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MP-08.01
Validation of NF-Kappab P65 as a Prostate Cancer Prognostic Marker on a Large European Cohort

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Introduction: Important advances in prostate cancer (PCa) diagnosis and treatment have resulted in increased patient survival. However, it remains difficult to identify accurately patients better suited for less-aggressive therapy (active surveillance) from those at higher risk of disease progression. Over the past several years, our group has published several studies supporting an association between the nuclear NF-kB p65 and poor prognosis. In this study, we present validating results confirming a direct association between NF-kB p65 and biochemical recurrence (BCR) in a large independent cohort.

Methods: Primary tumors from radical prostatectomy were obtained from a large European prostate cancer cohort and tumor specimens were spotted on tissue microarrays. NF-kB p65 expression was detected by immunohistochemistry on a final number of 1,850 cores containing suitable malignant tissue.

Results: We observed a significant correlation between an increase in the nuclear frequency of NF-kB p65 and overall BCR (p<0.001, T-Test), metastasis (p=0.001, T-Test) and mortality (p=0.008, T-Test). In univariate COX regressions, the nuclear frequency and the nuclear intensity of NF-kB p65 were both associated with overall BCR (p<0.001, each). For the multivariate analyses, the clinical model included the following parameters: preoperative PSA (continuous), Gleason score, extra-capsular extension, lymph node invasion, seminal vesicle involvement and surgical margin status. We found that the nuclear frequency of NF-kB p65 was retained in the multivariate model (p<0.001) and that it improved the fitness of the clinical model when performing a Likelihood ratio test and an Akaike Information Criterion (AIC) test. Furthermore, we observed that the fitness of the clinical model was improved in sub-cohorts of: (i) 1023 lymph node negative patients, (ii) 1242 patients with negative margins, (iii) 194 patients with Gleason score ≥ 4+3. Finally, the cytoplasmic intensity improved the clinical model in a sub-cohort of 72 lymph node positive patients.

Conclusions: Our study offers validating results linking the nuclear frequency of NF-kB p65 with disease progression using a large cohort of European man. These results, and others in the scientific literature, suggest that NF-kB p65 can be useful as a molecular marker in the clinical decision process for PCa patients.

MP-08.02
Impact of NF-Kb Relb Subunit Expression on the Biology of Prostate Cancer Cells

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Introduction: Our group previously observed the nuclear localization of the alternative NF-kB subunits RelB and p52 in tumor cells of prostate cancer (PCa) patients. These observations, based on immunohistochemistry analyses, suggest a constitutive activation of the alternative NF-kB pathway and a potential implication in PCa progression. The current study aims to define the role of the alternative NF-kB signaling in PCa by focusing on RelB, the alternative NF-kB subunit with a gene expression promoting transactivation domain. We used an over-expression system to assess its effects on the biology of a PCa cell line.

Methods: PCa cell lines (LNCaP, 22Rv1, DU145 and PC3) express RelB differentially. Indeed, Western blot analyses clearly show a higher expression of RelB in DU145 and PC3 cells, while LNCaP and 22Rv1 cells do not express it. Using a lentivirus-based expression system, we introduced the cDNA of RelB into the 22Rv1 cells. We then assessed RelB's effects on different cellular processes such as cell proliferation, migration, adhesion and 3D anchorage-independent cell growth (soft agar assay).

Results: By introducing RelB into 22Rv1 cells using lentiviral infection, we induced the expression of RelB at a level comparable to those observed in DU145 and PC3 cell lines. Our results indicate that the expression of RelB doubles the cellular proliferation rate in 22Rv1 cells after 5 days of culture (p<0.05). However, RelB expression decreases the ability of the 22Rv1 cells to grow in 3D (p=0.002). Moreover, RelB over-expression halves the migration rate of 22Rc1 cells (p=0.017), but did not affect their adhesion on a matrix coated with type I collagen (p=0.183).

Conclusions: This study reveals that in 22Rv1 cells, over-expression of RelB distinctly modifies proliferation, migration and 3D anchorage-independent growth. Ongoing *in vivo* experiments will determine how RelB expression affects the tumorigenic potential of 22Rv1 cells, thereby providing clues to its possible role in PCa.

