 1 and Gli2 ASO may be a potential new therapy to stop progression and invasion in bladder cancer improving the survival of bladder cancer patients.

MP-03.04

STAT1 inhibition restored chemotherapy sensitivity in chemoresistant bladder cancer

Hayashi, Tetsutaro^{1,2}; Seiler, Roland¹; Bell, Robert H.¹; Ettinger, Susan¹; Wang, Kendric¹; Goriki, Akihiro^{1,2}; Oo, Htoo¹; Awrey, Shannon¹; Altamirano-Dimas, Manuel¹; Matsubara, Akio²; Collins, Colin¹; Black, Peter C.¹

¹The Vancouver Prostate Centre and Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Department of Urology, Hiroshima University, Hiroshima, Japan

Introduction and Objectives: Cisplatin-based combination chemotherapy is the standard treatment for patients with advanced bladder cancer (BC). Although responses are observed in up to 60% of patients, these are rarely durable. Therefore, novel targets are essential for designing new treatments. Here, we studied to identify a genomic signature associated with resistance to cisplatin (CDDP) and gemcitabine (GEM), and explore novel therapeutic targets in BC.

Methods: Three chemosensitive BC cell lines (RT112, UM-UC3, UM-UC13) were treated serially with increasing concentrations of CDDP up to 10 μ M, 3.3 μ M, 3.3 μ M, or GEM up to 10 μ M, 10 μ M, 0.1 μ M, respectively in order to establish resistance (CDDP-R and GEM-R). Gene expression microarray analysis of the resistant cell lines compared to the sensitive parental lines was conducted.

Results: We ranked the top differentially expressed genes between the parental chemosensitive cell lines and the derived chemoresistant cell lines. Genes related to interferon (IFN) signaling made up 10 of the top 15 up-regulated genes. Pathway analysis revealed that IFN/STAT1 signaling is activated in chemoresistant cell lines. Knockdown of STAT1 in these chemoresistant cell lines decreased expression of IFITM1, IFI27, IFI44L, IFI6, IFITM1, and IFIT1, which are downstream target genes of IFN/STAT1 signaling. Next, we studied IFN/STAT1 effect on proliferation in chemoresistant cells. STAT1 knockdown without CDDP/GEM treatment increased cell growth by cell cycle progression. This is accompanied with increased SKP2 and decreased p27. However, STAT1 knockdown with CDDP/GEM treatment decreased cell growth and increased apoptosis, suggesting that STAT1 silencing combined with chemotherapy restored chemotherapy sensitivity.

Conclusions: IFN/STAT1 signaling is activated in chemoresistant BC cell lines and its inhibition can resensitize to chemotherapy. These genes warrant further study for their potential for rational cotargeting in the context of chemoresistant BC.

MP-03.05

Development of a bio-layer interferometry-based assay for studying insulin-like growth factor (IGF)/IGF-binding protein interactions

Alsabban, Abdulrahman^{1,2}; Cox, Michael E.¹; Ghaffari, Mazhar¹

¹The Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada; ²Department of Urology, King Abdulaziz University, Jeddah, Saudi Arabia

Introduction and Objectives: The IGF axis is comprised of two hormone growth factors (IGF-I and IGF-II), two cell surface receptors (IGF-IR and IGF-IIR), six high-affinity binding proteins (IGFBP-1 to -6), and several other factors that regulate IGF actions in essentially all tissues. Activation of IGF-IR promotes cell cycle progression and inhibits apoptosis, and has angiogenic and metastatic activities in various cancers. These effects depend on the bioavailability of the IGFs, which is regulated by IGFBPs. Increased IGFBP-2 and IGFBP-5 expression is observed in castration-resistant prostate cancer. We hypothesize that blocking the IGF binding site of IGFBPs can affect the bioavailability of IGFs to target tissues, and thus can be used for treatment of various IGF-responsive diseases, including prostate cancer.

Methods: We have developed a bio-layer interferometry (BLI)-based in vitro assay to study IGFBP-5/IGF-I binding kinetics. This will allow for high throughput screening of factors that can affect this intermolecular interaction for use as a platform to screen for compounds targeting this and other IGFBPs.

Results: The assay uses biotinylated IGF-I bound to streptavidin-coated biosensors, which in optimized buffer conditions, bind recombinant IGFBP-5 with high affinity (dissociation rate constant (Kdis) of 1.2×10^{-4} (1/s)). We found that recombinant IGF-I can efficiently disrupted this interaction (Kdis 1.5×10^{-3} 1/s), while the amino-terminal IGF-I mutant, E3R, exhibits an intermediate competitive activity (Kdis 5.51×10^{-4} 1/s) and insulin exhibits a lower competitive activity (Kdis 5.3×10^{-4} 1/s).

Conclusions: Results from the development of this BLI-based IGFBP-5/IGF-I interaction assay demonstrates the capacity to differentiate relative competitive activity of compounds that target the high affinity IGF-I binding site of IGFBPs. This assay will serve as a platform to screen for factors that might be developed as rationale therapeutics to disrupt sequestration of IGF-I by IGFBPs.

