

Moderated Poster Session II: Basic Research

Thursday, October 8, 11:00 a.m. — noon

P18 Evaluation of Hedgehog Signaling in Human Transitional Carcinoma Cell Lines

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Introduction and Objective: Hedgehog (Hh) is a ligand-driven signaling pathway that is involved in embryonic tissue growth and morphogenesis. Hh activates Gli transcription and increases cell proliferation and invasiveness. Aberrant Hh signaling is involved in human tumorigenesis and drugs related to cyclopamine, that block Hh signaling, are being tested as anti-cancer agents. Here, we describe efforts to characterize a potential role of Hh signaling in bladder cancer by quantifying expression of Hh signaling components in human transitional carcinoma cell (TCC) lines and by evaluating the effects of cyclopamine, on the growth of these cells.

Materials and Methods: Human TCC lines, RT4, 253JP and 253BV, UMUC 3 and 6, were characterized for their relative growth rate and invasiveness by cell counts and with a Matrigel invasion assay. Cells were tested for relative growth inhibition by cyclopamine (5 μ M) over 5 days. RNAs were extracted for quantitative RT-PCR evaluation of Sonic Hedgehog, Gli1/2, Smoothed and Patched gene expression.

Results: The different TCC lines were distinguished by a hierarchy of diminishing relative doubling times (DTs) that was inversely correlated with relative invasive behavior (Fig. 1). The cells with the shortest DTs and highest invasive behavior had the highest relative Gli2 expression (Fig. 2). Finally, cyclopamine had greater growth suppressive effects on the lines with the lowest DTs, highest invasion rates and higher Gli2 expression levels (Fig. 3).

Conclusion: The Sonic hedgehog pathway is active in bladder cancer. The invasive behavior of these cells correlates with basal Gli2 expression (Fig. 1, Fig. 2, Fig. 3).

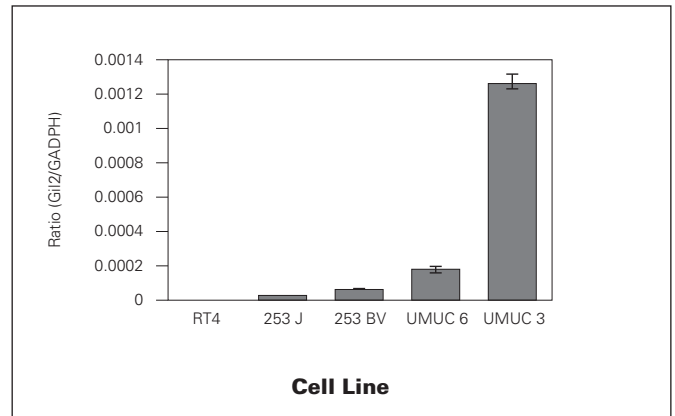


Fig. 2. Gli2 expression.

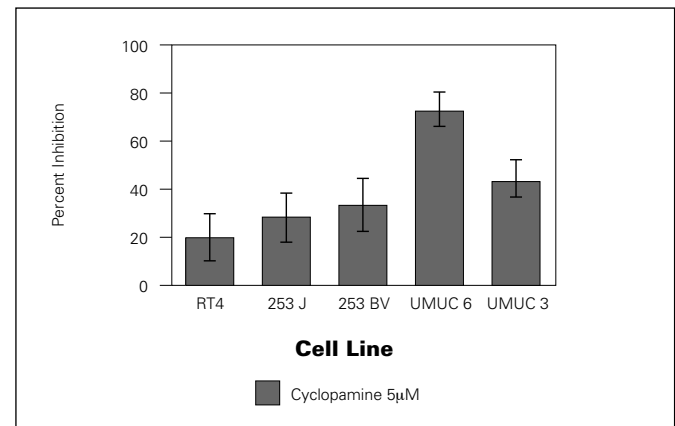


Fig. 3. The effects of cyclopamine on cell proliferation.

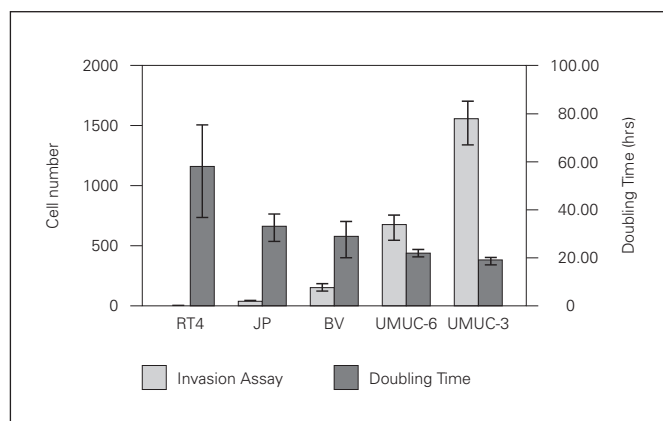


Fig. 1. Characteristics of invasiveness and growth of bladder cancer cell lines.

P19 Effect of Neoplasia in the Urinary Tract on Urine Levels of CC Chemokines: A Cross-Sectional Study

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Introduction and Objective: Inflammation plays a key role in diseases emanating from the upper or lower urinary tract. The chemotaxis of monocytes, eosinophils, lymphocytes and mast cells to the disease site is the key aspect of any grade of inflammation. Chemotaxis is mostly attributable to the activity of different members from CC class of chemokines produced by both immune and non-immune cells. We hypothesized that distinct members of the CC family orchestrate cancer-associated inflammation and inflammation associated with non-cancerous diseases.