MP-08.03
Mechanism of Action of a Novel Combi-Molecule on Erbb-Family Receptors Mediated Signaling in Prostate Cancer

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Introduction and Objective: Current chemotherapies for prostate cancer (PCa) remain palliative and lead to resistance, thereby further limiting treatment options. As such, novel therapeutic strategies are essential to broaden

the spectrum of remission-oriented chemotherapy while reducing adverse side-effects. ZR2003, a novel combi-molecule, is composed of a quinazoline core (as in Gefitinib) and a hemi-mustard tail (analogous to half the musard tail of chlorambucil). Current data suggests that the target of ZR2003 would be EGFR/ErbB1. PCa is known to be associated with the overexpression of members of the ErbB receptor family (ErbB1 to 4). Thus, the quinazoline core of ZR2003 would inhibit the potent anti-apoptotic activity of ErbB family of receptors, therefore potentiating cell death signaling initiated by the DNA damage induced by the hemi-mustard tail. This dual effect would enhance its potency in EGFR overexpressing cells. To test this hypothesis, we evaluated the cytotoxic effect of ZR2003 on genetically enginnered PCa cell lines *in vitro* and *in vivo* and studied the impact of modulating ErbB receptor expression on its cytotoxicity.
Methods: The expression of ErbB family receptors was evaluated on four PCa cell lines (LNPCa, 22Rv1, DU145, PC3) by Western blot. Silencing of ErbB-family receptors is conducted through the infection of PCa cells by lentiviruses containing inducible shRNA constructs. The cytotoxic effect of ZR2003 will then be evaluated in relation to ErbB expression.
Results: Our primary objective being to determine the role of ErbB family expression in cell sensitivity to ZR2003, we first characterized ErbB family receptor expression in PCa cells. The primary analysis of ErbB status would also guide us on which of the cell lines to select for silencing or fos overexpressing ErbB family receptors. The results showed that DU145 cells expressed the highest level of EGFR while very low levels were observed in LNCaP, 22Rv1 and PC3 cells. ErbB2 is highly expressed in LNCaP, 22Rv1 and DU145 cells while being barely detectable in PC3 cells. Finally, we were able to effectively silence EGFR by at least two of the shRNA expressed in DU145 cells.
Discussion: Our work will lead to a better understanding of the molecular targets of this interesting novel combi-molecule. *In vivo* experiments are currently underway to evaluate the cytotoxic activity of ZR2003 against PCa cell lines in xenograft mouse tumour models.

MP-08.04
Antagonist Effect of the Androgen Receptor Activity and IKKe Expression in Prostate Cancer Xenograft Growth
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Objectives: Advanced prostate cancer (PCa) remains one of the leading causes of cancer related death and is often due to the progression from a hormone sensitive (HS) to a castrate resistant (CR) state. The androgen receptor (AR) is central to the initiation and growth of PCa. We have previously shown that CR PCa cell lines lacking AR expression exhibit high constitutive IKKe expression, whereas only very low IKKe expression is observed in HS PCa cell lines. We also found that TNF-alpha-induced IKKe expression is inhibited by an androgen-analog (R1881) in HS PCa cells. Based on these results, we put forth the hypothesis that the IKKe-AR interaction is important in the control of PCa progression.
Methods: We developed a sub-cutaneous xenograft PCa model expressing IKKe following the induction with doxycycline (dox). This model makes use of the dox-inducible 22Rv1-pTrexIKKe cells, as well as pTrexLacZ-inducible controls. The cells were mixed with matrigel prior to injection into the flank of SCID mice to allow the formation of better-defined tumors. Half of the studied mice were castrated one week prior to tumor cell injection. Dox was delivered through dox-supplemented food (625 mg/kg) to minimize stress. Mice were sacrificed when the tumor volume reached 2,500 mm^3, in accordance with institutional guidelines.
Results: We performed a large-scale experiment (108 mice) to study the effect of androgen depletion on the *in vivo* proliferation of HS 22Rv1-pTrexIKKe cells. We did not find any difference in the proliferation rate of 22Rv1-pTrexIKKe and 22Rv1-pTrexLacZ cells injected in castrated mice fed with normal or dox-supplemented diet. As expected, 22Rv1-pTrexIKKe and 22Rv1-pTrexLacZ cells injected in castrated mice had a lower proliferation rate than cells injected in uncastrated mice fed with normal diet. Surprisingly, we observed an important decrease in the growth rate of the 22Rv1-pTrexIKKe xenografts in uncastrated SCID mice fed with the dox-supplemented diet compared to all the other groups.
Conclusions: These results provide the first evidence for the opposing role of IKKe and AR in HS PCa cell proliferation and survival. These observations may be significant in the development of CR PCa.

