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ABSTRACTS

12th Congress of the European Society for Urological Oncology and Endocrinology (ESUOE)

March 6–8, 1997, Essen, Germany

Congress chairman: Prof. Dr. H. Rübben, Essen, Germany

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EDITORIAL

The European Society for Urological Oncology and Endocrinology exists to promote communication between basic and clinical researchers. The increasing interest is reflected in the submission of 175 abstracts for this year's meeting. The programm committee had the difficult task to select 100 abstracts for presentation. On this base we have an exciting scientific program with oral sessions, poster sessions and social activities to promote maximum contact among attendees.

Main topics of the congress are

- molecular and cell biology
- new techniques
- cell interactions, cell identity
- growth factors
- development of new in vivo and in vitro models
- pre-/clinical research

Main topics will be addressed by distinguished speakers demonstrating application of the topic to problems in Urology. These sessions will be highly interactive which will be facilitated by poster sessions.

The scientific and social program should meet the goals of the ESUOE Meeting in providing scientific excellence in an environment which maximizes interaction between distinguished guests, society members and non-member attendees.

I am very much looking forward to welcome you in Essen.

Herbert Rübben, Chairman, Essen

Cell interaction & growth factors

0 1.1

TRANSFORMING GROWTH FACTOR BETA RECEPTOR EXPRESSION IN HUMAN BLADDER CARCINOMAS AND IN HUMAN BLADDER CELL LINES

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Introduction and objectives: Reduced or loss of expression of TGF-ß receptors (TGF-ßrI-III) are thought to play a critical role when cells become TGF-ß-resistant. Therefore we looked at receptor expression in various bladder tumors as well as in different bladder cell lines.

Methods: mRNA expression was determined by semiquantitative RT-PCR in relation to GAPDH. Protein expression was studied by Western blots. Proliferation studies were performed by a colorimetric MTT-assay. In order to seperate epithelial cells from fibroblasts we used selective culture media.

Results: Invasive bladder tumors (n=7) showed a higher risk for reduction or loss of TGF-ß receptors types I, II, or III as compared with normal urothelium (n=9) and superficial tumors (n=8), repectively.

Primary epithelial cells as well as fibroblasts were growth inhibited by TGF-ß1 to 34-87% of untreated controls whereas the permanent cell lines HT-1197 and RT-4/31 were stimulated by TGF-ß1. However, alterations in receptor expression between primary cells and permanent cell lines were detected neither on mRNA nor on protein levels.

Conclusions: Loss or downregulation of TGF-ß receptors seems to be a critical step in bladder tumor progression. However, TGF-ß resistance of cells in vitro does not correlate with loss or reduced receptor expression.

0 1.2

EXPRESSION AND FUNCTIONAL ANALYSIS OF TRANSFORMING GROWTH FACTOR β_1 AND ITS RECEPTORS IN NORMAL HUMAN UROTHELIUM AND HUMAN TRANSITIONAL CELL CARCINOMAS (TCC).

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INTRODUCTION: Previous studies suggested that Transforming Growth Factor β_1 ($TGF\beta_1$) has important regulatory functions in murine urothelium. An important role has also been suggested for several human cancers. However, its expression and functional effects in normal human urothelial cells or bladder cancer are not well known.

METHODS: The expression of $TGF\beta_1$ and $TGF\beta$ specific receptor was semi-quantified by immunocytochemistry and in situ hybridization with riboprobes in normal human urothelium and TCC. Functional effects of $TGF\beta_1$ on proliferation, differentiation and reepithelialisation of primary normal human urothelial cultures were evaluated in a model reflecting urothelial regeneration . The effect of $TGF\beta_1$ on the proliferation of four primary human TCC cell lines was determined

RESULTS: By immunocytochemistry, higher expression levels of $TGF\beta_1$ and $TGF\beta$ type II receptor were found in superficial and basal layers of normal urothelium compared to the intermediate layer. This distribution was conserved in superficial tumors. In invasive tumors, all tumor cells stained intensely for both proteins. $TGF\beta$ receptor type I was present in all layer of normal urothelial or TCC cells. In situ hybridization pointed out that all cell layers in normal urothelium contain similar mRNA levels. Functionally, $TGF\beta_1$ inhibited the proliferation of normal urothelial cells, but stimulated their migration during the first 24 hours after damage. In contrast, $TGF\beta_1$ barely affected the proliferation of TCC cells.

CONCLUSION: Our data show the differential distribution of $TGF\beta_1$ and its receptors in human urothelium. The functional data demonstrated that $TGF\beta_1$ affects migration and proliferation of normal urothelial cells. In contrast, a loss of negative $TGF\beta_1$ regulation of TCC growth was noted.

0 1.3

ANDROGEN-DEPENDANT SECRETION AND PROLIFERATION OF HUMAN OSTEOSARCOMA CELLS HOS TE85 (TE85): EXPRESSION OF PROSTATE-SPECIFIC ANTIGEN (PSA) AND TYPES I AND II 5α-REDUCTASE (5α-R) ACTIVITIES

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Introduction: We analyzed the potential role of bone in prostate cancer (Pca) metastasis using TE85 cells. [^{3}H]-testosterone (T) metabolism and the effect of PSA purified from seminal plasma was also evaluated.

Methods: TE85 cell [^{3}H]-T metabolism was characterized in the presence or absence of type I and II selective 5α-R inhibitors (5α-RIs). Type I and II 5α-R mRNA and PSA mRNA levels were evaluated using semi-quantitative competitive reverse transcriptase polymerase chain reaction. Immunoreactive PSA (iPSA) was quantified by ELISA before and after T stimulation.

Results: We observed time-dependent [^{3}H]-dihydrotestosterone (DHT) formation in TE85 cells. Type I 5α-R isozyme activity predominated as reflected by potent inhibition (IC_{50} = 9nM) of TE85 [^{3}H]-DHT formation by the type I selective inhibitor LY306089. The type II-selective 5α-RI SKF 105657 was much less potent (IC_{50} = 890nM). Levels of 5α-R I and II mRNA were not significantly different. After T stimulation (10^{-10}M), TE85 cell numbers increased. After treatment, iPSA was detected in the media (0.17 ng/ml) and PSA mRNA was stimulated 3.1-fold. Interestingly, PSA stimulated TE85 cellular proliferation at low (3-10ng/mlPSA) concentrations [max. response = 195 ± 22 % over control (p< 0.05)].

Conclusions: T stimulates TE85 cellular proliferation and PSA secretion. In addition, TE85 cells proliferate in response to PSA and express 5α-R enzymatic activity. To the extent that these cells express a bone phenotype, TE85 has potential ability to study cellular interactions mediating Pca osseous metastasis.

0 1.4

PHOSPHOLIPID REQUIREMENT OF 5α-REDUCTASE IN HUMAN BENIGN PROSTATIC HYPERPLASIA (BPH)

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Various studies indicated that membrane components are necessary for optimal 5α-reductase activity. Using two approaches, we investigated the relationship between 5α-reductase activity and membrane environment. First, epithelial and stromal cell homogenates of human BPH were treated with phospholipase A_2 (PLA_2) and C (PLC) to modify specifically the structure of cellular phospholipids. Second, the effect of phospholipids of known structure on 5α-reductase was investigated. BPH tissue was separated mechanically in epithelium and stroma. 5α-reductase activity was determined using various testosterone (12-585 nM), phospholipase (0.01-100 U/ml) and phospholipid concentrations (10-5000 µg/ml). The androgen metabolites were separated by HPLC. Results: (1) Both PLA_2 and PLC inhibited 5α-reductase activity dose-dependently, the inhibition being significantly (p<0.01) stronger by PLC. (2) In epithelium and stroma, PLA_2 and PLC gave rise to a significant (p<0.05) decrease of V_{max}. In epithelium, the K_m increased significantly (p<0.05) after adding PLC. (3) Neither in epithelium nor in stroma, phosphatidylethanolamine, phosphatidylinositol, and sphingomyelin had any effect on 5α-reductase activity. (4) In epithelium, 5α-reductase activity [relative activity ± SEM] was enhanced by phosphatidylserine (459% ± 66), phosphatidylcholine (137% ± 10), and phosphatidic acid (208% ± 25). (5) On the other hand, in stroma phosphatidylserine, phosphatidylcholine, and phosphatidic acid were unable to stimulate 5α-reductase. In conclusion, the impact of phospholipases and phospholipids on the activity of 5α-reductase is specific and structure related. Our data support the hypothesis that the 5α-reductase activity will be regulated by the interaction with phospholipids.

0 1.5

ANDROGEN RECEPTOR DELETION MUTANTS IN THE CROSS-TALK BETWEEN ANDROGEN AND POLYPEPTIDE GROWTH FACTOR SIGNALING PATHWAYS.