MP-03.06

Targeting heat-shock protein 27 inhibits tumour growth and enhances sensitivity to sorafenib treatment in human renal cancer cells

Frees, Sebastian¹; Chavez-Munoz, Claudia¹; Zhou, Betty¹; Raven, Peter A.¹; Fazli, Ladan¹; Chi, Kim¹; Lawson, Keith²; Finelli, Antonio²; Gleave, Martin E.¹; So, Alan I.¹

¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Department of Surgical Oncology, University of Toronto, Toronto, ON, Canada

Introduction and Objectives: Novel targeted therapies, tyrosine kinase inhibitors (TKI), have led to significant improvement in outcome of patients with metastatic renal cell carcinoma (mCCRCC). However, eventual drug resistance to TKI universally occurs due to upregulation of non-VEGF genes that allows for tumour progression. Heat shock protein 27 (Hsp27), a chaperone molecule involved in cell stress response, has been shown to be involved in treatment resistance in many malignancies. In this study, we assess the expression of Hsp27 in CCRCC and the effects of knocking down Hsp27 in CCRCC with or without TKIs.

Methods: Immunohistochemistry of a tissue microarray from 15 patients treated with sorafenib was stained for expression of Hsp27. Several renal cell lines (CAKI-1, CAKI-2, 7680 and ACHN) were treated with sorafenib and analysed for Hsp27 expression. After specific knockdown of Hsp27 with siRNA and antisense oligonucleotide (OGX-427) cell viability was measured using Presto Blue.

Results: Staining of the tissue microarray revealed a significant upregulation in the protein expression of Hsp27 after treatment compared to a historical untreated cohort. In human renal cancer cell lines, we were able to observe similar results. Targeting Hsp27 with siRNA and antisense oligonucleotide revealed a decrease in cell viability in combination with sorafenib treatment.

Conclusion: Treatment of renal cell carcinoma with sorafenib leads to an upregulation of the Hsp27 in-vivo and in vitro. Targeting Hsp27 in combination with sorafenib treatment might lead to better response rates in the treatment of advanced renal cancer.

MP-03.07

Orthotopic sunitinib-resistant renal cell carcinoma xenograft mouse model

Frees, Sebastian¹; Moskalev, Igor¹; Raven, Peter A.¹; Zhou, Betty¹; Chavez-Munoz, Claudia¹; Black, Peter C.¹; So, Alan I.¹

¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Introduction and Objectives: Current first-line treatments of renal cell carcinoma (RCC) are tyrosine kinase inhibitors (TKI), such as sunitinib (SU), which target angiogenesis through VEGFR pathway. After an initial response rate of 30-40%, it has been shown an acquired resistance after 6-11 months despite continuous treatment. Most commonly used TKI-resistant animal models are created in vitro, by exposing kidney cancer cells to increasing doses of the drug. To account for the fact that angiogenesis is crucial in the development and treatment of RCC, we developed an in vivo SU-resistant mouse model.

Methods: Luciferin expressing-CAKI-1 human renal cancer cells were injected under the renal capsule of athymic nude mice using ultrasound guidance. After initial tumour growth, mice were treated with SU at 40 mg/kg, which was increased sequentially (60, 80, and 100 mg/kg) at each tumour passage. Tumours were monitored twice weekly by ultrasound and bioluminescence (IVIS). Tumours exceeding 200 mm³ by ultrasound or 10¹⁰ photons/sec by IVIS were considered endpoints. For each passage, tumours were harvested, dissociated, and injected in single-cell suspension (5 x 10⁵ cells) into the following batch of mice, considering this a cycle.

Results: Initial treatment of SU at 40 mg/kg showed decrease in tumour size of about 30% using both methods. After 14 days of treatment, tumours started to regrow, despite continuous treatment. In the second cycle, tumours did respond to a subsequent treatment of 40 mg/kg, proving resistance. Even after increasing the dose of SU in the following cycles to 60 and 80 mg/kg, respectively, there was no response observed. 100 mg/kg had to be terminated due to severe side effects in the animals.

Conclusions: This study describes the first orthotopic xenograft RCC SU-resistant mouse model created in angiogenic in vivo environment, opening the possibility to study not only the mechanism of SU resistance, but also to find alternative therapies to overcome SU resistance.

MP-03.08

Towards understanding the interplay between androgen receptors, androgens, and IKK ϵ in the tumour growth control of prostate cancer cells

Huon, Yannick^{1,2,3}; Péant, Benjamin^{1,2,3}; Leclerc-Desaulniers, Kim^{1,2,3}; Delvoe, Nathalie^{1,2,3}; Mes-Masson, Anne-Marie^{2,3,4}; Saad, Fred^{2,3,5}

¹Université de Montréal, Montreal, QC, Canada; ²L'Institut de Cardiologie de Montréal, Montreal, QC, Canada; ³Centre de recherche, Centre hospitalier de l'Université de Montréal, Montreal, QC, Canada; ⁴Department of Medicine, Université de Montréal, Montreal, QC, Canada; ⁵Department of Surgery, Université de Montréal, Montreal, QC, Canada

Introduction and Objectives: Following androgen-depletion therapy, prostate cancer (PCa) can progress from hormone-sensitive (HS) to a castrate-resistant state. We have demonstrated the specific high expression of IKK ϵ in advanced stages of PCa. We are performing experiments to determine whether androgen and hormone dependence influence IKK ϵ function.