MP-08.05
Potential Immunotherapeutic Applications of Phosphodiesterase Inhibition for Prostate Cancer
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Introduction: The cyclic nucleotide phosphodiesterases (PDEs) are a family of enzymes that play a central role in controlling cyclic nucleotide action and subsequent regulation of cell function. Specifically in tumor biology, previous studies have ascribed a protective role of cyclic guanosine monophosphate (cGMP) mediated signaling on hypoxia-mediated cancer progression. Herein we describe the expression levels of the known PDE variants in prostate cancer as well as explore the functional role that they play in cancer immune escape.
Methods: cGMP phosphodiesterase assays were used to measure the PDE activity in human prostate cancer cell lines DU145 and PC3. Western Blot analysis was performed to determine the presence of the PDEs in human tissue samples. The effect of PDE inhibitors on cancer cell apoptosis was measured using a flow cytometry-based TUNEL assay. The effect of PDE inhibition on hypoxia-mediated immune escape mechanisms was determined after pre-incubating cells in 0.5% or 20% O_2 in the absence or presence of Zaprinast (10^{-7} – 10^{-6} M) and subsequent fow cytometric analysis of MICA, an essential tumor-associated antigen required for innate immune responses. A NK-competent murine model was used to measure the in vivo effects of Zaprinast on the growth of human prostate cancer xenografts.
Results: PDE activity assays indicated that the majority of cGMP PDE activity in prostate cancer cell lines is made up from a combination of PDE5 and PDE11 (64-86% of PDE activity). The same two PDE variants also predominated in human malignant prostate tissue as detected using Western blot. TUNEL assays revealed some increased apoptosis when prostate cancer cells were treated with a PDE inhibitor in both hypoxic and standard oxygen conditions. MICA expression was reduced when prostate cancer cells were exposed to hypoxia; however, immunogenicity could be restored by incubating hypoxic samples with a PDE inhibitor (see figure, $p<0.01$). Finally, growth of human prostate tumor xenografts in mice was inhibited by PDE inhibition compared to controls ($p<0.05$).
Conclusion: PDE 5 and 11 are present in human prostate cancer tissue and contribute to the majority of cGMP PDE activity in prostate cancer cell lines. Inhibition of PDE activity, reestablishing cGMP cell signaling in the hypoxic tumor environment, would appear to have beneficial effects including modulating hypoxia-induced cancer immune escape. These results indicate that PDE inhibition may represent a novel therapeutic or adjuvant target for men with prostate cancer.

MP-08.06
A Mechanism of Hypoxia-Induced Immune Escape in Prostate Cancer Cells
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Introduction: We previously showed that hypoxia induces resistance to NK cell-mediated lysis in prostate cancer cells through a mechanism that involves the shedding of NK cell-activating ligands (MICA) from the cell surface. Furthermore, we were able to block this hypoxia-induced shedding of MICA, as well as resistance to NK-mediated lysis, by activating NO signalling in the cancer cells. Here we explore the mechanisms of this hypoxia-mediated prostate cancer immune escape.