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The activity of the androgen receptor (AR) can be up-regulated by stimulators of protein kinases in the ligand-dependent and ligand-independent manner (Culig et al: Cancer Res 54:5474, 1994; Nazareth and Weigel: J Biol Chem 271:19900, 1996; Reinikainen et al: Endocrinology 137:4351, 1996). This cross-talk between androgen and growth factor signaling pathways may be operative in advanced prostate cancer. We studied molecular mechanism of this type of receptor activation by generating 5'-truncated AR expression vectors. Prostate cancer cells DU-145 were cotransfected with a reporter gene which was driven by a promoter consisting of two androgen-responsive elements in front of a TATA box and with either wild-type or one of the truncated plasmids. The reporter gene activity was measured after incubation with increasing concetrations of the synthetic androgen methyltrienolone and/or a growth factor. Effects of insulin-like growth factor (IGF-I) and epidermal growth factor (EGF) were assessed. Increase in the reporter gene activity was measured with the wild-type receptor after stimulation by methyltrienolone, by IGF-I and EGF even in the absence of androgen, and by low doses of androgen and growth factors in a synergistic manner. When truncated ARs were expressed in DU-145 cells, the AR transactivation function induced by growth factors was affected stronger then that induced by androgen alone. This reduction of the reporter gene activity was observed with the mutant receptors δ440 AR, δ473 AR, and δ488 AR. In the presence of the mutants δ510 AR and δ536 AR neither androgen nor growth factors induced an increase in the reporter gene activity. Our data suggest that the AR amino-terminal sequences have an important role in the cross-talk between androgen and peptide growth factor signaling pathways.

0 1.6

IGF-I AND IGF-II ARE POTENT MITOGENS FOR BENIGN PROSTATIC EPITHELIUM AND STROMA.

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Primary epithelial cells derived from BPH, prostate cancer and normal tissues do not secrete detectable levels of either IGF-I or IGF-II (1). In contrast prostatic stromal cells produce readily detectable levels of IGF-II in vitro (2). Furthermore it has been observed that IGF-II gene expression and secretion is elevated in BPH-derived stroma compared with cells derived from normal and cancer tissues. These results elude to a potential role for IGFs in the pathogenesis of BPH.

RT-PCR analysis has demonstrated that cultured benign epithelial cells (BE; PO) and stromal cells (BST; P1-4) exhibit high level expression of the IGF-I receptor. The presence of IGF-I receptor protein in these cells was confirmed by Western blotting. Under serum-free conditions, IGF-I (500ng ml^{-1}) and IGF-II (5 ng ml^{-1}) induce a 35% and 80% stimulation in epithelial cell numbers respectively compared to control. Likewise IGF-I (500 ng ml^{-1}) and IGF-II (500 ng ml^{-1}) stimulate an 80-113% increase in stromal cell numbers. To the best of our knowledge this is the first report demonstrating the stimulation of benign prostate cell growth by IGF-I and IGF-II . These findings suggest that the IGFs may operate as paracrine and autocrine growth factors within the prostate and that they may be involved in the cellular proliferation characteristic of BPH.

1. Cohen et al. 1991 J Clin Endocrinol Metab 73: 401-407.
2. Cohen et al. 1994 J Clin Endocrinol Metab 79: 1410-1415.

0 1.7

NEUROENDOCRINE DIFFERENTIATION IN THE PC-295 HUMAN PROSTATE CANCER XENOGRAFT MODEL

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Introduction: It is supposed that neuroendocrine (NE) cells form an androgen independent subfraction of the prostatic epithelium. These cells may play a role in prostate cancer (PC) growth by the secretion of neuropeptides with growth modulating properties in a paracrine way. In the androgen dependent PC xenograft model PC-295 NE differentiation can be induced by androgen withdrawal (Am.J.Pathol, 149: 859-71, 1996). PC-295 tumors continuously labelled with bromo-deoxy-uridine (BrdU), an S-phase cell cycle marker, 48 hours prior to castration showed no double labeling of NE cells with BrdU or proliferation marker MIB-1, indicating that NE cells differentiated from G_0-phase arrested cells. We further examined if the NE cells are G_0-phase arrested cells indeed.

Materials & Method: A time-course castration experiment with PC-295 tumor bearing nude mice was performed. Mice were sacrificed at day 0, 0.5, 1, 1.5, 2, 4 and 7 after castration. Mice from all the different time points received BrdU one hour prior to sacrifice, whereas some of the mice received BrdU continuously as of the moment of castration. BrdU labeling, apoptotic index by using TUNEL method, MIB-1 score and expression of Chromogranin A (CgA), the general NE marker, were analyzed by immunohistochemistry or Western analysis.

Results: The tumor regressed 50% in 4 days. Androgen receptor expression decreased from 90% to 0% within 2 days. BrdU incorporation and MIB-1 expression decreased, from control levels of 10% and 20%, respectively, to almost 0% within 4 to 7 days. The number of neuroendocrine cells increased from $0.1*10^4/mm^2$ (n=3) to $1.4 \pm 0.7*10^4/mm^2$ (n=6) after 4 days and $6.0 \pm 1.2*10^4/mm^2$ (n=4) at day 7. The apoptotic index was maximally 20% at day 4. Double labeling of CgA with BrdU showed that after 2 and 4 days of continuous BrdU labeling, 5% and 10% of the NE cells were BrdU positive, respectively. In non-NE cells these figures were 40% to 70%, respectively. Tumors labelled with BrdU 1 hour prior to sacrifice showed no double labelled NE cells.

Conclusion: Androgen withdrawal in the PC-295 model leads to a rapid tumor regression and induction of NE differentiation, which was shown to be a proliferation independent process. The NE differentiated cells are for the greater part G_0-phase arrested cells. The NE cells that were BrdU positive are probably NE differentiating cells that entered S-phase shortly after castration. At the moment we are testing the expression of growth modulating neuropeptides in NE cells and looking for early events in NE differentiation.

Supported by the Dutch Cancer Society (Grant EUR 95-1029)

0 1.8

PRIMARY PROSTATIC EPITHELIAL CELL BINDING TO HUMAN BONE MARROW STROMA AND THE ROLE OF α2β1 INTEGRIN

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Prostate cancer selectively metastasises to the bone. To look at the importance of prostate epithelial cell adhesion to bone marrow cells in this process we examined the binding of human primary prostatic epithelial cells to human bone marrow stromal cultures. Prostatic epithelia (PEC) and fibroblasts (PFB) were prepared by collagenase and trypsin digests from samples of benign prostatic hyperplasia and prostate cancer. Bone marrow stromal (BMS) cultures were grown from the bone marrow of patients with benign disease. BMS, or PFB, or extracellular matrix molecules (10 ug/ml of collagen I, fibronectin, vitronectin or laminin) were plated in 96 well plates. PEC were seeded into these wells, washed off at increasing time intervals (1 to 48 hours) and then immunohistochemically stained for cytokeratin. The number of epithelial cells bound/well was then counted. The role of specific integrins were examined by inhibition studies, preincubating the BMS for 1 hour with disintegrin or integrin monoclonal antibody before completing a further 24 hour binding assay. Maximum binding of primary epithelial cells was achieved at 24 hours. Prostate epithelia from both malignant (mPEC) and benign (bPEC) tissue showed increased adhesion to BMS (340% and 200% respectively) in comparison to benign PFB or uncoated culture plates. Adhesion was also increased by type I collagen and fibronectin but not vitronectin and laminin. Disintegrins significantly inhibited 63% of mPEC samples and 13% of bPEC binding to BMS ($p<0.05$). Specifically, β1 and α2 integrin antibodies consistently inhibited PEC binding to BMS, whereas α3 and α5 integrin antibodies did not. In conclusion, BMS is adhesive for PEC derived from both malignant and benign tissues. The integrin α2β1 is a major contributor to this interaction.

O 1.9

ENHANCED CYTOSTATIC EFFECT OF POLYAMINE SYNTHESIS BLOCKADE BY SIMULTANEOUS INHIBITION OF POLYAMINE TRANSPORT IN DU-145 PROSTATE CANCER CELLS.

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Polyamines (putrescine, spermidine and spermine) are cellular components that are essential for mammalian cell growth. Inhibition of polyamine biosynthesis has therefore been considered as a potential anti-cancer treatment. One of the problems encountered, however, is that intracellular polyamine depletion activates a transmembrane polyamine transport system, leading to compensatory polyamine uptake from the extracellular environment. Effective therapeutic use of polyamine synthesis inhibitors would therefore require simultaneous inhibition of the transport system. Although the molecular identity of polyamine transporters has not been elucidated, a potent inhibitor of polyamine uptake, a polymeric spermine conjugate (pSPM), was recently described (Aziz et al., J. Pharmacol. Exp. Ther., 274:181-186, 1995). We have investigated the effects of pSPM, in combination with the polyamine synthesis inhibitor DFMO, on DU-145 prostate cancer cells.

Exposure of DU-145 cells to 1 mM DFMO in medium containing 7.5% fetal calf serum inhibited its proliferation (51% of control, determined by MTT-assay). Silica treatment of the serum (which removes polyamines), resulted in substantially larger growth inhibition (20% of control) though the growth of control cells was not affected. The effect of DFMO on DU-145 cells was completely reversed by the addition of spermidine at concentrations as low as 1 μM. Exposure to pSPM resulted in a dose-dependent growth inhibition under standard culture conditions and partially prevented the reversal of DFMO-induced inhibition by exogenous spermidine. The interference of pSPM with the polyamine transport system was demonstrated by the inhibition of MGBG uptake (95% inhibition by 1 μM pSPM) and by a shift in the sensitivity to MGBG (10-fold reduction in the presence of 1 μM pSPM).