Methods: We used HS 22Rv1 PCa cells that overexpress IKK ϵ in an inducible manner. We injected these cells into SCID mice and compared 72 castrated and 72 uncastrated animals with or without IKK ϵ induction. In order to study the implication of the androgen receptor (AR) itself, we studied the effect of AR knockdown in relation to differential IKK ϵ expression in 22Rv1 cells. Cell proliferation and clonogenic capability were performed.

Results: Growth of xenografts is decreased when IKK ϵ is expressed, and thus mice survival is increased (p=0.0002). Interestingly, this effect is weakened upon castration, with a reduce effect on mice survival (p=0.0017). In vitro experiments don't reveal any effect of concomitant IKK ϵ expression and AR knockdown on proliferation and clonogenic ability. In order to more faithfully mimic in vivo castration, we are repeating these results through the chemical inhibition of the AR.

Conclusions: Our results demonstrate that the presence of androgen concomitant with IKK ϵ expression had a positive effect on the survival of mice. Unexpectedly, this benefit is reduced upon castration, suggesting that the positive tumour growth activity of IKK ϵ is contextual and influenced by androgen. On the other hand, in vitro experiments fail to reveal any differences in oncogenic capability of cells upon IKK ϵ and AR knockdown, although AR signalling inhibition may have a different effect. Alternatively, immune regulatory factors, such as NK cells and macrophages, may contribute to the results seen in xenografts. To address this, we are studying the presence of infiltrating immune cells in our xenografts by immunohistochemistry.

MP-03.09

CCN3 is a prognostic biomarker and functional mediator of prostate cancer bone metastasis

Danker, Matthew R.¹; Ouellet, Veronique²; Perkins, Dru¹; Annis, Matthew G.¹; Schmitt, Estelle²; Dong, Zhifeng¹; Mes-Masson, Anne-Marie²; Saad, Fred²; Siegel, Peter¹

¹Goodman Cancer Research Centre, McGill University, Montreal, QC, Canada; ²Centre de recherche and Urology, Centre hospitalier de l'Université de Montréal, Montreal, QC, Canada

Introduction and Objectives: CCN3 has been implicated in promoting the formation of osteolytic prostate cancer (PCa) bone metastases. The C-terminal domain (CT) of CCN3 binds growth factors, heparin sulfate proteoglycans, activates Notch signaling, and promotes dimerization of CCN family members. We hypothesize that the CT domain is required to promote osteolytic PCa bone metastasis and that CCN3 represents a prognostic biomarker in primary PCa tumours to predict recurrence to bone.

Methods: LNCaP C4-2 cells were first tagged with a ZSGreen-Luciferase reporter construct. Tagged LNCaP C4-2 cells were then transduced with control, CCN3 wild-type (WT) or CCN3 CT domain mutant (Δ CT) retrovirus. The cell populations were subjected to intra-cardiac injection in immune-compromised mice. Animals were imaged bi-weekly for bioluminescence (IVIS 1000) and at endpoint by live animal micro-computed tomography scan. To evaluate the expression of CCN3 in human prostate cancer tissue, we accessed the Canadian Prostate Cancer Biomarker Network tissue microarray test series composed of adjacent benign and tumour cores from 250 radical prostatectomy specimens. Immunohistochemistry was performed followed by a semi-automated image analysis using VisiomorphDP software.

Results: Intra-cardiac injection revealed bone metastases exclusively in the mandibles, with incidence of 60% in mice injected with vector control cells, 90% with CCN3WT-expressing cells, and 60% with CCN3 Δ CT mutant-expressing cells. Ex vivo micro-CT scans have revealed a significant decrease in bone mineral density in the mandibles of mice injected with CCN3WT expressing cells compared to vector control or CCN3 Δ CT mutant cells. Meanwhile, immunofluorescence staining with anti-CCN3 antibody of primary prostate tumours was significantly associated with recurrence to bone.

Conclusions: We conclude that CCN3 promotes the formation of bone metastases in a CT-domain fashion and is a valid biomarker for predicting PCa recurrence to bone.

MP-03.10

Biomarker validation by immunofluorescence: A novel approach to follow prostate cancer progression

Clairefond, Sylvie¹; Ouellet, Veronique¹; Péant, Benjamin¹; Barrès, Veronique¹; Frago, Gabriela¹; Mes-Masson, Anne-Marie^{1,2}; Saad, Fred^{1,3}

¹Centre de recherche, Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada; ²Département de médecine, Université de Montréal, Montreal, QC, Canada; ³Département de chirurgie, Université de Montréal, Montreal, QC, Canada

Introduction and Objectives: Prostate cancer (PCa) is the most frequently diagnosed form of cancer and the third leading cause of cancer-related mortality in Canada. As PCa patient management varies widely, there is a need to find putative biomarkers for clinical use to identify patients with poor prognosis, where treatment options would be impacted. In this context, it is necessary to validate biomarkers for stratification of PCa. Our aim is to evaluate several biomarkers by immunofluorescence (IF) and correlate their expression with patient clinical data in order to identify the best prognostic biomarkers, either alone or in combination.