Methods: To determine whether tumour cell resistance to lysis by NK cells is dependent on HIF-1transcriptional activity we knocked down HIF-1a using validated siRNA. We investigated hypoxic regulation of the metalloproteinase disintegrins ADAM 10 and 17 utilizing Western blot, qPCR and confocal immunofluorescence and their role in hypoxia-induced shedding of MICA (on flow cytometry) with siRNA knockdown experiments. Standard 4-hour chromium release assays were used to determine effects on NK-mediated lysis. Finally, we examined whether endogenous nitric oxide signalling regulates the hypoxia-induced up-regulation of the ADAMs and HIF-1a using nitroglycerin or 8-bromo-cGMP.
Results: Knockdown of HIF-1a in DU145 prostate cancer cells abrogated the hypoxia-induced down-regulation of surface MICA levels ($p<0.001$(ANOVA) as well as hypoxia-induced resistance to NK cell-mediated lysis. ADAM17 expression decreased in hypoxic conditions but ADAM10 increased and was confirmed by confocal immunofluorescence and qPCR ($p<0.05$). Down-regulation of ADAM10 expression significantly attenuated the hypoxia-induced release of MICA on flow cytometry and forced down-regulation of HIF-1a expression prevented the hypoxia-induced increase of ADAM10 transcript levels ($p<0.01$). Activation of nitric oxide signalling with either nitroglycerin (1 μM) or 8-bromo-cGMP (10 nM) effectively attenuated the hypoxia-induced increases in ADAM10 transcript levels. Also, Western blot analysis revealed that nitroglycerin (1 μM) was able to block the accumulation of HIF-1a in DU145 cells incubated in hypoxia.
Conclusions: Our findings demonstrate a novel mechanism by which hypoxia contributes to immune escape in prostate cancer cells. Furthermore, they reveal that activation of nitric oxide signalling interferes with this pathway. These findings are important because they indicate that nitric oxide mimetics could potentially be used as immunosensitisers in the treatment and/or prevention of cancer.

MP-08.07

Incidence of Vitamin D Defficiency in Patients Presenting with Urolithiasis to Tertiary Stone Clinic

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Introduction and Purpose: Replacement of vitamin D in deficient patients has risen in popularity in recent years. However, its incidence in patients with urolithiasis is not known. The aim of the present study was to assess vitamin D deficiency in patients presenting with urolithiasis to tertiary stone clinic**.**
Patients and Methods: A retrospective review of prospectively collected data on patients presenting to stone clinic from August 1st, 2009 to January 31st, 2010 was performed. Demographic data including previous medical and surgical histories were collected. Metabolic stone work-up included serum 25-hydroxy vitamin D (D_1) measuring vitamin D stores. Vitamin D deficiency was defined as D_1 <20 ng/mL (50 nmol/L) while 21 to 29 ng/mL (52 to 72 nmol/L) indicated vitamin D insufficiency.
Results: Out of 101 patients having D_1 levels measured, 81 (80.2%) patients were found to have inadequate vitamin D stores. Thirty-four (33.7%) patients were deficient and 47(46.5%) had insufficient stores of VD. For vitamin D inadequate patients, the mean age was 50.4±15.8 years (range: 14-87), 42% were smokers, 51% were recurrent stone formers and 54% had positive family history of urolithiasis. Secondary hyperparathyroidism was detected in 25.3%.
Conclusions: Vitamin D deficiency and insufficiency is common in patients presenting with urolithiasis. The safety of vitamin D supplementation in this population needs to be further evaluated.

MP-08.08

Comparing Different Decellularization Protocols of Animals' Bladders for Mesenchymal Stem Cell-Based Tissue Engineering

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Introduction and Objective: Artificial bladder tissue is a constant need in reconstructive surgery. Intestine can be used but it has several deleterious complications. We are aiming to produce xenogenic decellularized bladder seeded with autologous mesenchymal stem cells for bladder replacement/augmentation in human. We are reporting the first stage of this work which is decellularization protocol for efficient removal of cells and nuclear debris in three species. We also demonstrate the adherence and differentiation of mesenchymal stem cells on the decellularized scaffolds.
Methods: This study compared four decellularization protocols for bladders of three species: rat, rabbit and porcine. We first determined which detergent (1% SDS or 1% Triton X-100 in hypotonic Tris-HCl) was more efficient at removing cytoplasmic debris while preserving the structural anatomy. This was done by H&E staining of paraffin sections. We also determined the optimal duration of decellularization. After extensive washes, we verified whether deoxyribonuclease (DNase) digestion of nuclear debris was necessary and adequate using 4',6-diamidino-2-phenylindole (DAPI) staining. We then analyzed the resultant decellularized extracellular matrices for evidence of preserved active growth factors (VEGF, TGFβ1, EGF, TGFβ and matrix proteins collagen (type 1, 2, 3, 4), laminin, and elastin by histology, immuno-fluorescence staining and confocal microscopy. We then tested the ability of mesenchymal stem cells to adhere and differentiate into smooth muscle cells on these scaffolds.
Results: Both detergents were equally efficient at removing cytoplasmic debris. The duration of detergent treatment proved to be critical here. Triton X-100 appears to preserve the extracellular matrix better. DNase digestion was always necessary for complete removal of nuclear debris. Mesenchymal stem cells adhered well on the scaffolds.
Conclusions: The use of Triton X-100 in hypotonic buffer followed by nuclease digestion is efficient for production of decellularized bladder tissue. This method preserves the structural anatomy, extracellular matrix proteins, and growth factors within bladder tissue. Moreover, mesenchymal stem cells adhere well on these scaffolds and remain viable after 2 weeks in vitro.