Materials and Methods: Urine samples were obtained from 53 patients with either non-diseased urinary tracts (n=11) or with various urologic

conditions, including active bladder cancer (CaB; n=4), interstitial cystitis/painful bladder syndrome (IC/PBS; n=8), neurogenic overactive bladder (NOAB; n=13), prostate cancer (CaP; n=7), status-post robot-assisted laparoscopic prostatectomy (RALP; n=6), and nephrolithiasis (n=4). The Luminex 100™ IS multiple antigen bead assay (MiraiBio, Alameda, CA) was used to measure 5 CC chemokines (MIP-1, MIP-1, MCP-1, RANTES and Eotaxin), 1 CXC chemokine (IP-10), and 7 cytokines (IL-1ra, IL-2R, IL-5, IL-6, IL-8, IL-10, IL-12p70/p40). Chemokine levels were normalized to the creatinine (Cr) in urine and compared pairwise to healthy controls using Bonferroni correction for multiple comparisons.

Results: The presence of neoplasia in the bladder of CaB patients was associated with distinguishing elevation of MIP-1 in urine, whereas absence of neoplasia in pathology of IC/PBS and NOAB was remarked by a rise of MIP-1β (p<0.005). Urine elevation of MCP-1 levels distinguished IC/PBS from other diseases and a distinct elevation of another CC chemokine RANTES was noticed only in CaP patients (p<0.005). The CaB and CaP patients also showed an elevation of IL-12p70/p40 and IL-10 (p=0.006), respectively, and rise of IL-5 was seen in NOAB (p<0.005). There was a universal elevation of IL-6 and IL-12p70/p40 (p=0.006) in all patients and none were significantly elevated in the RALP group.

Conclusion: Lack or presence of cancer-associated, urine-detectable inflammation was characterized by elevation of distinct members of the same CC chemokine gene family. RANTES, MIP-1α and MCP-1 were distinctly associated with patients of CaP, CaB and IC/PBS, respectively. These 3 CC chemokines bind to the same CCR5 receptor and their genes are encoded on the same chromosome 17q12, which could explain the underlying reasons for very subtle differences in inflammation linked to cancer or visceral pain.

P20 Cannabinoid Receptors in Human Urothelial Carcinoma

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Introduction and Objective: The endocannabinoid system may serve as new therapeutic targets for various genitourinary diseases including overactive bladder and interstitial cystitis, or be used as prognostic markers for cancer severity and outcome. To date, cannabinoid receptors have yet to be characterized in human bladder cancer.

Materials and Methods: Under IRB protocol, human bladder tissue from organ donors (n=7) or from patients with bladder cancer (n=14) undergoing cystectomy were collected for PCR analysis and/or muscle strip studies. qRT-PCR with CB1 and CB2 primers (SAbioscience) was performed using an ABI 7300 RT-PCR (Applied Biosystems). The copy number per μg RNA was determined according to standard curves generated by CB1 or CB2 plasmids and represented as means.

For muscle strip studies, tissue sections were mounted using stainless steel hooks between two electrodes in organ baths containing 25 mL Krebs buffer, gassed continuously with carbogen. Changes in isometric force were detected by Grass FT03c transducers and recorded using Chart5 software. Electrical field stimulation (EFS) was delivered at frequency of 5Hz and 10Hz. CB1 or CB2 agonists, GP 1a or ACEA, (100nM or 1μM) were added to the baths and the effect on the EFS-induced muscle strip contraction was measured. Vehicle only (ethanol) served as negative control. The percent inhibition from baseline or control EFS was calculated.

Results: CB1 receptor copy number in normal bladder detrusor and urothelium (n=7) (mean 20,000 and 10,000 copies) was approximately 3-fold higher compared to tissue from cystectomy patients (n=14) (mean 6,000 and 3,000 copies, respectively). CB2 receptor expression was also higher in normal detrusor and urothelium compared to the diseased group (mean of 30,000 and 55,000 copies versus 20,000 and 5,000 copies). At 100 nM, GP1a markedly inhibited EFS-induced contractions in normal bladder (n=3) at 5hz and 10hz (average reduction: 63% and 62%, respectively) compared to the cancer group (n=6) (average reduction: 12% and 17%, respectively). ACEA also inhibited normal bladder contractions at 5 and 10 Hz (average reduction: 57% and 56% respectively), but not those in the cancer group (average reduc-

tion: 12% and 3%, respectively). A similar effect was observed at 1μM.

Conclusion: CB1 and CB2 agonists inhibit neurally-evoked human detrusor contractions. This effect is diminished in bladder cancer tissue, correlating with the downregulation of cannabinoid receptor expression in bladder tissue from bladder cancer patients. This further supports that the endocannabinoid system plays a role in cancer biology and may serve as a diagnostic tool for invasive urothelial bladder cancer.

P21 Prostate Cancer Progression through the Fer Kinase, a Potent Regulator of Androgen Receptor Signaling

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Introduction and Objective: Several mechanisms involving the androgen receptor (AR) were proposed to explain the transitory response of prostate cancer (PCa) patients to endocrine therapy and progression of the disease. Our earlier results and recent reports suggest a new form of AR activation via tyrosine (Y) phosphorylation in response to diverse growth promoting factors, including interleukin-6 (IL-6). In this study, we aimed to investigate if the Fer kinase, which is overexpressed in PCa and controls STAT3 activation in response to IL-6, may also control AR activation.

Materials and Methods: LNCaP cells were transfected with: siRNA (fer and AR), wild-type and double mutant fer cDNA, and a reporter construct of the PSA promoter (gift: Dr M. Sadar, B.C.). Transactivation of the PSA gene was tested using luciferase assays. Survival/growth in response to IL-6 and the synthetic androgen, R1881 was monitored by MTT. Proteins (Fer, AR, STAT3) and their extent of Y-phosphorylation were analyzed by immuno-fluorescence, -precipitation (IP) & Western blotting (WB). Pull-down assays were performed using the Fer-SH2 domain fused to GST (vs. control GST). The Fer kinase domain was used for in vitro phosphorylation of recombinant AR. PCa specimens were immunostained for Fer and AR expression.