These results indicate that inhibition of exogenous polyamine uptake can enhance therapeutic efficacy of polyamine biosynthesis inhibitors.

O 1.10

RE-ESTABLISHMENT OF THE CADHERIN-CATENIN PATHWAY IN PC3 CELLS LEADS TO NON-TUMORIGENIC PHENOTYPE AND REORGANIZATION OF CYTOSKELETAL FILAMENTS

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Introduction: PC-3 is an α-catenin deficient prostate cancer cell line, which is losing the tumorigenic potential after re-establishment of the cadherin-catenin pathway. These α-cat positive PC3 cells demonstrate a dramatic change in morphology, which we investigated in the present study.
Methods: α-cat deficient PC-3 cells (wt) were treated by microcell transfer of chromosome 5, resulting in the adhesive phenotype (adh). Re-establishment of the cadherin-catenin pathway is confirmed by northern and western analysis. Cytoskeletal filaments were evaluated by Immunofluorescense for actin, microtubular and intermediate filaments. Nuclear morphology was assessed by morphometry of nuclear roundness, form and cross-area. Time lapse video experiments were performed to study motility and cell-cell interaction in wt and adh PC3 clones.
Results: E-cad positive PC3 cells lack α-cat, whereas adh PC3 cells demonstrate expression of the E-cad/α-cat complex by northern and western analysis. Immunofluorescense demonstrates expression of cytoskeletal filaments in both, wt and adh clones, but the filament architecture is completely reorganized in the adh, whereas the wt demonstrates non-organized cytoskeletal proteins. The nuclear area of the wt and adh was similar (142vs139), but the adh demonstrated a more homogeneous size population. A significant difference was found in the ferret diameter, a measure of the elliptical nature of nuclei, demonstrating uniform elliptical nuclei in the adh (ratio 1.55) compared to heterogeneous nuclei of the wt (1.34). Time lapse experiments demonstrate uncoordinated cell movement and minimal cell-cell contacts in the wt, but coordinated cell-cell interaction in the adh, suggesting a re-established cell-signaling pathway after microcelltransfer.
Conclusion: Our results demonstrate the importance of the cad-cat pathway not only for the cell adhesion but also for signal transduction and cell signaling. We conclude that reorganization of the cytoskeleton and nuclei in adh PC-3 cells provide a biological model of tensegrity, a theory of mechano-transduction pathways of non-compressional cell elements influencing nuclear matrix and gene expression.

O 1.11

CADHERIN EXPRESSION IN PROSTATE CANCER.

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Changes in cell-cell interactions are critical in the process of cancer progression. Likewise, it was shown that loss of expression of the cell adhesion molecule E-cadherin is associated with grade, stage, and prognosis in many carcinomas, including bladder and prostate cancer. Impaired cadherin-mediated interaction results in an invasive phenotype which most likely explains the correlation with a more aggressive biological behaviour. It is not clear, however, how the cells that detach from the primary site, migrate and establish themselves at certain preferential secondary sites.

In order to investigate whether cadherins can also play a role in cell-cell interactions in the migration of tumor cells and in the establishing of metastases, we used a cloning strategy with degenerate primers for sequences that are highly conserved between the various cadherins. Primers for both the extracellular repeats as well as for the catenin-binding domain were used. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using mRNA from the human prostate cancer cell lines PC-3 and TSU-Pr1 as template.

We thus identified E-cadherin to be expressed in PC-3 cells (as was already known) but also several other cadherins (N-, R-, K-, OB-, and proto-cadherin) are expressed in PC-3 and/or TSU-Pr1 cells. Next, we analyzed the expression of the different classical and proto-cadherins as well as of the catenins in seven human prostate cancer cell lines. Interestingly, the cell lines that lack expression of α-catenin, ie, PC-3, PPC-1, and ALVA-31 and thus have an impaired E-cadherin-function, express several other cadherins. Also the cell lines that lack expression of E-cadherin, ie, TSU-Pr1 and JCA-1, show expression of other cadherins.

These data suggest that acquisition of an invasive phenotype may not only be associated with loss of E-cadherin function, but also with (upregulation of) expression of other classical- and protocadherins. Further studies will have to elucidate the role of these cadherin molecules in prostate cancer invasion and metastasis.

O 1.12

ENDOCRINE REGULATION OF CYTOSKELETON AND APOCRINE SECRETION IN THE RAT DORSAL PROSTATE

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Apocrine secretion is a highly specialized form of release of secretory material from glands, especially from the prostate. Its most peculiar form is found in the rat dorsal prostate, where blebs with secretory proteins are released. The participation of the cytoskeleton in apocrine secretion and during the formation of the so-called prostasomes have not been studied so far.
We have used semi- and ultrathin sections of dorsal prostates from intact, carbamylcholine-stimulated, castrated and estrogen-treated rats which were processed for the co-localization of particular secretory proteins (65, 70 and 115 kD, respectively) with cytoskeletal proteins (ß-actin, α-actinin, myosin, gelsolin, villin, fodrin and tubulin).
In the intact rat, the secretory blebs contain 65 kD and 70 kD proteins in apocrine blebs, along with myosin, gelsolin and ß-actin. The 115 kDa protein is restricted to secretory granules in the Golgi region. In the blebs, the cytoskeletal and secretory proteins are homogeneously distributed. Only ß-actin is often concentrated at nipple-like protrusions, perhaps the former stalk from where the blebs derive. In estrogen-treated animals the prostatic epithelium is low and apocrine blebs are usually lacking. At the apical cell pole, ß-actin is concentrated at the junctional system. After castration, no apical blebs are left. α-actinin is the only cytoskeletal protein that is present and is concentrated in the Golgi region. Our findings indicate that (i) apocrine secretion is a physiological secretory mechanism in the rat dorsal prostate and (ii) that secretory, cytoskeletal and membrane proteins involved in this process are hormonally regulated in a differential manner.

O 1.13

HYPOXIA INDUCES APOPTOSIS IN THE RAT VENTRAL PROSTATE

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The mechanisms involved in the castration-induced involution of the ventral prostate (VP) are not fully clarified. In an earlier experiment, we have seen a blood flow decrement that precedes the apoptotic involution of the VP. One theory could be that the blood flow decrement causing a relative hypoxia might be a factor of importance for the development of apoptosis. We therefore examined if transient ischemic hypoxia could induce apoptosis in the VP.

Methods: Male rats of 350-450 g were laparotomized and the right iliacal artery was dissected and clamped for 60 minutes. This decreased the blood flow to the ipsilateral lobe of the VP to less than 25 %, as measured with radioactive microspheres. Sham-operated rats were used as controls and another group were given a bolus dose of testosterone to counter any stress-induced decrease in testosterone blood levels. The VPs were taken out 12 or 24 hours after the hypoxic period, frozen in liquid nitrogen or fixed in formalin, and stained with ISEL-immunohistochemistry. The amount of apoptosis was quantified by light microscopy on a grid under 400x magnification and given as a percentage of ISEL-stained epithelial cells. Apoptosis was also examined by DNA laddering and Northern blotting against TRPM-2.

Results: Animals subjected to ischemia of the right ventral prostatic lobe showed a significant increase in the ratio of apoptotic to normal glandular epithelial cells. Administration of a bolus dose of testosterone did not prevent the apoptotic development.

Conclusion: The results of this study support the notion that hypoxia is a factor that affects the development of apoptosis in the ventral prostate of the rat. This relationship has also been seen in other organs, such as brain, and kidney. The importance of this finding in relation to the castration-induced apoptosis needs to be further clarified.

O 1.14

INDUCTION OF APOPTOSIS IN LNCAP HUMAN PROSTATE CANCER CELLS BY THE PHORBOLESTER TPA IS RELATED TO A PROLONGED TRANSLOCATION OF PROTEIN KINASE C α

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LNCaP androgen sensitive human prostate cancer cells undergo apoptotic cell death in response to 12-0-tetradecanoyl-phorbol-13-acetate (TPA). A class of proteins that bind to and are activated by TPA have been identified: protein kinase C (PKC) isozymes. In the absence of TPA, LNCaP cells express mRNAs for PKC α, δ, ε and η, a very low amount of β2-chimaerin mRNA, but no detectable PKC β, γ θ or α-chimaerin.

Six to twelve hours after addition of continuous TPA (10nM), a strong induction of PKC α mRNA expression was found, which decreased by 24 to 48 hours. No changes in PKC δ, ε or η mRNA levels were observed after addition of TPA. Immunoblot analysis revealed partial translocation of PKC α from cytoplasm to plasma membrane fractions in LNCaP cells within 54 minutes after addition of TPA. LNCaP cells exposed to a two hour pulse of TPA underwent cell death over the next 3 days to the same degree as cells in the continous presence of TPA. Under these conditions PKC α levels remained high in both cytoplasm and plasma membrane at least 48 hours after TPA addition, 24 hours after DNA fragmentation was observed. A sub-population of LNCaP cells was selected for normal growth in the presence of 10 nM TPA. After withdrawal of TPA from these cells for 16 hours, re-addition of TPA yielded undetectable PKC α protein in both cytoplasm and membranes and normal growth, whereas removal of TPA for 10 days with re-addition of TPA for 4 hours was related to recovery of PKC α and subsequent cell death.