Methods: Biomarker antibodies (PUMA, NOXA, EGFR, and ErbB3) were verified for specificity using western blots and optimization tissue microarrays, which contain cell lines and xenografts created using human PCa cell lines injected in mice. Quantification of potential biomarker expression was performed on radical prostatectomy samples (286 patients) arrayed as 0.6 mm cores in duplicate. The analysis of biomarker expression was semi-automated using the VisiomorphDP software. Followed-up data (90.5 months) are available for statistical analyses.

Results: Analysis has been completed for PUMA. As expected from previous results, we found no significant association with the prognosis of PCA patients. The predictive PUMA/NOXA combination that has previously been shown to be prognostic remains under investigation. Two commercial NOXA antibodies were non-specific and a third is in study. The EGFR antibody has been validated and the staining with epithelial markers (CK8 and CK18) and the basal cell marker (p63) is being optimized. The biomarker ErbB3 has been validated and the analyses of expression levels are ongoing.

Conclusions: Our multimarker approach allows us to quantitatively compare the expression of individual markers within the tumour micro-environment, as well as at the sub-cellular level, providing rich datasets for biomarker selection and validation.

MP-03.11

Development of a urine-based inflammatory test for prostate cancer

Gevariya, Nikunj^{1,2}; Robitaille, Karine^{1,2}; Bergeron, Alain^{1,2}; Ben-Zvi, Tal^{1,2}; Allard, Marc-Andre^{1,2}; Fradet, Yves^{1,2}; Bisson, Nicolas¹; Fradet, Vincent^{1,2}
¹Cancer Research Centre, Université Laval, Quebec, QC, Canada; ²Laboratoire d'uro-oncologie, CHU de Québec Research Centre, Quebec, QC, Canada

Introduction and Objectives: Chronic inflammation is a potential causal factor of prostate cancer (PCa). However, a non-invasive test assessing prostatic inflammation is inexistent. The aim of this project is to develop a non-invasive urine-based test to measure prostate inflammation and stratify risk of PCa. Specifically, here, we sought to identify proteins, inflammatory-related in particular, only present in post-digital rectal exam (DRE) urine.

Methods: We collected urine samples before and after DRE in 10 consenting men presenting for prostate needle biopsy. A total of 22 urine samples were passed through shotgun mass spectrometry protocols. First, we optimized conditions in order to specifically analyze secreted proteins. Then, we compared global secreted protein expression of pre- and post-DRE urine samples (total 18 samples) of nine patients (three of each: Gleason (G)8, G6, and without cancer groups) to discover cancer-specific biomarkers of inflammation. Protein abundance were compared using the Welch t-test with p values <0.1 declared significant.

Results: The condition optimization process defined our protocol to use of 5 µg of protein for a two-hour run duration from undepleted urine samples. We identified 69 proteins greater than two-fold more abundant in post-DRE urine as compared with pre-DRE urine. We identified 50 proteins found only in PCa versus patients without cancer. Of these, four proteins were involved in inflammatory pathways, including known cancer identifiers CD-74 and CD-276, the latter also being an immune-checkpoint. We identified 24 proteins differentially found in G8 versus G6 patients.

Conclusions: We identified candidate protein signatures of PCa and specifically of high-grade PCa. These must be validated in larger cohorts. Only few of these proteins are implicated in inflammatory pathways making shotgun proteomics not relevant to identify them. Targeted proteomics will be needed to measure inflammatory-related cytokines.

MP-03.12

A comparative analysis of uropathogen prevalence and susceptibility profiles in a prospectively managed HIV-positive cohort in Dublin, Ireland

O'Kelly, Fardod¹; Landon, Dara¹; Tinago, Willard^{2,3}; Macken, Alan²; Daly, Padraig¹; O'Malley, Kiaran^{1,2}; Mallon, Patrick²

¹Department of Urological and Reconstructive Surgery, Mater Misericordiae University Hospital, Dublin, Ireland; ²Division of Infectious Diseases, Mater Misericordiae University Hospital, Dublin, Ireland; ³Department of Biostatistics, University of Zimbabwe, Harare, Zimbabwe

Introduction and Objectives: Multiple factors impact resistance profiles of uropathogens. The rates vary greatly between countries, where resistance rates to certain antibiotics have reached critical levels. There is no data on the prevalence of urinary tract infections (UTIs) or resistance in HIV-positive populations within the U.K. or Ireland.

Methods: The institutional prospective database was analyzed, in addition to clinical data for 539 HIV-positive patients. Prevalence, pathogenicity, and clinicodemographic characteristics were collated. Statistical analysis was carried out with SPSS (IBM).