Results: AR was rapidly Y-phosphorylated in response to IL-6 and R1881. Under these stimuli, AR was found to localize with Fer in the nucleus within 30 min. In contrast, STAT3 was only translocated into the nucleus under IL6 where it was found activated and in close proximity with both AR and Fer. Complexes between Fer, AR and STAT3 as well as AR and STAT3 were observed. Interestingly, Fer expression and activation regulated IL-6 induced AR Y-phosphorylation, nuclear translocation, transactivation of the PSA promoter and growth. Moreover the Fer kinase phosphorylates the AR and AR was pulled-down by the Fer-SH2 domain, supporting direct activation and interaction. However Fer and AR knock down revealed a strict requirement of Fer and only partial of AR for IL-6 growth response, whereas the converse was observed for R1881. Clinical relevance was shown by Fer and AR expression and similar distribution in the cytoplasm and nucleus of the same subsets of tumor cells.

Conclusion: The overexpression of Fer in PCa together with its ability to activate AR and control its nuclear translocation under IL-6 may explain, at least in part, how tumor cells adapt and grow despite endocrine therapy.

P22 Effect of Combined EGFR Inhibition and PPAR Gamma-Agonist in Bladder Cancer Therapy

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Introduction and Objective: Bladder cancer is one of the most common types of cancer in the world, ranking fourth in men and ninth in women. As understanding of the biology of urothelial carcinoma improves, novel therapeutic approaches need to be studied including novel targets for therapy. The two signaling molecules that are extremely attractive for targeted therapy are the epidermal growth factor receptor (EGFR) and the peroxisome proliferator-activated receptor γ (PPARγ). We evaluate the integration of combined drugs against these targets in the management of bladder cancer therapy.

Materials and Methods: The effect of EGFR inhibitor (Gefitinib) and PPAR γ -agonist C-DIM, on cell growth, were screened in a panel of 9 human urothelial carcinoma cell lines derived from well-differentiated superficial bladder tumors as well as from high-grade invasive tumors. Cell proliferation was determined, by using MTT assay, after incubation with varying concentrations of the targeted agents (10^{-3} to 10^2 μ m) for 72 hs. Antiproliferative effects of combined therapy (Gefitinib and C-DIM) compared to each drug alone were also monitored *in vitro*, by MTT assays and *in vivo*, on nude mice inoculated with the more resistant cell lines (KU-7 and UM-UC13). Baseline levels of expression of EGFR and PPAR γ were evaluated by Western Blot analysis as well as after of pre-treatment with EGFR inhibitor (Gefitinib). Immunofluorescence was then used to determine PPAR γ nuclear accumulation mediated by Gefitinib.

Results: Gefitinib and C-DIM inhibit growth of bladder cancer cell lines in a dose-dependent manner but with variable sensitivity. Induction of PPAR γ expression was observed in response to different concentrations of Gefitinib for 24 h. Moreover, nuclear accumulation of PPAR γ was observed followed its upregulation. MTT assay shows that maximum inhibition of cell proliferation was observed when cells were pre-treated with Gefitinib for 24 h followed by C-DIM as compared to C-DIM followed by Gefitinib or each treatment alone. Tumors weight from mice treated with combined therapy were significantly reduced versus each treatment alone compared to control ($p < 0.02$).

Conclusion: Preliminary results suggest that PPAR γ -agonist C-DIM can render bladder tumor sensitive to EGFR inhibition and combination efficacy might be achieved in a schedule-specific manner.

P23

The FGFR3 Mutation is Related to Favorable pT1 Bladder Cancer

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Introduction and Objective: Fibroblast growth factor receptor 3 (FGFR3) mutations have been recently reported at a very high frequency in pTa bladder cancer (BC) whereas these mutations are rare in high grade muscle-invasive BC. pT1-BC comprises a heterogeneous group of tumors for which different management options are advocated depending on the risk of progression to muscle-invasive disease. We have determined the frequency of FGFR3 mutations in a group of primary pT1-BC and correlated the FGFR3 mutation to various histo-pathological variables.

Materials and Methods: We included 132 patients from two academic centers (n=60 in Rotterdam / 72 in Toronto) with primary (first diagnosis) pT1-BC. Mean age was 68.7 years (SD: 9.9 y). An experienced uro-pathologist reviewed the slides for grade (1973 and 2004 classification systems) and determined if the tumor was micro-invasive (<0.5 mm; pT1m) or extensive invasive (multiple spots with invasion and/or >0.5 mm; pT1e). The FGFR3 mutation status was examined by SNaPshot analysis and correlated to pathological parameters.

Results: FGFR3 mutations were detected in 37/132 (28%) pT1-BC. The most frequent mutation in the FGFR3 gene (S249C) was observed 24 times whereas other FGFR3 mutations, R248C, G372C, Y375C, G382R were observed 5, 2, 5 and 1 time(s), respectively. Table 1 shows that presence of a FGFR3 mutation was highly correlated with favorable disease characteristics in pT1-BC.

Conclusion: The FGFR3 mutation selectively identifies pT1-BC patients with favorable disease characteristics. Further study may confirm that this molecular marker is able to select patients who will benefit from a conservative approach to their disease.