Thus, translocation, and presumably activation of PKC α is associated with TPA induction of apoptosis in LNCaP cells, whereas down-regulation of this isozyme correlates with resistance to TPA.

Molecular biology & genetic

O 2.1

PRELIMINARY MOLECULAR CHARACTERISATION OF A PSA VARIANT WITH REDUCED BINDING TO α1-ANTI-CHYMOTRYPSIN.

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A patient has been identified whose PSA was not recognized in some assays but readily detected in others (Van Duijnhoven et al; Clin. Chem. 1996; 42: 637-41). Based on the very high proportion of free PSA detected, it was hypothesized that this PSA species is hampered in the formation of complexes with α1-antichymotrypsin (ACT) because of a mutation in the PSA molecule. To test this hypothesis, rt-PCR was performed on archival paraffin material with three partially overlapping primer sets, spanning the entire PSA coding sequence. The transplantable human prostate carcinoma line PC-82 (courtesy Dr. G.J. van Steenbrugge, Erasmus University Rotterdam), known to synthetize PSA was used as reference.

Preliminary **results** indicate that: 1] for the PC-82 tumour line the entire cDNA has been sequenced and the sequence is identical to the published PSA sequence. 2] For the patient's PSA, initial attention has focussed on exon 3, since the coding sequence for the ACT binding site resides in this exon. To date the nucleotides encoding amino acids 75-164, representing approximately one third of exon 3 and a small part of exon 4 have been cloned and sequenced. In addition to the wild type PSA sequence, a product missing 123 nucleotides has been found. The predicted protein encoded by this sequence would miss amino acids 94-134. 3] The occurrence of such an aberrant PSA mRNA is not restricted to this patient since in other prostatic tissues, benign and malignant, comparable transcripts were detected. It is tentatively **concluded** that apart from wild type PSA in this patient indeed a mutated PSA molecule prevails. The (patho)-fysiological consequences of this observation need to be evaluated.

O 2.2

DD3: A NEW PROSTATE-SPECIFIC MARKER, STRONGLY OVEREXPRESSED IN PROSTATIC TUMORS.

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Using differential display analysis we identified a cDNA, DD3, that by Northern blot analysis detects two major transcripts that are highly overexpressed in 45 out of 48 prostatic carcinomas studied whereas no (or very low levels of) expression of these transcripts was detected in normal prostate or benign prostatic hyperplasia (BPH) tissue from the same patients. The level of overexpression of DD3 in prostatic cancers shows a trend towards a positive correlation with tumor grade.

Until now, we have not found any homology of DD3 with known genes and we have not yet been able to determine the open reading frame. Currently, we are analyzing 200 DD3-related cDNA clones. Initial results showed that we are dealing with a complex transcription unit: several alternatively spliced exons and at least four different poly-A-addition signals have been found.

Using RNA in situ hybridization (RISH) we have been able to show that the DD3 mRNA is specifically expressed in the epithelial cells of the prostate. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using DD3-specific primers showed that DD3-related products can be amplified from normal prostate and BPH tissue. More importantly, we found that DD3 expression is very prostate specific since no DD3-related product could be amplified under the same conditions in normal human artery, brain, breast, bladder, colon, duodenum, heart, liver, lung, ovary, pancreas, placenta, seminal vesicles, skeletal muscle, skin, spinal cord, spleen or testis. Also in the human prostate cancer cell lines ALVA-1, DU-145, JCA-1, PC-3, PPC-1 and TSU, no DD3-related products could be detected. Only in LNCaP cells could a product be amplified.

Our results indicate that DD3 is the most prostate-cancer-specific gene described to date and this makes it not only a promising new marker for prostate cancer but it may also provide us with new approaches for the treatment of advanced prostate cancer.

0 2.3

OSTEOPONTIN (OPN) IN PROSTATE CANCER: BIOLOGICAL ACTIVITY, IMMUNOHISTOCHEMICAL AND MOLECULAR ANALYSIS OF EXPRESSION AND DISTRIBUTION. G.N. Thalmann[1], P.Troncoso[2], R.E. Devoll[3], R.A. Sikes[4], R.Ball[1], U.E. Studer[1], M.C. Farach-Carson[3], L.W.K. Chung[4] [1]Dept. of Urology, Berne, Switzerland, [2]Dept. of Pathology, M.D. Anderson Cancer Center, Houston TX; [3]Dept. of Biochemistry, U.T. Dental Branch, Houston TX, [4]Molecular Urology and Therapeutics Program, Charlottesville VA.

Prostate cancer (PCa) invariably progresses to an androgen-independent state and forms nonrandom metastases in the axial skeleton. OPN, a noncollagenous acidic bone matrix protein, which has been associated with malignant transformation, has recently been shown to be a CD44 ligand. We determined the biologic activity of OPN in the LNCaP model of PCa in vitro and the expression and distribution of OPN in human PCa specimens by immunohistochemistry and Northern blot analysis. Growth curves of LNCaP and C4-2 PCa cells were established with rOPN and affinity column-purified antibody (OPN-AB). mRNA was extracted from 10 PCa cell lines and OPN expression determined. Archival paraffin-embedded tumor specimens of 31 patients undergoing radical prostatectomy and 7 undergoing palliative transurethral resection (pTURP) were deparaffinized and stained with OPN-AB using a biotin-streptavidine-peroxidase system. Tissue specimens of patients undergoing pTURP were snapfrozen, RNA extracted and RNA levels assessed by RNA blot analysis for OPN and GAPDH.

OPN stimulates growth of hormone-dependent PCa cells in vitro. Hormone-refractory PCa cells express elevated OPN mRNA levels. 18/31 (58%) radical prostatectomy specimens with peripheral zone cancer Gleason grade ≥ 6 stained focally positive for OPN. Three of 4 prostate cancers in the transitional zone stained positive. High grade PIN stained positive in 1/3 of cases (n=12). 2/7 of pTURP specimens evidenced strong positive staining. Northern blot analysis of pTURP specimens of patients with metastatic disease demonstrated distinctly elevated levels of OPN mRNA.

OPN is expressed in peripheral and transitional zone prostate cancer. OPN is highly expressed on the mRNA level in primary tumors of patients with metastatic disease.

0 2.4

ALTERED FIBRONECTIN PRE-mRNA SPLICING PATTERN UPON METASTATIC PROGRESSION IN DUNNING RAT PROSTATE TUMOR LINES

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Cellular changes that lead to metastatic behavior of cancer cells have only incompletely been characterized. Metastatic progression of several tumors is accompanied with altered production of extracellular matrix components. These micro-environmental changes may affect attachment, migration and differentiation of the cells, and may play a role in metastasis.

In the Dunning rat prostate tumor sub-lines, G (well-differentiated, non-metastatic), AT2.1 (anaplastic, non-metastatic), and MAT-LyLu (anaplastic, metastatic), fibronectin (Fn) mRNA is differentially expressed. On Northern blots, a high expression level is observed in AT2.1 cells, whereas the levels in G and MAT-LyLu cells are approximately four times lower. Unfortunately, identification of specific fibronectin splice variants on Northern blots is not possible, and we decided to investigate the mRNA expression of alternative exons EIIIA, EIIIB, and V in these cell lines using RT-PCR assays. An identical expression pattern of fibronectin splice variants is observed in G and AT2.1, where Fn-EIIIB/V, Fn-EIIIA/V, and Fn-V are the major transcripts. In the MAT-LyLu cells, however, Fn-EIIIB/V and Fn-V transcripts are down-regulated, and Fn-EIIIA/V remains as the major transcript.

These results show that fibronectin splice variants are differentially expressed in metastatic and non-metastatic Dunning sub-lines. Whether this differential expression pattern is involved in the metastatic behavior of MAT-LyLu cells needs to be established.

0 2.5

DIFFERENTIAL DISPLAY PCR FOR THE DETECTION OF ALTERED GENE EXPRESSION IN PROSTATE CANCER

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INTRODUCTION AND OBJECTIVES: Although prostate cancer (CaP) is one of the most common neoplasms in Europe, the genetic basis of this disease is not well understood. For the identification of genes related to the development of prostate cancer we used differential display PCR (DD-PCR).

METHODS: Prostatic tissue obtained during surgery for CaP were diagnosed by a reference pathologist. Specimen were used only if more than 80% of the assumed cancerous tissue consisted of tumor and no tumor was detectable in the corresponding benign tissue. RNA was extracted from frozen tissue according to the protocol of Chomczynski and Sacchi. The RNA of 9 patients with untreated CaP was analysed for differentially expressed genes by using the RNAimage Kit from GenHunter Co. Differentially expressed bands were reamplified, cloned and sequenced. RT-PCR with sequence specific primers was performed for 20 cycles with 7 tumor and 13 benign tissue samples (pairs of tissues from 5 patients) using the incorporation of α^{32}P-dATP for detection. Control RT-PCRs were performed with DNAse and RNAse digested cDNA. Northern-blot analysis was applied to identify the expression patterns of the cloned band in different tissues.