Results: The cohort comprised of 539 patients, 314 (58.26%) of whom were male, with females occupying significantly more of the culture-positive MSU (+MSU) group (76.7%) (p<0.001). The median age (at the time of anti-retroviral treatment (ART) initiation) was 40 years, and there was no significant age difference between groups. Controlling for other factors, a positive MSU was statistically more likely in Caucasians (adjusted odds ratio (OR) 3.54, 95% CI 1.31; 9.57), females (OR 11.01, 95% CI 4.67; 25.94), with advancing age (OR 1.75/10-year increment, 95% CI 1.10; 2.77), and with intravenous /injecting drug use (IVDU) (OR 5.96, 95% CI 1.54; 23.14). There was no significant difference observed in the +MSU rate, and the incidence in the general population over this period was 7.037%. *E. coli* was the most common causative organism present in all culture specimens and *Klebsiella pneumoniae* co-infection in 0.23%. Repeat infection was seen in 11.6% individuals, all of whom were co-infected with HIV/hepatitis C.

Conclusions: This study represents the largest of its kind in Europe. Females in general are at increased risk regardless of ethnicity. Caucasians are more at increase than non-Caucasians. The hepatitis C and treatment effects are likely driven by a greater proportion of immunocompromised patients having hepatitis C and being on protease inhibitors rather than either the treatment or hepatitis C infection contributing directly.

1. Eccles-Radtke C, Rector TS, Cutting A, et al. Urinary tract infections in male veterans with human immunodeficiency virus. *Open Forum Infect Dis* 2014;1:ofu100. <http://dx.doi.org/10.1093/ofid/ofu100>
2. Padmavathy K, Padma K, Rajasekaran S. Extended-spectrum β-lactamase/AmpC-producing uropathogenic *Escherichia coli* from HIV patients: Do they have a low virulence score? *J Med Microbiol* 2013;62:345-51. <http://dx.doi.org/10.1099/jmm.0.050013-0>
3. Breyer BN, Van den Eeden SK, Horberg MA, et al. HIV status is an independent risk factor for reporting lower urinary tract symptoms. *J Urol* 2011;185:1710-5. <http://dx.doi.org/10.1016/j.juro.2010.12.043>

MP-03.13

Angiotensin I-converting enzyme gene polymorphism in Iraqi patients with chronic renal failure

Al-Jebouri, Mohemid¹

¹Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

Introduction and Objectives: The renin-angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and their interaction. In such situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin-converting enzyme-1 (ACE-1) is an important component of RAS, which determines the vasoactive peptide angiotensin-II.

Methods: In the present study, we have investigated 100 end-stage renal disease (ESRD) patients and 50 normal healthy controls to deduce the association between ACE gene polymorphism and ESRD. A total of 50 normal healthy controls were also genotyped for ACE I/D polymorphism. The criterion of defining control sample as normal was totally based on the absence of any kidney disease determined from the serum creatinine level. Genotyping of ACE I/D was assayed by polymerase chain reaction (PCR) based DNA amplification using specific flanking primers.

Results: Of 100 patients with chronic renal failure with different causes, the DD genotype was 56%, II genotype 13%, ID was 28% and negative results was 3%. The control group results was DD 26%, II 26%, ID 22%, and negative results was 26%.

Conclusion: The results in this study of patients with chronic renal disease indicate that presence of the D allele in the ACE genotype may be of particular importance as a predictor of high rate of progression to ESRD.

MP-03.14**Isolated hypoxia-induced inflammation and fibrotic pathways in human bladder smooth muscle cells**Wiafe, Bridget¹; Adesida, Adetola¹; Churchill, Thomas¹; Metcalfe, Peter¹¹Surgery, University of Alberta, Edmonton, AB, Canada

Introduction and Objectives: Several studies have implicated hypoxia in the etiology of partial bladder outlet obstruction (pBOO). However, the isolated effects of hypoxia on bladder cells as far as the initiation and progression of pBOO is concerned are not yet known. Therefore, we aimed to characterize the progressive effects of pure hypoxia on bladder smooth muscle cells.

Methods: Sub-confluent normal human bladder smooth muscle cells (hbSMC) were cultured in 3% O₂ tension for two, 24, 48, and 72 hours. RNA was extracted for gene expression analysis using reverse transcription polymerase chain reaction (RT PCR) and protein produced in culture medium was analysed with enzyme-linked immunosorbent assay (ELISA).

Results: Transcription of HIF 1 and 2 were transiently induced after two hours of hypoxia ($p < 0.05$) whereas HIF 3 was upregulated after 72 hours ($p < 0.005$). VEGF mRNA increased significantly after 24 and 72 hours ($p < 0.005$). The inflammatory cytokines; TGF β 1 (protein and mRNA), IL 1 β , IL6, and TNF α (mRNA) demonstrated a time-dependent increased expression. Furthermore, the anti-inflammatory cytokine, IL-10, was downregulated after 72 hours ($p < 0.05$). Evidence of myofibroblast activation and EMT included increased α SMA, vimentin, and desmin. Evidence of pro-fibrotic changes included increases in CTGF, SMAD 2 and 3, as well as collagens 1, 2, 3, 4, fibronectin, aggrecan and TIMP 1 transcripts ($p < 0.05$). Total collagen proteins also increased time-dependently ($p > 0.05$).