Table 1. Correlation of a FGFR3 Mutation

No. of rat with positive NF- κ B staining		FGFR3 mutant	FGFR3 wild type	P-value (chi-square)
Grade 1973	G2	25	31	<0.001
	G3	12	64	
Grade 2004	Low-grade	15	11	<0.001
	High-grade	22	84	
Sub-stage	pT1m	18	22	=0.004
	pT1e	19	73	
Total		37	95	

P24

Role of NF- κ B in Bladder Carcinogenesis in the Rat

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Introduction and Objective: NF- κ B regulates anti-apoptotic genes and cell cycle regulators. Evidence has been presented that suggests an important role of NF- κ B in the carcinogenesis of several human neoplasms. NF- κ B was activated in 80% of human urothelial carcinomas and it has been hypothesized that activation of this gene may be involved in the promotion/progression of this neoplasm. The objective of the present study was to test this hypothesis in an animal model.

Materials and Methods: Male Fischer rats which had been implanted with a heterotopic bladder were instilled once a week for 20 weeks with 0.5 ml PBS or this solution containing 1 μ mol N-hydroxy-2-aminofluorene (N-OH-N-GI-AF) or the N'-glucuronide of N'-hydroxy-N-acetylbenzidine (N'-OH-N-GI-ABZ) into this bladder. The heterotopic bladders were then instilled with PBS once a week for an additional 30 weeks. The experiment was terminated at the end of 50 weeks. Formalin-fixed normal bladders from the control group and urothelial carcinomas from the treated groups were sectioned for immunohistochemical staining with an antibody against NF- κ B p105/50.

Results: The results of NF- κ B staining are presented in Table 1. The staining of normal bladders in the control group was very weak at best and it was interpreted as negative. NF- κ B was activated only in one urothelial carcinoma which had positive staining in both cytoplasm and nucleus. Normal and hyperplastic urothelial cells in the treated group were not stained.

Conclusion: Since the activation of NF- κ B is induced by inflammation and oxidative stress, we conclude that these two processes and NF- κ B activation are not involved in bladder carcinogenesis in the rat model.

Table 1. No. of rat with positive NF- κ B staining

Treatment	Total no. of rat	Cytoplasm	Nucleus
Control	6	0	0
N-OH-N-GI-AF	9	6	0
N'-OH-N-GI-ABZ	5	3	1

P25**Soy Isoflavone G-2535 and Bladder Cancer: Molecular Effects in Tumor Tissue and Urine**

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Introduction and Objective: Isoflavones, phytoestrogens present in soy beans, are known to have anti-growth factor, anti-proliferative and proapoptotic effects in various cancer cell targets including human bladder cancer (BC). To assess whether G-2535, an oral preparation of unconjugated genistein (62%), daidzein (33%) and glycitein (5%) had similar effects in BC *in vivo*, patients scheduled to undergo BC surgery were randomized to receive none (placebo), 300 mg/day or 600 mg/day of G-2535 for 14-21 days immediately before surgery.

Materials and Methods: Patients were stratified according to suspected depth of tumor invasion ($\leq T1$, $\geq T2$). The primary endpoint was reduction in activation of the epidermal growth factor receptor (EGF-R) as determined by reduced phosphorylated (p) EGF-R on tyr 992 and tyr 1068 using a pair of monoclonal antibodies to these epitopes and grading with the quantitative German immunoreactive score (IHS) which accounts for percentage positively stained cells and intensity of staining. Secondary endpoints included changes in expression of Ki67, Caspase 3, Survivin, AKT, pAKT and COX-2 in tissue (using IHS), and urinary Survivin. In addition, toxicities were monitored throughout the trial.

Results: 60 patients were randomized from 7 sites: 52 men, 8 women; median age of 70.5 years; 80% stage $\leq T1$. No toxicities could be attributed to G-2535, which was well tolerated. 300 mg/day of G-2535 (n=17) reduced pEGF-R in BC tissue compared to placebo (n=15) ($p=0.02$), and 300 or 600 mg of G-2535's (n=34) reduction of pEGF-R approached statistical significance ($p=0.078$). Results were similar for $\leq T1$ and $\geq T2$ BCs. G-2535 also effected a non-significant reduction in urinary Survivin from baseline levels compared with placebo after 8 ($p=0.10$) and 14-21 ($p=0.18$) days. The other parameters were not affected by G-2535.

Conclusion: 300 mg/day G-2535 is well tolerated and reduces EGF-R activity in BC tissue. This natural compound warrants further testing, alone or in combination with other interventions, as a preventive and/or therapeutic agent.

P26**Reconstruction of an Autologous Urethral Model by Tissue Engineering**

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Introduction and Objective: Several diseases, like hypospadias and strictures, cause urethral disorders. To correct these anomalies, acellular bio-materials or non-urolologic tissues are used when necessary, but it often leads to post-surgical complications. Our objective is to reconstruct, by tissue engineering and self-assembly method, an autologous urethral model that can be grafted and is viable. For our test, a porcine model was used because of its similarity to the human urinary tract. But the biggest challenge of the model is the production of a porcine fibroblasts sheet, because it is difficult to obtain extracellular matrix from animal fibroblastic cells and no research teams have been able to produce one.

Materials and Methods: We develop a tubular model composed of fibroblasts, extracted from the dermal layer of the skin and urothelial cells stem from the bladder. The fibroblasts are cultured four weeks to allow secretion of their extracellular matrix and to obtain an easy-to-handle sheet. This sheet is rolled around a mandrel to obtain a tubular form and then it matures for three weeks to gain superior adhesion between the fibroblasts layers. Urothelial cells are seeded inside the tubular model and placed under perfusion in a bioreactor for one week to promote proliferation and maturation of the urothelial cells. To compare the urethral equivalent with a native urethra, we characterized our model by

histology, immunofluorescence (IF), Western Blot (WB) and we also performed a viability test.