RESULTS: One band which was only expressed in the benign tissue has been identified by DD-PCR. RT-PCR with sequence specific primers revealed that this band N7 is expressed in 2 tumors and all benign tissues analysed. No expression was observed in the control RT-PCR. Northern blot analysis revealed that the N7 fragment is expressed in various tissues including prostate, testis, kidney and lung. Homology search in the GenBank database revealed a strong homology of the N7 fragment to an EST from senescent fibroblasts.

CONCLUSIONS: The data seem to indicate that DD-PCR could be used to identify tumor suppressor genes in prostate cancer. However, further studies are needed to identify the potential biological role of this gene in the development of CaP.

0 2.6

IMMUNOHISTOCHEMICAL DETECTION OF MOLECULES INVOLVED IN G1-RESTRICTION POINT CONTROL IN HUMAN UROTHELIAL LESIONS.

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Aim: To better understand the regulation of the restriction point of the cell cycle as a major target of tumor initiation in human bladder cancer, the expression of three important molecules, cyclinD1, p16 and pRB, known to be involved in cell cycle regulation at this point, was investigated with immunohistochemical staining techniques.

Methods: The three molecules were visualized in adjacent sections of 176 biopsies (antibodies against cyclin D1 [Progen], pRB [Oncogene Sciences], p16 [Pharmingen] and expression was compared among the three molecules and also in relation to tumor grade and stage in the area of staining. Cyclin D1 expression was quantitated with a true colour image analysis system in addition to semiquantitative scoring

Results: Correlation of image analysis values and semiquantitative scoring was very high. Cyclin D1 was markedly overexpressed in the group of dysplastic lesions and carcinomata in situ, paralleled by strong pRB staining. A different pattern with prominent p16 staining and low numbers of cyclin D1 positive nuclei was found in inflammatory lesions. In papillary tumors high cyclin D1 values were dominating, while negative cyclin D1 in combination with negative pRB were found in about 20 % of muscle invasive tumors. The other prominent subgroup in invasive tumors was solely negative for p16 (20%).

Conclusion: Data obtained strongly indicate a role of cyclin D1 in carcinogenesis of bladder cancer. By looking at the related oncogene and tumor suppressor gene products an important screening method to focus on specific mutational analysis to further understanding of bladder cancer initiation and growth control is provided.

O 2.7

CLONING OF A YEAST GENE CONFERRING CISPLATIN RESISTANCE ON TESTIS TUMOUR CELLS.
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In contrast to prostate and bladder cancer, testicular germ cell tumours are highly sensitive to DNA-damaging agents. To determine the genetic basis for the curability of testis tumours we have attempted to complement cisplatin sensitivity using yeast and human DNA libraries. Testis tumour cells are difficult to grow *in vitro* and have low transfection frequencies, making conventional expression cloning strategies impractical. To overcome this problem, we have expressed the EBNA-1 gene in a testis tumour cell line, SuSa, which has increased the transfection efficiency of a selected clone, SuSa E46, to 0.15%. The EBNA-1 gene enables EBV-based vectors to be stably transfected at a high rate (in this case 75-fold higher than the parental line) and because the plasmid is maintained episomally, it is much easier to recover than DNA integrated into the host genome. We have transfected a *S cerevisiae* genomic library into SuSa E46 cells, selected transfectants that are resistant to hygromycin and cisplatin, and rescued the plasmids using Hirt extraction. Between 3 and 10 plasmids containing different-sized inserts have been recovered from individual clones. Following secondary and tertiary transfection, one of these inserts has been cloned and confers cisplatin resistance and morphological changes to SuSa E46.

O 2.8

CHROMOSOME 13Q May CONTAIN TUMOR SUPPRESSOR GENES OTHER THAN BRCA2 AND RB1 INVOVLVED IN THE DEVELOPMENT OF PROSTATE CANCER.
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(Presented by Dr Li).

Loss of chromosome 13q12-14 is frequent in prostate cancers. Tumor suppressor genes, BRCA2 and RB1, are located on this region. In this study, the genotypic and phenotypic analyses on the region were performed in 36 prostate cancers, of which 25 were from primary localized tumors, 7 from local lymph node metastases and 4 from brain metastases. The tumor DNA was derived from tissues histopathologically estimated to contain more than 60% tumor cells. The corresponding paraffin blocks were used for RB immunostaining with a monoclonal antibody PMG-245. We used 8 polymorphic microsatellite markers to detect loss of heterozygosity (LOH) of BRCA2 and RB1 loci. Single strand conformational analysis (SSCP) was used to detect mutations in the BRCA2 coding region.

In total, 18 out of 36 (50%) prostate cancers displayed LOH on 13q12.2-14.3. Deletion mapping with 8 markers revealed two frequently (31%) deleted regions: one between D13S218 and D13S153 (intragenic of RB1); another between D13S319 and D13S137. SSCP screening the BRCA coding region did not reveal any mutations in 18 tumors with 13q LOH including 5 with LOH in BRCA2 locus. Eight out of 27 tumors displayed absent nuclear RB staining. Interestingly, 7 other tumors displayed intensified ectopic cytoplasmic RB localization. The RB aberrant expression was not in concordance with RB1 intragenic LOH. The chromosome 13q LOH as well as aberrant RB expression was in association with early clinical onset ($p<0.05$).

Our results demonstrate that inactivation of BRCA2 by mutation is less common in prostate cancer. RB1 LOH is not consistent with aberrant expression. The ectopic cytoplasmic RB localization may indicate accumulation of functionally disrupted RB proteins. The deletion mapping data suggest that other tumor suppressor genes than BRCA2 or RB1 are located on 13q and may be involved in the pathogenesis of prostate cancers.

O 2.9

PROGNOSTIC MARKERS IN TRANSITIONAL CELL CARCINOMA: DELETION AT THE *del-27* TUMOR SUPPRESSOR LOCUS ON 5p13-12, BUT NOT IN THE *APC* AND *MTS1* GENES CORRELATES WITH TUMOR PROGRESSION

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Inactivation of relevant tumor suppressor genes by allelic or homozygous deletion is a chracteristic event in tumor cells. Here, the prognostic value of allelic deletions on 5p13-12 at the putative *del-27* tumor suppressor locus and in the *APC* tumor suppressor gene on 5q21, as well as homozygous deletions of the *MTS1* (*p16*^{INK4}, *CDKN2*) tumor suppressor gene on 9p21 was assessed in 87 transitional cell carcinomas using microdissection and PCR-based assays. Tumor-specific LOH was detected in 10 of 38 (26 %, *del-27*), and 15 of 30 (50 %, *APC*) informative specimens, respectively. Homozygous deletion of the *MTS1* gene was detected in 33 % of 84 tumors investigated. These deletion frequencies suggest an implication of the three tumor suppressor regions in the genesis of transitional cell carcinoma. In contrast to deletions of the *APC* or *MTS1* genes, LOH at the *del-27* locus correlated with tumor progression. This suggests that loss of the putative tumor suppressor gene *DEL-27* is involved in a more aggressive behaviour of the tumor cells and appears to be a prognostic marker for the clinical outcome of patients with transitional cell carcinoma.

O 2.10

MOLECULAR CYTOGENETICS OF BLADDER CANCER PROGRESSION.
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Tumor progression is driven by genetic changes. To learn more about the role of cytogenetic alterations for bladder cancer progression, 76 bladder cancer specimens (28 pTa, 39 pT1 and 17 pT2-4) were examined by comparative genomic hybridization (CGH). CGH is based on the simultaneous hybridization of differentially labeled tumor and normal DNA's to normal metaphase chromosomes. CGH allows the detection of all DNA sequence copy number changes (deletions, amplifications) of a tumor in one examination. Losses of the Y chromosome (22%), deletions at 9p (43%), 9q (54%), and gains at 1q (18%) were frequently found in pTa tumors with no significant further frequency increase in higher stage tumors (pT1-4). These alterations may therefore constitute early alterations in bladder carcinogenesis. pT1 carcinomas differed markedly from pTa tumors. The total number of aberrations per tumor was 2.5 in pTa but 8.1 in pT1 carcinomas ($p<0.0001$). Aberrations being significantly more frequent in pT1 than in pTa tumors included deletions at 2q (33% in pT1), 4q (15%), 8p (45%), 13q (11%), and 16p (25%) as well as gains at 3q (15%), 6p (18%), 8q (38%), and 12q (15%). At least some of these alterations may play a role for pTa-pT1 progression. Only a few changes were significantly more frequent in pT2-4 than in pT1 carcinomas. These altertions which migh play a role in further tumor progression of pT1 carcinomas include deletions at 3p (found in 23% of pT2-4 carcinomas), 5q (47%), and 6q (24%) and gains at 3p (12%), 5p (35%) and 8p (18%). Conclusion: The striking genetic difference between pTa and pT1 carcinomas argues against grouping these tumors together as "superficial bladder cancer".

O 2.11

CHROMOSOMAL NUMERICAL ABERRATION DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION ON BLADDER WASH FROM PATIENTS WITH BLADDER CANCER.