Conclusions: Together, these data show that the exposure of hbSMC to low oxygen tension results in intense hypoxic cascade, including inflammation, myofibroblast activation, pro-fibrotic changes, and increased extracellular matrix expression. This elucidates mechanisms of hypoxia-

driven bladder deterioration after pBOO and may result in tailored in vivo experiments and, ultimately, translate into improved clinical outcomes.

MP-03.15**Erythropoietin accelerates the recovery of normal ureteral function following complete unilateral obstruction**Janssen, Claudia^{1,2}; Jäger, Wolfgang^{1,2}; Moskalev, Igor³; Fazli, Ladan³; Thüroff, Joachim²; Lange, Dirk¹¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Urology, University of Mainz, Mainz, Germany; ³Vancouver Prostate Centre, University of British Columbia, Vancouver, BC, Canada

Introduction and Objectives: Obstructive uropathy facilitates ureteral aperistalsis and ascending pyelonephritis. While physiologic smooth muscle contractions are lost in the dilated hydronephrotic ureter during unilateral ureteral obstruction (UUO), the functional recovery of the ureter following obstruction reversal remains unclear. Erythropoietin (EPO) is protective in non-hematopoietic organs and restores peristalsis in hypocontractile intestinal smooth muscle cells. Here, we investigated the role of EPO in the (patho-) physiology of ureteral peristalsis and determined its therapeutic value in obstructive uropathy.

Methods: Abdominal laparotomy and UUO were performed in female CD-1 mice for 24 hours (Group 1; n=22), 48 hours (Group 2; n=22), or 72 hours (Group 3; n=22) using a non-traumatic vascular micro clip. Expression of EPO, EPO receptor (EPOR), and β -common receptor (β CR) in obstructed and unobstructed ureters was determined via reverse transcription polymerase chain reaction (RT PCR) in group 2 animals. Ten animals per group received 200 IU EPO on four consecutive days; controls received saline. The duration from removal of the obstruction until regression of hydronephrosis was assessed by ultrasonography. Peristaltic activity was tracked microscopically pre- and post-obstruction via open laparotomy.

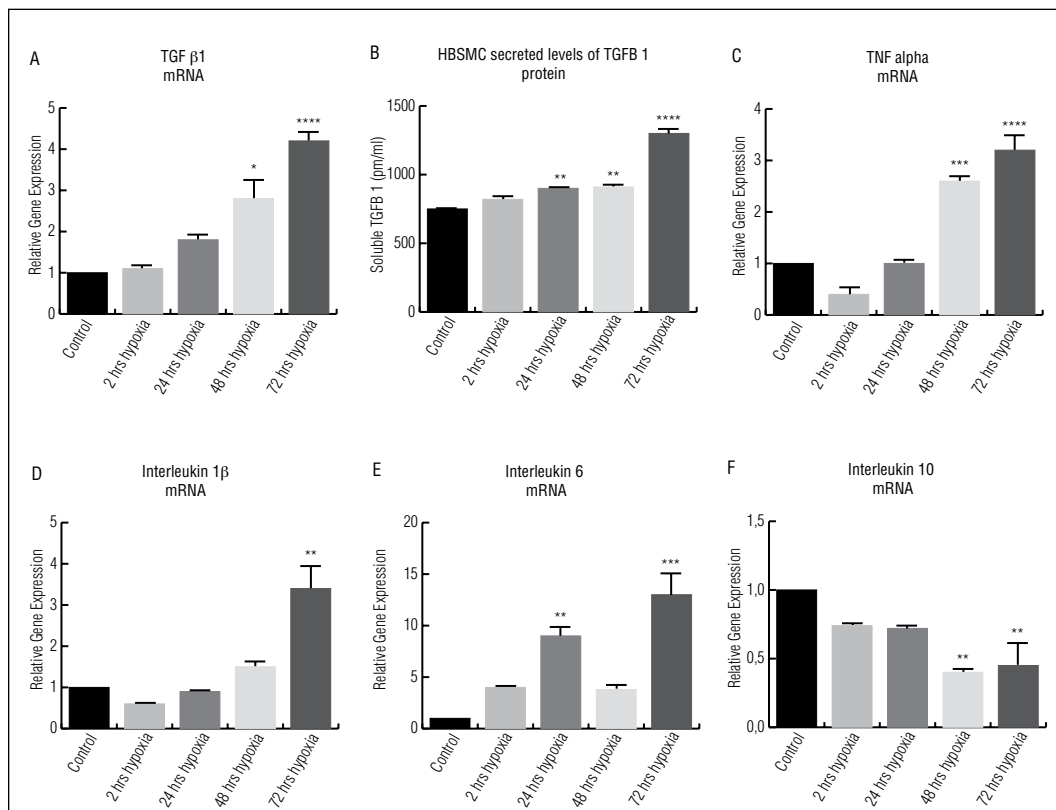


Fig. 1. MP-03.14.