Results: Our tubular model, macroscopically uniformly assembled, provided suture resistance and was easily handled. Histologically, we obtained a thick layer of fibroblasts in an abundant extracellular matrix and the urothelium was similar to a native urethra. The urethral model characterization with IF and WB confirmed the presence of a well-differentiated and pseudostratified urothelium.

Conclusion: Our porcine autologous urethral equivalent is a first of its kind in tissue engineering and will allow us, in a near future, *in vivo* implantation on the pig. It will also help us to go further with human urological researches. Furthermore, the great advantage of our model will be the use of the patient's cells, which would decrease the inflammatory reaction and post-surgical complications.

P27**Effect of Varicocelectomy on Sperm Chromatin and DNA Integrity**

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Introduction and Objective: The effect of adult varicocelectomy on male fertility remains controversial largely because of the paucity of randomized and controlled trials. As well, using the improvement in conventional semen parameters as an outcome measure after varicocelectomy is limited by virtue of the high degree of biological variability of these parameters. An improvement in sperm DNA integrity would provide more credibility as to the therapeutic effect of varicocelectomy because compared to standard semen parameters, measures of sperm DNA damage exhibit a lower degree of biologic variability and may be better predictors of male fertility potential. We sought to further evaluate the effect of varicocelectomy on sperm chromatin and DNA integrity.

Materials and Methods: All data were collected in a prospective manner. Twenty consecutive infertile men with clinical varicocele were included in the study. Preoperative and postoperative semen samples were obtained and analyzed for conventional and sperm chromatin parameters. Sperm chromatin integrity was assessed using a flow cytometry-based assay with the results expressed as (i) % DFI (DNA fragmentation index — an index of DNA fragmentation) and (ii) %HDS (high DNA stainability — an index of chromatin compaction), and, by sperm nuclear single-stranded (ss) DNA immunostaining (with the results expressed as the % of cells with diffuse ssDNA stain). All men signed an informed consent before participation in the study.

Results: Varicocelectomy resulted in a significant improvement in sperm concentration (43 ± 37 to 106 ± 122 million sperm per ml, $p=0.013$) and progressive motility (25 ± 15 to $41 \pm 23\%$, $p=0.0009$). As well, sperm DNA integrity and sperm chromatin compaction improved significantly after surgery (%DFI decreased from 19 ± 11 to $12 \pm 6\%$, and %HDS decreased from 10 ± 6 to $6 \pm 5\%$, respectively, $p<0.01$). Furthermore, ssDNA immunocytochemistry studies demonstrated that varicocelectomy was associated with a decrease in the proportion of spermatozoa exhibiting diffuse ssDNA (10 ± 6 to 5 ± 5 , respectively, $p=0.02$).

Conclusion: These data show that varicocelectomy is associated with an improvement in conventional sperm parameters, and, in sperm DNA integrity and chromatin compaction. The data suggest that varicocelectomy may improve sperm DNA integrity by enhancing spermatogenesis, particularly spermiogenesis (this is the stage in spermatogenesis where nuclear histones are replaced by protamines, and, where the normal compaction and stability of the DNA and chromatin occur). The data provide further evidence in support of the beneficial effect of varicocelectomy on male fertility potential.

P28**Pilot Study on WST11 Safety and Efficacy for Vascular Targeted Photodynamic Therapy in the Dog Prostate**

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Introduction and Objective: Vascular targeted photodynamic therapy (VTP) with the bacteriochlorophyll derivative WST09 as the photosensitizer was proven efficient for the treatment of localized prostate cancer (PCa). However solubility in aqueous solutions limits the scope of clinical applications. The aim of this study was to assess safety and efficacy of the water soluble bacteriochlorophyll, WST11, for VTP in the dog prostate model.

Materials and Methods: WST11-VTP was done at laparotomy on 25 dogs: 1 to 2 optic fibers of 1 cm were inserted in each prostate lobe. WST11 (1-15mg/kg) was infused i.v. for 10 min. The light (laser power: 150-250mW/cm) was turned on at 5-10min to deliver energy at doses of 100-400J/cm/fiber. Blood pressure was recorded. Blood was withdrawn to assay diverse biochemical or hematological parameters and WST11 kinetics. Dogs were followed for 8 days. The prostate, lung, and liver were harvested for pathology.

Results: The VTP-procedure was well tolerated with no change in blood pressure and biochemical or hematological parameters. Blood levels of WST11 increased in parallel with infusion and declined rapidly to undetectable levels by 60 min. Subjects experienced only mild lower urinary tract symptoms, resolved within 1-2 days. There was neither macro- nor microscopic changes in the liver and lung, but hemorrhages were detected in all prostates. Necrosis was consistently associated with endothelial cell damages in blood vessels. It was optimal at 5.0 mg/kg WST11 and 400J/cm. The average destroyed area per section of a lobe was 2.24cm². This represented significant volumes of treated lobes (50-80%), according to the prostate size.

Conclusion: These pre-clinical findings support the assessment of WST11-VTP in patients with localized PCa.

P29**Regulation of Apoptosis in Renal Epithelial Cells by Annexin A1 During Mechanical Stress**

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Introduction and Objective: Obstruction of the urinary tract subjects renal epithelium to forces of mechanical trauma. Epithelial cells undergoing such stress may undergo apoptosis in a highly regulated fashion, with the protein Annexin A1 (AnxA1) thought to play a central role. We have previously demonstrated through an *in vivo* rodent model for urinary tract obstruction that urinary AnxA1 is increased. The apoptotic response of renal epithelium to mechanically induced cellular stretch can be recreated *in vitro*. This work attempts to further define the role for AnxA1 in the regulation of apoptosis in renal epithelial cells subjected to external mechanical stresses.

Materials and Methods: NRK-52E renal epithelial cells were grown to 95% confluence and exposed to 25% maximal radial stretch, for 12 hours at 0.25 Hz. Cells were exposed to stretch in the presence or absence of an AnxA1 mimetic (Ac2-26). A cell death detection ELISA kit was used to quantify apoptosis.