Yi Pan, Bernard Tribukait, Chunde Li, Peter Ekman, Ulf SR Bergerheim, Stockholm, Sweden; Alexander Marano, Bari, Italy (presented by Dr. Alexander Marano).

INTRODUCTION AND OBJECTIVES: Numerical and structural aberrations of chromosomes 1, 3, 5, 7, 8, 9, 11 and 17 have be seen in transitional cell carcinoma of bladder by fluorescence in situ hybridization (FISH). Monosomy 9 and trisomy 7 have also been suggested to be of importance for the progression of bladder cancer. Cytological examination of bladder wash is routinely used in diagnosis and to monitor the recurrence of bladder cancer. The possibility to asses the malignant potential of tumors by direct molecular genetic analyses of the cytological sample, could improve the sensitivity of diagnosis and staging of bladder cancer.

MATERIAL AND METHODS: Fifteen bladder wash samples and tumor tissue specimens were collected from patients with superficial transitional cell carcinoma. We used chromosome 7, 8, 9 and 11 centromeric probes for FISH analyses. The corresponding samples were also analysed with DNA ploidy flow cytometry.

RESULTS: Of 15 superficial bladder cancers, trisomy of chromosome 7, 8, 9 and 11 were detected in 30%, 20%, 27% and 20% respectively. Monosomy of chromosome 9 were seen in 20% of these tumors. DNA aneuploidy were related to numerical aberration of chromosomes 7, 8 and 11, but not to chromosome 9. Although better hybridization efficiency was achived from touch biopsies the results from bladder wash were in good concordance with touch biopsies samples.

CONCLUSION: Comparable results by using material from bladder washings as well as on specimens from touch biopsie are obtain by FISH technique. FISH analyses on specimens from bladder wash can in the future improve the diagnostic capacity of cytology.

O 2.12

XENOTRANSPLANTATION OF THE ACELLULAR MATRIX GRAFT PROMOTES FUNCTIONAL RAT BLADDER REGENERATION

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INTRODUCTION: Recently the homologous bladder acellular matrix graft (BAMG) has shown to serve as a scaffold for bladder regeneration in the rat model. The present study was designed to demonstrate the decreased antigenicity of the BAMG through xenotransplantation and to evaluate the *in vivo-* and *in vitro-* functional properties of the BAMG-regenerated rat urinary bladder.

MATERIALS AND METHODS: Following partial cystectomy (>50%), BAMGs prepared from hamster, rabbit and dog bladders were grafted to 20 male and female Sprague-Dawley rats; 10 control rats underwent partial cystectomy only. Urinary storage and voiding function were monitored *in vivo*, using a specially designed "micturition cage" and cystometry. After 4 months, organ bath studies and histologic techniques were employed for the *in vitro* evaluation of bladder regeneration in the augmented bladders.

RESULTS: Clinically relevant antigenicity was not evident: no animal died of rejection, and all bladder wall components regenerated in all BAMG xenografts. The degree and quality of regeneration varied, however. Muscularization, peak pressures, and bladder capacity were higher in the hamster BAMG-grafted animals, whereas *in vitro* contractility and compliance were best in the dog BAMG-regenerated bladders. In terms of capacity and compliance, all grafted bladders were significantly superior to the autoregenerated bladders after partial cystectomy alone.

CONCLUSIONS: The present *in vivo-* and *in vitro-* studies showed that augmentation cystoplasty with the BAMG can lead to morphologic *and* functional regeneration of the rat urinary bladder, preserving its low-pressure reservoir function. Because BAMG-regenerated bladders exhibit functional innervation that is similar to normal, they can work in coordination with the host bladder components, thus generating adequate intravesical pressure to produce sustained voiding. The decreased antigenicity makes heterologous BAMG transplants feasible without immuno-suppression

O 2.13

A NOVEL IN-VITRO MODEL FOR BENIGN PROSTATIC HYPERPLASIA

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Introduction and objectives: Investigation of the processes underlying BPH has been hampered by the limitations of current models available. To overcome these short comings we have developed a new in-vitro model.

Methods: Epithelial and stromal cells were cultured from hyperplastic tissue and these cells were then grown in co-culture using Millicell™ inserts. Verification of the purity of the cells was established and the expression of 5 alpha reductase types I and II, and androgen receptors (AR) was investigated as was the production of PSA.

Results: Electron microscopy of the epithelial cells demonstrated the presence of tonofibrils and microvilli whilst the stroma was characterised by the presence of golgi, endoplasmic reticulum and cilia. Epithelial cells grown in co-culture expressed PSA and the media conditioned by these cells demonstrated measurable concentrations of the antigen; following treatment with testosterone the PSA concentrations in the media doubled. RT-PCR analysis of the co-cultured cells demonstrate mRNA expression for 5 alpha R-I and 5 alpha R-II in both cells. Furthermore the functional activity of these isoenzymes was established with both tissue types demonstrating activities for the type I and type II isoenzymes but the activity was higher in the epithelial cells. Additionally the presence of androgen receptors were confirmed by Western blot analysis. These results differ from those obtained for primary cultured epithelial cells which show no PSA production, no response to androgens, no expression of AR a 5 alpha R-II.

Conclusion: This model represents a significant improvement over current cell lines and other human cultured cell model in its representation of BPH in-vitro allowing for a better understanding of the underlying processes involved.

O 2.14

THE LNCaP MOUSE MODEL OF HUMAN PROSTATE CANCER (PCa): ANDROGEN-INDEPENDENCE AND OSSEOUS METASTASIS

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Clinically the lethal phenotypes of human PCa progression are characterized by their androgen-independence and their propensity to form osseous metastases. A series of androgen-independent (AI) human PCa cell lines were derived from androgen-dependent (AD) LNCaP cells. One of the sublines, C4-2, acquired a high tumorigenic and metastatic potential and metastasized to the bone (Cancer Res. 54: 2577). We further characterized this animal model of human PCa metastasis and the cell lines isolated from osseous metastases.

1×10^6 C4-2 cells were injected s.c. or orthotopically into male athymic balb\c nude mice and tumor incidence, formation, and incidence of osseous metastases assessed. PSA secretion was measured by the Abbott IMX system. Prostate epithelial cells were cultured from bone metastases and were characterized after purification. Cytogenetic analysis was performed as described previously. We assessed in vitro tumorigenicity, invasiveness and intrinsic growth. PSA mRNA expression and 10^{-9} M DHT induction and responsiveness were determined by RNA blot analysis.

1. The LNCaP model closely mimics human PCa and its metastatic pattern to form osteoblastic bone metastases. 2. Biochemical characterizations also indicate similarities between human PCa and the LNCaP model. In the absence of androgenic stimuli, AI sublines expressed 2-4 fold higher basal steady-state levels of prostate-specific antigen (PSA) than the parental AD LNCaP cells. DHT varied PSA mRNA expression from induction to suppression. 3. Additional LNCaP sublines derived from C4-2 bone metastases showed cytogenetic changes as in PCa.

The LNCaP model of human PCa closely mimics the biologic, cytogenetic, biochemical, and molecular properties of AD, AI and metastatic human PCa.

Clinical research

O 3.1

FLOW-CYTOMETRIC THREE-PARAMETER DNA-STAINING TO QUANTIFY EGFR IN BLADDER CANCER.

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Aim: Although the Epidermal Growth Factor (EGFR) is known to be a relevant molecule in human bladder cancer initiation and progression, EGFR content determination has not found its way into routine as a prognostic tool. With the hypothesis of a better definition of EGFR content by selection of tumor cells and ploidy, a method was established to quantify EGFR in bladder cancer cell lines (Cytometry, 17:75, 1994, Analyt Cell Pathol, 11:55, 1996), transferred for the first time to bladder tumors and compared with immunohistochemical staining patterns.

Methods: 25 mechanically dissociated human bladder carcinomas were stained with an indirect immunofluorescence technique using EGFR (Oncogene Science, PE), Uro5 (Signet, FITC,) and PI for DNA staining, and measured on a FACScan flow cytometer (Becton Dickinson). A sensitive three-step immunoperoxidase for EGFR staining was applied to sections from the same tumor.

Results: Adequate compensation resulted in reproducible data on a single laser cytometer. Additional absolute quantitation of EGFR by using microbeads with defined antigenic sites (Quantum Simply Cellular) stained with the samples was possible. Clear cut differences in receptor content between stromal cells and tumor cells could be shown, and correlated well with immunohistochemistry, however flow cytometry was more sensitive in the low receptor range (2-3x10^4), often found in high differentiated tumors.

Conclusions: The method represents an example for the practicality of flow cytometry to define and quantify tumor subpopulations of clinical relevance, which can hardly be achieved with other methods of analysis.

O 3.2

TELOMERASE ACTIVITY IN BLADDER CANCER AND BLADDER WASHINGS

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INTRODUCTION: With only a few exceptions, the ribonucleoprotein telomerase has been found in malignant, but not in benign tissues. Telomerase is thus a potentially new diagnostic marker. A non-invasive or minimally invasive method for detection of urothelial carcinomas of the urinary bladder would open new vistas in the diagnosis and follow-up of patients with bladder carcinoma

METHODS: In order to evaluate the diagnostic capabilities of telomerase in bladder carcinomas, four cell lines derived from human urothelial carcinomas of the bladder, 75 tissue samples from bladder carcinomas, eight tissue samples of normal bladder urothelium and 40 bladder washings were examined for telomerase activity. Telomerase activity was detected by the highly sensitive TRAP-assay (telomeric repeat amplification protocol), based on the polymerase chain reaction (PCR).