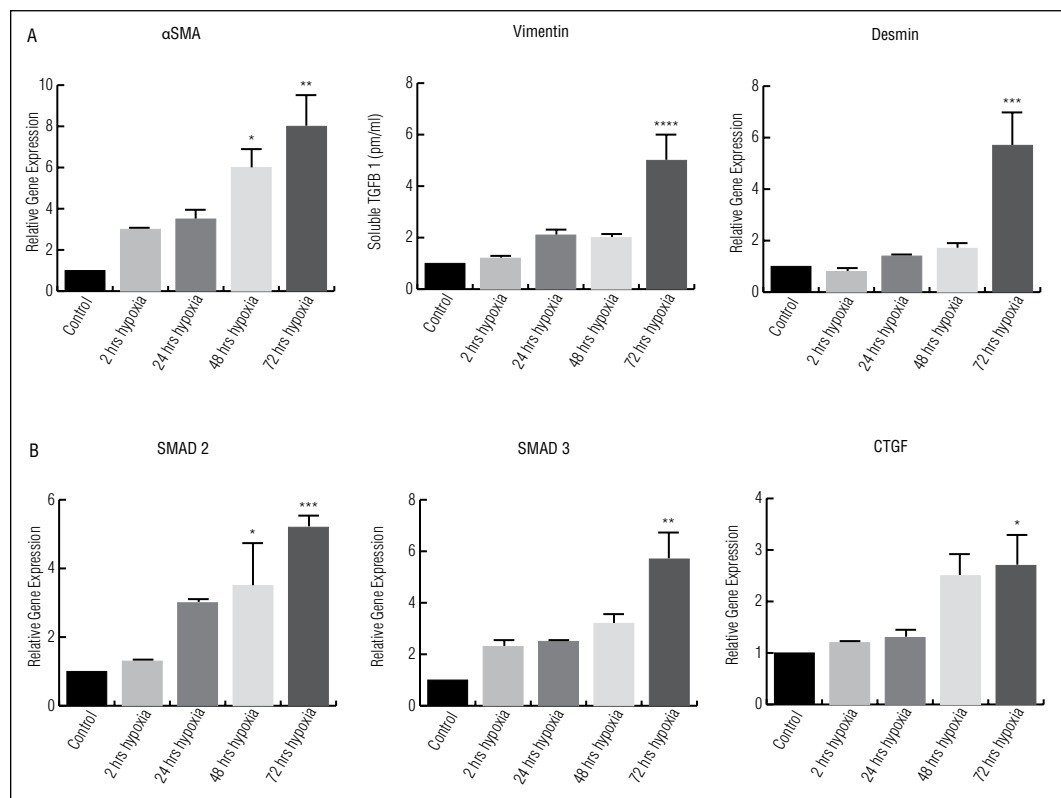


Fig. 2. MP-03.14.

Results: Both EPOR and β CR are expressed in murine ureters. Expression of endogenous EPO was remarkably up regulated in obstructed ureters compared to unobstructed ureters in untreated animals. The time until complete resolution of hydronephrosis after removal of the obstruction was found to depend on the duration of obstruction. Despite complete resolution of hydronephrosis after two, six, and eight days (Groups 1, 2, 3), the return of peristalsis was significantly delayed. EPO significantly accelerated regression of hydronephrosis and restoration of coordinated ureteral peristalsis in all groups.

Conclusions: EPO signaling may present a novel target for future therapeutic agents for obstructive uropathy and motility disturbances of the ureter.

MP-03.16

Urothelial cells express a functional succinate receptor GPR91

Velasquez-Flores, Monica¹; Cammisotto, Philippe¹; Campeau, Lyianne¹

¹Lady Davis Institute for Medical Research, Montreal, QC, Canada

Introduction and Objectives: Lower urinary tract symptoms (LUTS) are associated with the metabolic syndrome (MetS). Increased succinate production is detected in the presence of hyperglycemia and hypoxemia, as with diabetes mellitus and metabolic syndrome, which is strongly associated with overactive bladder syndrome. The aim of our study is to determine how succinate modulated bladder contractility.

Methods: Urothelial cells were isolated from Sprague-Dawley rat bladder using a collagenase IV method and grown in collagen IV-coated petri dishes. After confluency, cells were exposed to succinate then assessed by microscopy and immunoblotting analysis.

Results: Immunohistochemistry revealed that cells were confluent and express cytokeratin 17 and the receptor of succinate SUCNR1 (GPR91). Immunohistochemistry confirmed expression of GPR91. Incubation of cells with succinate (10-2 M) results in phosphorylation of Erk and c-Jun amino-terminal kinases (JNKs) JNK, with no effect on Akt-308P, Akt 473P, enos-1177P, or enos-405P. Erk phosphorylation was not observed with exposure to alpha-keto glutarate and citrate, two other intermediates of the

citric acid cycle without affinity to the GPR91 receptor, or malonate, which increases intracellular succinate. On the other hand, succinate dose-dependently decreased the concentrations of intracellular cyclic AMP stimulated by forskolin. Finally, succinate dose-dependently increased the secretion of prostaglandin E2 (PGE2).