Results: Cells treated with Ac2-26 showed a 3.0 fold higher apoptotic rate than unstretched cells, compared with a 1.8 fold higher for stretched cells with no AnxA1 mimetic. The addition of Ac2-26 alone without stretch showed no significant difference in apoptosis compared to controls.

Conclusion: Apoptosis of renal epithelial cells exposed to an AnxA1 analogue is greatly increased when exposed to mechanical stretch forces. This indicates that AnxA1 may function as a key regulator in the apoptotic cascade in renal epithelium during urinary tract obstruction. Further studies to elucidate the effect of transcriptional regulators of AnxA1 or the associated binding partners of this protein would be of great inter-

est. Numerous avenues of investigation are readily available to help elucidate the key components of apoptosis using this *in vivo* model of urinary tract obstruction.

P30**Specific NADP(H) Oxidase Inhibition Abrogates HIF Transactivation and the Tumorigenic Phenotype of Renal Cancer Cells**

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Introduction and Objective: Inactivation of the von Hippel Lindau tumor suppressor (VHL) is an early event in sporadic clear cell kidney cancer. VHL targets hypoxia-inducible factor alpha (HIF- α) for ubiquitin-mediated degradation, and VHL loss results in accumulation and activation of HIF. We previously reported that generation of reactive oxygen species (ROS) by the Nox4 NADPH oxidase is critical for expression and activity of HIF2- α . Nox4 knockdown results in dramatic reduction of HIF transcription even in the absence of VHL. Further, Nox4 silencing abrogated branching morphogenesis and invasion, and completely inhibited RCC xenograft growth. GenKyoTex compound GKT136901 is a novel Nox inhibitor with activity against Nox4. To determine the anti-tumor activity of GKT136901, we exposed 786-0 human kidney cancer cells to the compound and assayed for HIF activity, branching morphogenesis and invasion.

Materials and Methods: 786-0 cells with stable expression of non-specific shRNA (NS) were plated in DMEM with 10% FBS with serial dilutions of GKT136901 ranging from 10 μ M to 0.1 nM. Cells expressing Nox4 shRNA (KD) served as a positive control for Nox4 inhibition. A luciferase reporter construct containing the minimal promoter region of VEGF was used to measure HIF-transactivation. To assay branching morphogenesis, the same cells were suspended in matrigel and incubated with recombinant HGF-SF at 37°C for 72 hours. Triplicate wells were evaluated by measuring the percentage of branching cells in 3 wells. Invasive potential was quantitated by measuring the mean number of cells to cross an 8 mm pore filter membrane coated with basement membrane matrix toward an HGF-SF gradient, in triplicate. Significance was determined by a Mann-Whitney Rank Sum Test. Cell viability was measured in parallel cultures with CellTitre Blue (Promega).

Results: VEGF-luciferase expression was decreased 53% in untreated KD relative to untreated NS cells ($p=0.01$), whereas GKT136901 demonstrated a dose-dependent inhibition of VEGF-luciferase to a maximum of 56% at 1 μ M ($p=0.004$). Branching morphogenesis was inhibited by GKT136901 in a dose-dependent fashion, and the inhibitor significantly reduced the mean number of cells to invade through a basement membrane at 1 μ M relative to no drug (48 vs. 156, $p=0.02$). No cytotoxicity was seen at any concentration of GKT136901.

Conclusion: GKT136901 Nox inhibition mimics the anti-RCC impact of Nox4 silencing, suggesting a role for GKT136901 in the treatment of RCC. Ongoing studies are measuring the efficacy of GKT136901 for suppression of RCC xenograft growth.

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P31**Frequency Dependent Pudendal Neuromodulation and Opiate Receptor Involvement in the Pudendal-to-Bladder Reflex in Cats**

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Introduction and Objective: Different frequencies of pudendal nerve stimulation can evoke bladder contraction (20-40Hz) or promote bladder relaxation (0.5-10Hz) in spinal intact and spinal transected cats. However, the neurotransmitters involved in pudendal neuromodulation have not been determined. This study investigates the role of opiate receptors in the pudendal-to-bladder pathway utilized by pudendal neuromodulation.

Materials and Methods: Spinal intact cats anesthetized with alpha-

chloralose were tested by unilateral pudendal nerve neuromodulation (0.5-40Hz) via cuff electrodes. At volumes of 100-120% of bladder capacity, different stimulation frequencies were applied to either inhibit or facilitate isovolumetric contractions. The optimal inhibitory and excitatory frequencies were subsequently tested during cystometrograms (CMG) to change bladder capacity. Burst stimulation (duration 30 seconds) at the optimal excitatory frequency was also assessed during CMG (saline infusion rate 1-2ml/min). Incremental doses of naloxone, an opiate receptor antagonist (0.01, 0.03, 0.1, 0.3, and 1.0mg/kg), were then intravenously administered with repeat CMGs, isovolumetric conditions, and stimulation of the pudendal nerve.

Results: Maximal inhibition was seen at 3-10Hz during isovolumetric contractions. The strongest bladder contractions (50-70cmH₂O) were observed at 20-30Hz stimulation with bladder volumes above 60% capacity ($p < 0.05$). Bladder capacity increased to 142 \pm 6.5% ($p < 0.01$) of control capacity with 3-10 Hz continuous stimulation during CMG, but it was not significantly changed with 20-30Hz continuous stimulation. Administration of naloxone enhanced isovolumetric bladder contractions and decreased bladder capacity by 53 \pm 15% ($p < 0.01$). Stimulation frequencies between 5 to 10Hz in naloxone-treated cats suppressed bladder activity and increased bladder capacity by 220%. Higher frequency (20-40Hz) and lower frequency (0.5-3Hz) stimulation enhanced bladder contractions.