RESULTS: The four cell lines derived from urothelial carcinoma of the bladder (F975, J82, SCaBER, UM-UC-3) all exhibited high telomerase activity and were thus used as positive controls. Telomerase activity was found in nearly all (96%) tissue samples obtained from histologically confirmed urothelial carcinoma of the bladder. None of the normal tissue samples examined showed telomerase activity. Telomerase activity was similarly found in 73% of bladder washings in patients with histologically confirmed bladder carcinoma. There were no false positive results.

CONCLUSIONS: The enzyme telomerase is detectable in nearly all cases of bladder carcinoma. Determination of telomerase activity in bladder washing samples represents a new diagnostic method with high sensitivity and specifity for detection of tumor cells in rinsing media.

O 3.3

P53-EXPRESSION IN HISTOLOGICALLY BENIGN LOOKING BLADDER MUCOSA IN PATIENTS WITH TRANSITIONAL CELL CANCER OF THE BLADDER

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OBJECTIVES: Nuclear overexpression of p53 has been shown for superficial and invasive transitional cell cancer (TCC) in several reports. P53 accumulation has also been shown in urothelial dysplasia. The aim of our study was to evaluate if immunohistochemical staining of histologically benign looking bladder mucosa reveals p53-overexpression indicating premalignant p53-genomic alteration.

METHODS: In 60 consecutive patients (PTS) (47 superficial TCC, 13 muscle-invasive TCC) deparaffinized tissue sections of representative tumor and histologically "normal" bladder mucosa were stained with the p53 monoclonal antibody DO7 using a biotine streptavidine-peroxidase system. Antigen exposure was enhanced by boiling the slides in the microwave oven. 20 patients with histologically normal bladder mucosa who underwent radical prostatectomy served as controls. p53 staining of the specimens was rated by counting the number of stained tumor cells. Samples with less than 5% positive staining were considered negative.

RESULTS: Evaluation of histological normal bladder mucosa showed positive p53 staining (5-30%) in 10/60 patients (16.7%): 8 pTa/T1 tumors and 2 >= pT2 tumors. After a median follow up of 20 months 4 /8 pTa/T1 patients suffered from recurrent disease.

CONCLUSION: We are the first to describe p53 overexpression in histologically normal bladder mucosa in patients with TCC possibly identifying premalignant alterations in tumor surrounding areas. We conclude that p53 accumulation in histologically benign bladder mucosa might be a usefull tool to detect patients with a high risk for disease recurrence.

O 3.4

P53 NUCLEAR OVEREXPRESSION IS ASSOCIATED TO BLADDER TUMOR RECURRENCE AND PROGRESSION AFTER BCG TREATMENT.

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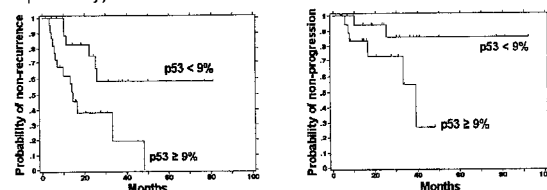
INTRODUCTION AND OBJECTIVES: To evaluate prognosis value of p53 expression in predicting recurrence and progression in bladder tumor patients treated with BCG

METHODS: Fresh frozen samples from 43 TCC obtained before BCG treatment were immunostained with a monoclonal antibody, pab 1801, which recognizes a resistant denaturation epitope present on wild type and mutated p53 (Oncogne Science). Scores were determined for each case counting at least 500 nuclei per slide in 3-5 selected regions. Kaplan Meier curves were used for correlation between recurrence, progression and p53 expression.

RESULTS: The mean p53 expression, in nonrecurrent patients, was 5,7% and 20,7% in recurrent patients (t student test p=0.002). Patients with P53 tumor expression higher than 9% exhibited significantly more recurrences and progressions than those who expressed less than 9% (Log Rank test p=0.007 and 0.03 respectively).



CONCLUSIONS: p53 immunostaining evaluation, on tumor samples before BCG treatment, could be a useful tool to select high risk patients for recurrence and progression after a single course of BCG treatment.

O 3.5

P53 IS NOT A PREDICTOR OF RESPONSE TO BACILLUS CALMETTE-GUERIN (BCG) THERAPY FOR SUPERFICIAL BLADDER CANCER.

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INTRODUCTION AND OBJECTIVES: BCG therapy has been demonstrated to be an effective treatment in the prevention of bladder tumor recurrences. However, a significant percentage of patients do not respond to BCG therapy and unfortunately there are currently very few objective markers which can predict patients who will fail to this treatment. P53 alterations have been demonstrated to be a factor of chemo- and radioresistance. We have investigated in the present study whether p53 overexpression could be a predictor of response to BCG therapy.

METHODS: An homogenous group of 36 patients with superficial bladder cancer at moderate or high risk for tumor recurrences (patients with pTa, pT1 or carcinoma in situ (Cis) with at least one tumor recurrence in the last year, multiple tumors or large tumors) and treated with 6 weekly BCG intravesical instillations (120 mg, Pasteur strain) were included in the study. Expression of p53 (DO7) was determined by immunohistochemistry in paraffin-embedded tissues.

RESULTS: Tumor grade was G1, G2, G3 and Cis in 11, 19, 4 and 2 patients respectively while tumor stage was Ta and T1 in 11 and 23 patients respectively. Average follow-up was 28.2 months. Twenty-two patients had a tumor recurrence (61%) with a mean time to recurrence of 12.4 months. Seven out of 22 patients with recurrences had a later cystectomy (31.8%). P53 positive staining (cut-off 10%) occurred in 7/36 patients (19.4%), 2 without and 5 with recurrences (not statistically different). A cut-off of 20% for p53 positive staining resulted in the same absence of statistically significant difference. P53 was not a significant predictor of time to recurrence or progression to invasive disease neither.

CONCLUSIONS: Overexpression of p53 is not a common event in superficial bladder cancer and is not a predictor of tumor recurrence or tumor progression to invasive disease in patients who received BCG therapy.

O 3.6

MHC-class II transfectants of mouse renal cell carcinoma induce potent anti-tumor immunity (in vitro and in vivo study)

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The renal cell carcinoma line (Renca) of Balb/c mice is lethal in autologous host, although it has been demonstrated repeatedly that the tumor is weakly immunogeneic. In view of the latter we explored wether facilitating peptide presentation by transfection of the tumor cells with MHC-class II genes would support an activation of immune effector cells even without additional supply of helper factors or supporting costimulatory molecules.
Renca cells transfected with MHC-class II showed unaltered growth characteristrie in vitro as compared with untransfected Renca cells. The expression of MHC-class II was insufficient to induce tumor rejection even at the TD 100 threshold dose. Nevertheless the untransfected Renca cells injected s.c. in a dosis of $1x10^6$ developed a tumor of 400 mm^2 in crossection area at day 30 while MHC-class II transfectats tumors arised just 160 mm^2. Futhermore, after immunisation with MHC-class II transfected Renca cells ,the survival time of animals subsequently challenged with Renca cells was prolonged as compared to mice immunised and challenged with untransfected Renca cells (day 20 - day 56). We established tumor-specific lymphocyte clones against MHC-class II transfected and control transfected tumor cells. After two months of in vitro cultivation, lymphocytes were composed of 40% CD4 and 60% CD8 positiv cells. The proliferation response of lymphocytes established against MHC-class II transfected cells or against control transfected cells was significantly better towards MHC-class II transfected tumor cells as compared to control cells. The cytotoxic pontential of lymphocytes cultured with untransfected or control transfected tumor cells was demonstrated by lysis of 10% of parental Renca cells and 55 % of MHC-class II positiv tumor cells at a ratio of 8:1, while lymphocytes stimulated with the MHC-class II transfected tumor cells lysed parental Renca cells in 20% and MHC-class II positiv tumor cells in 80% at a ratio of 4:1.
Conclusions: MHC-class II transfected tumor cells facilitate mounting of anti-tumor immunity.The „in vivo“ growth rate of MHC-class II positiv tumor cells is retarded as compared to wild-typ tumor cells. In vitro the MHC-class II transfected cells are far more efficiently killed and provoke a strong proliferative response of TILs.

O 3.7

GENERATION OF HYBRID CELL VACCINE FOR THE TREATMENT OF PATIENTS WITH ADVANCED RENAL CELL CARCINOMA

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Introduction: Renal cell carcinoma (RCC) is an immunogenic malignancy. Anti tumor immune responses are primarily mediated by T cells. MHC class II antigens are required for initiation of CD4 positive T cells. These T cells induce proliferation of CD8 positive cytotoxic T lymphocytes (CTL) acting as effectors on tumor target cells. The CTL require MHC class I antigens to achieve sufficient antitumor activity. RCC frequently presents MHC class I antigens but lacks MHC class II antigens.