MP-08.09

Bone Marrow Mesenchymal Stromal Cell Therapy for External Urethral Sphincter Restoration in a Rat Model of Stress Urinary Incontinence

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Introduction and Objective: To assess the effect of intra-sphincteric injections of bone marrow mesenchymal stromal cells (MSCs) on Valsalva leak point pressure (VLPP) changes in an animal model of stress urinary incontinence (SUI).
Methods: 24 female Sprague-Dawley rats underwent bilateral pudendal nerve section for SUI induction. Six rats were SUI controls, 6 received periurethral injection of Plasma-Lyte (SUI placebo control) and 12 were given periurethral injection of PKH26-labelled MSCs. Four weeks after injection, conscious cystometry was undertaken in animals and VLPP recorded. All groups were sacrificed, and frozen urethra sections were submitted to pathology assessment.
Results: Rat MSCs were positive for the cell surface antigens CD44, CD73, CD90, and RT1A, and negative for CD31, CD45, and RT1B, confirming their stem cell phenotype. *In vitro,* differentiated MSCs expressed α-smooth muscle actin (SMA) and desmin, markers of smooth and striated

muscles. Immunohistochemistry of rat urethras revealed PKH26-labelled MSCs *in situ* and at the injection site. LPP was significantly improved in animals infused with MSCs. Mean LPP was 24.28 ± 1.47 cm H_20 in rats transplanted with MSCs and 16.21 ± 1.26 cm H_20 in SUI controls injected with Plasma Lyte ($p<0.001$).

Conclusions: Bone marrow rat MSCs have the ability to differentiate and skew their phenotype towards smooth and striated muscles, as demonstrated by SMA up-regulation and desmin expression. Periurethral injection of MSCs in an animal model of SUI restored the damaged external urethral sphincter and significantly improved VLPP

MP-08.10

Metabolic Abnormalities Of Vitamin D Deficient Patients Presenting With Urolithiasis

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Introduction and Objectives: Vitamin D deficiency is common in the general population and in patients presenting with history of urolithiasis. Currently, there are no data regarding metabolic abnormalities of these vitamin D inadequate patients with history of urolithiasis. The aim of the present study was to determine urinary and serum abnormalities in patients presenting with urolithiasis and found to have deficient or insufficient vitamin D stores.

Methods: A retrospective review of prospectively collected data on patients presenting to stone clinic from August 1st, 2009 to January 31st, 2010 was performed. Patients with vitamin D_1 levels <74 nmol/L indicating insufficient or deficient vitamin D stores were included in the study. Demographic data including previous medical and surgical history were recorded. Metabolic stone workup including two 24-hour urine collections and serum normalized ionized calcium and PTH was performed.

Results: Sixty-nine patients were included in the study with mean age of 52.8±14.6 years (range: 14-87) and a mean BMI of 28.7± 5.8 kg/m^2. Hyperparathyroidism was detected in 25.3%. Hypocalcemia was found in 36.2% and hyperuricemia was found in 11% of patients. Ninety-three percent of the 24-hour urine collections had at least one abnormality. The most prevalent pattern of urinary abnormalities in decreasing frequency were: suboptimal volume (<2 L/day) in 45%, hypocitaturia in 24%, hypocalciuria in 33%, hyperuricosuria in 16%, cystinuria in 5%, and hyperoxaluria in 3% of patients. Most importantly, hypercalciuria was observed in 20% of patients.

Conclusion: Patients with inadequate stores of vitamin D have high incidence of abnormalities on metabolic stone workup. These need to be taken into account prior to commencing any vitamin D replacement.