Conclusion: This study reveals the frequency dependence of pudendal nerve stimulation on bladder activity: 3-10Hz can promote storage and increase bladder capacity, and 20-30Hz can enhance bladder contractions. Furthermore, pudendal neuromodulation was not altered by naloxone, indicating that opiate receptors have a minor role in the pudendal-to-bladder reflex.

P32

Early Induction of Erectile Dysfunction by Angiotensin II in the Rat

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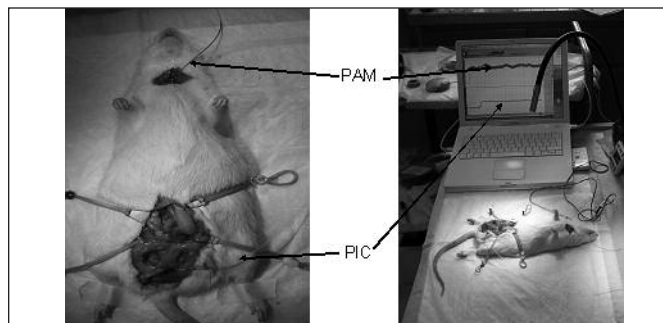


Fig. 1. Anesthetized rats responding to electrical stimulation of the cavernosal nerve.

Introduction and Objective: Erectile dysfunction (ED) is an early indicator of cardiovascular disease, of which a major contributing factor is hypertension. Furthermore, there is evidence to suggest that angiotensin II (AngII) receptor (AT1) blockers improve erectile function in hypertensive patients. The aim of this study is to determine whether the development of ED precedes systemic cardiovascular damage after a continuous infusion of AngII in rats.

Materials and Methods: Sprague-Dawley rats (250g) were randomized to receive a continuous infusion of either AngII (200 ng/kg/min s.c., n=5) or saline (n= 6) by osmotic minipumps implanted subcutaneously for a total of 7 days (Fig.1). The mean arterial pressure (MAP) and intracavernous pressure (ICP) were measured simultaneously, and erectile function was estimated by the ICP/MAP ratio measured in response to elec-

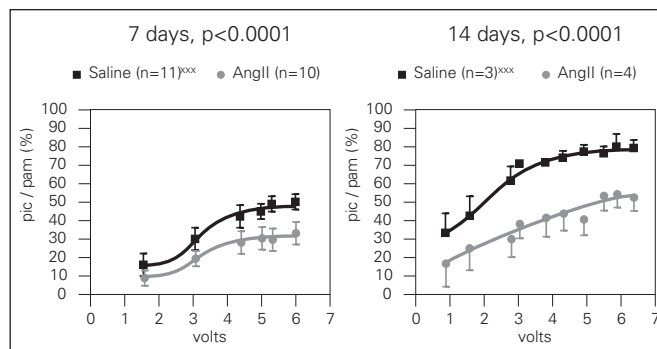


Fig. 2. Continuous AngII infusion caused a significant decrease in ICP/MAP ratio.

trical stimulation of the cavernosal nerve (1 - 5.5 volts) in the anesthetized rats. At the end of this procedure, the rats were sacrificed, the hearts excised and the ventricles separated and weighed. The aorta and penis were also excised and cleaned, and a section of each was fixed in formalin for histological analysis. Aortic rings were used to evaluate vascular relaxations induced by acetylcholine or sodium nitroprussiate (SNP).

Results: A continuous infusion of Ang II for 7 days did not significantly affect the MAP (saline 97 \pm 9mmHg vs. AngII 86 \pm 4mmHg, NS) prior to stimulations and 118 \pm 7mmHg vs. 109 \pm 10mmHg (NS), after heart/body weight ratio (saline 1,94 \pm 0,07 mg/g vs. AngII 1,95 \pm 0,22 mg/g, NS), aortic cross-sectional area (saline 0.47 \pm 0.020 mm² vs. AngII: 0.48 \pm 0.061 mm², NS) or aortic cell number (saline: 174 \pm 32.4 cells/m vs. AngII: 177 \pm 38.9cells/m, NS) (Fig. 2). The ratio ICP/MAP, on the other hand, was significantly diminished in the group treated with AngII in a voltage-dependent manner (at 5.5 volts, saline 26 \pm 9 vs. AngII 57 \pm 6, $p = 0.003$). Functional studies showed no evidence of endothelial dysfunction in the aorta.

Conclusion: This study, the first to examine the impact of chronic AngII administration on erectile function, suggests that ED precedes the development of arterial hypertension and left ventricular hypertrophy at 7 days of infusion. Our results support the hypothesis that erectile function is an early gauge of cardiovascular complications.

P33

Peroxi-redoxins: Novel Antioxidant Enzymes of Human Spermatozoa

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Introduction and Objective: Peroxi-redoxins (PRDXs) are a recently discovered family of thiol peroxidases. They play an important role in cell peroxide and peroxy-nitrite detoxification and in the regulation of hydrogen peroxide (H₂O₂) signaling. They are acidic proteins with one or two cysteine (Cys) residues required for their activity. The aim of this study was to characterize PRDXs and whether oxidative stress modifies their expression in human spermatozoa.

Materials and Methods: Semen samples were obtained from healthy volunteers. Localization of PRDXs in spermatozoa was done by immunocytochemistry using anti-PRDX antibodies. Sperm proteins from Triton X100-treated and non-treated sperm and from spermatozoa challenged with a mild (0.05 and 0.1 mM H₂O₂) or a strong (0.35-5 mM H₂O₂) oxidative stress for 30 min at 37°C were electrophoresed under non- and reducing conditions.