Conclusion: A functional succinate receptor is expressed in urothelial cells. Succinate triggers activation of Erk and JNK by binding to its receptor GPR91, and increasing release of PGE2. These results suggest that succinate might be a major regulator of bladder contractility through its actions on urothelial cell signaling and secretion.

MP-03.17

A high-throughput minimally invasive murine model for the study of indwelling urinary biomaterial-associated urinary tract infections

Janssen, Claudia^{1,2}; Jäger, Wolfgang^{1,2}; Moskalev, Igor¹; Chew, Ben H.¹; Lange, Dirk¹

¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Urology, University of Mainz, Mainz, Germany

Introduction and Objectives: Indwelling urinary device-associated infections are one of the most common healthcare-associated infections frequently complicated by encrustation, causing potential blockage of the device. Preclinical research is limited by the lack of relevant high-throughput and cost effective animal models. Current models are restricted to female mice, associated with major transurethral loss of catheter materials during micturition, highly invasive and complex. We present an ultrasound-guided, minimally invasive model enabling the testing of novel antimicrobial biomaterials for indwelling urinary devices in a realistic environment.

Methods: Implantation of 4 mm catheter segments into murine bladders was performed percutaneously (n=15 males; n=5 females) or transurethrally (n=15 females) using the Seldinger technique under ultrasound guidance. Proteus mirabilis was instilled intraluminally. Catheter encrusta-

tion was monitored by ultrasound. Bacteria were quantified from urine and catheters and encrustation analyzed on Days 6 or 21.

Results: Percutaneous and transurethral catheter implantations were performed efficiently (mean time: 3.6 ± 0.8 min vs. 2.5 ± 0.5 min) in all animals. Ultrasound confirmed that 100% vs. 66%, of implanted catheters remained indwelling over the study period. Catheter encrustation developed in *P. mirabilis*-infected urine 48 hours post-instillation and its increase over time was detectable with ultrasonography. Fourier Transform Infrared Spectroscopy (FTIR) analysis of the encrustation confirmed a typical struvite spectrum. Control catheters remained sterile over 21 days.

Conclusions: Our minimally invasive and reproducible percutaneous technique is suitable to study device-associated infection in both genders. Infecting urine with *P. mirabilis* generates a preclinical model of catheter encrustation within three days. The progression of encrustation can be monitored in vivo using ultrasonography, making this image-based model suitable to assess novel antibacterial and anti-encrustation therapies.

MP-03.18

Novel antimicrobial peptide coating to prevent catheter-associated urinary tract infections

Lo, Joey¹; Yu, Kai²; Haney, Evan³; Janssen, Claudia^{1,4}; Hancock, Robert E.³; Kizhakkedathu, Jayachandran²; Lange, Dirk¹

¹Urologic Sciences, University of British Columbia, Vancouver, BC, Canada; ²Pathology and Lab Medicine, University of British Columbia, Vancouver, BC, Canada; ³Microbiology & Immunology, University of British Columbia, Vancouver, BC, Canada; ⁴Urology, University of Mainz, Mainz, Germany

Introduction and Objectives: Urinary catheters are ideal surfaces for bacterial biofilm formation, making them the main source of hospital-

acquired infections. With the increase in antibiotic resistance, there is a push for non antibiotic-based measures to prevent catheter-associated urinary tract infections (CAUTI). We are pursuing the use of novel antimicrobial catheter coatings using polymer-linked broad-spectrum antimicrobial peptides (AMPs). Here, we present the efficacy of tethered AMPs against common uropathogens in vitro and in a relevant in vivo model.

Methods: Peptides Tet20, Tet26, and E6 were linked to surfaces at varying densities using polymer brushes PDMA and PSBMA. The antimicrobial activity was determined via colony counts following exposure of uropathogens for up to 48 hours. To evaluate the in vivo efficacy, CAUTI models were developed in mice and rats using 5 mm and 10 mm catheter segments respectively, introduced transurethral or transcutaneously via ultrasound guidance, followed by transurethral inoculation using 10^5 *S. aureus* or *P. mirabilis*.

Results: E6-PSBMA and E6-PDMA were shown to be the most effective peptide-brush combinations decreasing the bacterial load up to 10^8 -fold. Tet20-PSBMA and Tet20-PDMA were less effective at up to 100-fold less bacterial load than controls, while all combinations with Tet26 were ineffective. Transcutaneous ultrasound-guided introduction of catheter pieces was more efficient in mice than rats, in which the transurethral method was more successful. Using the transurethral model, coated catheter pieces were found to contain significantly less bacteria compared to uncoated controls.

Conclusions: Based on our in vitro data, the AMP E6 tethered to surfaces using polymer brushes may be an effective antimicrobial coating to prevent CAUTI. Further testing of these novel coatings using a novel in vivo CAUTI model developed in our laboratory will be important to test the efficacy of these coatings in relevant environments over time.