Results: PRDX 1, 2, 5 and 6 were immunolocalized in the sperm head and in the tail. PRDX 3 was found in the post-acrosomal region and in the principal piece. PRDX 4 was highly expressed in the acrosome region. PRDX 1, 4 (secreted form), and 6 were found in human spermatozoa and seminal plasma. The membrane bound form of PRDX 4 was also found in spermatozoa. PRDX 1 and 6 were present as protein doublets under non-reducing conditions. PRDX 4, 5 and 6 were found in both Triton-soluble and -insoluble sperm fractions, while PRDX 1 was found

only in the Triton-insoluble fraction. Under non-reducing conditions, PRDX 1 and 6 were found as bands of high molecular weight (>170 kDa) in spermatozoa incubated with 0.35-5 mM H₂O₂ (strong oxidative stress). PRDX 6 became a strong single band due to a mild oxidative stress (0.05 mM H₂O₂, a condition that promotes sperm capacitation). H₂O₂ (0.35-5 mM) caused a decrease of the signal for PRDX 4 and 5 compared to non-treated cells.

Conclusion: PRDXs are present in human spermatozoa and differentially modified by oxidative stress. These results suggest a role of PRDXs as antioxidants and as potential modulators of H₂O₂ action in human spermatozoa.

P34

Androgen Receptor (AR) and Bladder Cancer: A Large Bi-Institutional Study on 473 Patients

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Introduction and Objective: Bladder cancer is approximately three times more common in men than in women but the reasons behind this observed difference in incidence is largely unknown. A role for androgen receptor (AR) signaling has been suggested. Data on very limited number of patients showed that loss of androgen receptor expression was associated with invasive bladder cancer. We decided to further investigate the AR expression in a large series of bladder cancer patients.

Materials and Methods: A total of 473 patients have been analyzed. The study included specimens from the University of Toronto/University Health Network, Canada (n= 120) and the University of Texas Southwestern Medical Center, Dallas, Texas, US. Tissue microarrays comprising both non-muscle invasive (n=126) and invasive bladder (n=347) tumors have been accrued and immunohistochemical staining for AR was performed on tissue microarrays using a monoclonal mouse anti-AR antibody. We used bright field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System, ChromaVision Medical Systems Inc., San Juan Capistrano, CA) to detect, classify, and count stained cellular objects based on predetermined color morphology. Results obtained in Dallas were blindly reviewed and validated by one uro-pathologist in Toronto in a manual non-automated fashion.

Results: Androgen receptor was positively expressed in a total of 60/473 (12.6%) patients, 13/120 (10.8%) in Toronto and 47/353 (13.3%) in Dallas (p>0.05, NS). There was not statistically significant difference between AR expression in men and women. Overall, 8.7% of superficial tumors (pTa + pT1 + carcinoma in situ) expressed the AR compared with 16.3% of invasive tumors (pT2 + pT3+ pT4; P < 0.05). The highest percentage of AR (28.8% of cases) was found in T2 tumors.

Conclusion: In contrast with previous reports on limited number of patients, based on our large bladder cancer series, we did not observe a decrease in AR protein expression in tumors with increased pathologic stage and our data do not suggest that the loss of AR expression is associated with invasive bladder cancer. AR positivity remains a rare event in bladder cancer (12.6%) and is not gender-related.

Moderated Poster Session III: Robotics, Endoscopy and Laparoscopy Thursday, October 8, 4:00 – 5:00 p.m.

P35

A Multi-Centre Randomized Trial Comparing Bipolar vs. Monopolar Transurethral Resection of the Prostate

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Introduction and Objective: Monopolar transurethral resection of the prostate (TURP) is the gold standard therapy for men with lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH). Inherent with the use of monopolar transurethral electro-surgery however, are the risks of bleeding, tissue burns, dilutional hyponatremia and irrigant toxicity. Although generally considered safer, the experience with bipolar electro-surgery for TURP application is limited. The objective of this trial was to evaluate both monopolar and bipolar (Gyrus-VISTA platform) TURP outcomes in a multicentre, single-blinded trial.

Materials and Methods: Forty-four patients from four Canadian sites were randomized to undergo TURP with either the bipolar or monopolar devices. All patients underwent baseline determinations of AUA symptom score, peak urinary flow rate, post void residual bladder volume and transrectal ultrasound prostate volume. The primary outcome measure was improvement in AUA symptom score (AUA SS), quality of life assessment (AUA QOL) and bother assessment (AUA B) questionnaires and secondary measures included procedural times, duration of urethral catheterization, length of hospitalization, complications and the degree of thermal artifact in the tissue specimens. Patients were followed for six months after surgery.

Results: 22 patients were randomized to each treatment arm, pre-operative demographic data were not statistically different between the groups. AUA SS, AUA QOL, AUA B and sexual function assessments at all data collections times were no different for either group. The only differences observed were in the procedure time (60.7 for bipolar vs. 47.4 min for monopolar, p=0.042) and the duration of urethral catheterization (1.5 for bipolar vs. 1.1 days for monopolar, p=0.03). There was no statistically significant difference in the pathological degree of thermal artifact or the rate of complications between groups. There was no difference in the change of post operative hemoglobin among groups, and no patient required blood transfusion.

Conclusion: This trial suggests equivalent short term outcomes for men undergoing monopolar or bipolar TURP. Although admittedly monopolar TURP is associated with a relatively low risk of complications, eliminating the need for a return electrode pad and the risk of dilutional hyponatremia would appear to be added safety features favoring the bipolar technology.

P36

Development and External Validation of a Highly Accurate Nomogram for the Prediction of Perioperative Mortality After Transurethral Resection of the Prostate for Benign Prostatic Hyperplasia

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