Methods: Tumor tissue from patients with metastatic RCC is acquired during nephrectomy and a cell line is established. So far all cell lines express MHC class I by FACS analysis while MHC class II could not be detected. Allogene lymphocytes are isolated from buffy coat, stimulation for three days with lipopolysaccharide is performed and expression of MHC class II is documented. Cell suspensions from the tumor cells are fused with activated allogenic B cells by electromediated fusion.

Results: The hybrid cells express MHC class I and class II, ICAM-I, and B 7. The vaccine is irradiated and injected intra and subcutaneously. Induction of specific immunity is monitored as intracutaneous DTH reactions to the tumor cells. In addition peripheral blood leucocyte counts and CD4/CD8 ratio is evaluated before and after vaccination. Skin biopsies are taken and staining for immune competent cells is performed. Routine follow up using CT scans is administered to the patients. So far 6 patients have been vaccinated.

Conclusion: The generation of this vaccine provides a sophisticated drug for active specific immunization in renal cell carcinoma.

O 3.8

EFFECT OF ANTIBODY PROTEIN DOSE OF ANTI-RCC MONOCLONAL ANTIBODIES IN NUDE MICE WITH RCC XENOGRAFTS.

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For optimal radioimmunotherapy the choice of an adequate antibody protein dose is essential. Therefore, the effects of antibody protein dose on tumor uptake were investigated in nude mice with renal cell carcinoma (RCC) xenografts. The biodistribution of increasing protein doses (0.3-100 μg) of radioiodinated anti-RCC MAbs G250 and RC 38 (directed against different RCC-associated antigens) was studied in mice with NU-12 or SK-RC-52 RCC xenografts.
In NU-12 tumors the %ID/g G250 was very high at protein doses of 0.3 and 1 μg (125 %ID/g), but decreased at higher doses, suggesting tumor saturation. Saturation of G250 antigen occurred at 3 μg protein, with approximately 2.3 μg G250 specifically bound per gram NU-12 tumor. In this model only 0.3% of the available G250 sites were targeted at saturated dose levels (9,200 of available 2,900,000 sites per NU-12 cell). In contrast, in SK-RC-52 tumors G250 uptake was very low at low antibody dose (4 %ID/g at 1 μg) and increased with increasing protein dose. Additionally, very low blood levels were observed. These differences in G250 biodistribution are most likely related to differences in the processing of MAb G250 by the tumor cells. The RC 38 uptake in NU-12 tumors remained constant up to the 10 μg dose level (40 %ID/g) and decreased at higher doses. RC 38 antigens were saturated at 25 μg (7.5 μg/g NU-12 tumor specifically bound). With RC 38 1.3% of the available RC 38 antigens per NU-12 tumor cell were targeted.
The biodistribution data of G250 in the NU-12 model are strikingly in line with our observations in RCC patients that were injected i.v. with ^{131}I-G250. At doses exceeding 10 mg tumor uptake decreased 10-fold in terms of %ID/g, also suggesting saturation of G250 antigens. At saturated dose levels (25-50 mg) 1500-3000 G250 antigenic sites were targeted per tumor cell.
Our studies indicate that some RCC tumors can be saturated with anti-RCC MAbs at low (25 μg) to very low (3 μg) protein doses. At non-saturated dose levels very high tumor uptake can be achieved. Surprisingly, in NU-12 tumors only 0.3 and 1.3% of the available antigenic sites are targeted at the saturated dose levels.

O 3.9

IN VIVO MEASUREMENT OF OXYGEN IN RENAL CARCINOMA AND NORMAL KIDNEY TISSUE

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INTRODUCTION AND OBJECTIVES: The tumoral oxygen concentration is a clinically relevant parameter. The newly developed near infrared reflexion spectroscopy based system MULTISCAN OS 10 is able to measure oxygen saturation (from 0 to 100 %), oxygenated and deoxygenated hemoglobin and total hemoglobin (total Hb).
MATERIAL AND METHODS: We examined 16 patients with organ confirmed renal carcinoma under general anaesthesia intraoperatively. During surgical preparation of the kidney oxygen-measurements were taken from the tumour itself and the healthy kidney.
RESULTS: Saturation values were in kidneys (68 % ± 12) and tumours (92 % ± 7, p< 0.001). Total Hb was 2.08 ± 0.7 mg/ml tissue in kidney versus 4.8 ± 0.3 in tumours (p<0.001). After ligation of the renal artery and vein only a slight decrease of oxygen-saturation could be measured in the tumor, while normal kidney tissue decreased to 0 % saturation within 90 seconds.. These findings were controlled in a human renal carcinoma xenotransplanted on nude mice where we get the dame results as seen in patients. Additionally energy metabolism measurements in tumour biopsis were performed (glycolysis, lactate). The data showed a strong tendency to anaerobic metabolism.
CONCLUSION: These clinical measurements show that kidney tumors have a significant higher Total-Hb concentration and a better oxygenation than normal kidney tissue. Oxygen measurements after ligation of the blood supply must be interpreted as a reduced oxygen consumption of tumor tissue compared to normal kidney tissue with a compensating glycolytic metabolism .

O 3.10

MICROVESSEL DENSITY AS A PROGNOSTIC MARKER IN PATIENTS WITH PROSTATE CANCER.

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INTRODUCTION AND OBJECTIVES. The extent of tumor vascularization might correlate with metastatic disease and prognosis in solid neoplasms. Aim of our study was to analyze the preoperative microvessel density in prostate biopsies and to correlate these results with histological stage and prognosis.
METHODS: In 64 patients six systematic biopsies were available for immunohistochemistry with an antibody directed against endothelial cells (CD31, Biogenex, MA232-5c). All patients underwent radical prostatectomy for biopsy proven prostate cancer. Microvessel density was determined in the biopsy specimens in prostate cancer tissue and benign prostatic hyperplasia (BPH) by counting positive blood vessels in 6 high power fields . Results were calculated by microvessel density per mm^2.
RESULTS: In the biopsies mean microvessel density was 59,33 per mm^2 (SD 28,64) in prostate cancer areas and 23,94 per mm^2 (SD 16,65) in BPH areas.
No correlation was found between cancer grade or T-stage and microvessel density. However in patients with postoperative positive PSA mean microvessel density was 96 per mm^2 (SD 50,6) compared to 57,7 per mm^2 (SD 23,4) in patients with negative postoperative PSA (p= 0,043 (Mann-Whitney U Test).
CONCLUSION: Microvessel density seems to be an independent prognostic marker for tumor progression after radical prostatectomy.

O 3.11

THE ASSOCIATION BETWEEN CD44 EXPRESSION AND THE PROGNOSIS OF PROSTATE CANCER PATIENTS TREATED BY RADICAL PROSTATECTOMY

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The expression of splice variants of CD44, a trans-membranous adhesion molecule, has been linked to the metastatic potential of several malignant tumors. The prognostic value of the standard (CD44s) and v6 (CD44v6) variants was studied in surgically treated prostate cancer.

From 1980 to 1988 97 patients underwent pelvic lymph node dissection and subsequent radical prostatectomy for $T_{1-3}N_xM_0$ (TNM 1992) prostate cancer. The patients were followed prospectively for a mean period of 84 months. Immunohistochemically defined CD44 expression was determined semiquantitatively and was correlated with pathological and clinical data. In addition, 12 lymph node metastases were included and CD44 mRNA expression was studied by the reverse transcriptase polymerase chain reaction (RT-PCR) in 9 malignant and 8 benign prostatic tissues.

Intense CD44s and CD44v6 immunostaining was observed in benign prostatic glands. The expression decreased from benign to low-grade to high-grade prostate cancer and was absent in lymph node metastases. RT-PCR confirmed the expression of CD44s and CD44v6 by benign and malignant prostatic cells. Loss of CD44s and CD44v6 was statistically significantly correlated with Gleason grade and pT-stage. Decreased CD44s expression predicted an increased risk of clinical progression and tumor death, whereas loss of CD44v6 was associated only with the risk of clinical progression (Kaplan-Meier method). The independent prognostic markers identified by Cox's regression analysis were: Gleason grade and loss of CD44s for prostate-specific antigen based progression; Gleason grade, pT-stage and loss of CD44s for clinical progression; Gleason grade for tumor specific survival.

CD44 expression is down regulated in prostate cancer. Loss of CD44s is an independent prognostic marker for the prediction of progression of surgically treated prostate cancer patients.

O 3.12

SHORT CAG TRINUCLEOTIDE REPEAT IN THE ANDROGEN RECEPTOR (AR) GENE IS LINKED TO PROSTATE CANCER.

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The AR gene is mapped to chromosome Xq 11.2-12. The exon 1 of the gene contains a CAG trinucleotide repeat encoding a polymorphic polyglutamine tract. In vitro study has shown that decreasing the tract length increases the AR transcriptional activation on target genes. Varying frequencies of short CAG repeat have been implicated in the different incidence of prostate cancer among different populations. Polymerase chain reaction (PCR) with specific primers covering the repeat can distinguish the varying sized alleles in individuals.

We randomly selected 51 subjects who underwent transurethral resection of the prostate, of whom 32 had benign prostate hyperplasia (BPH) and 19 had prostate cancer. We also included 36 prostate cancer patients who underwent open surgery. All the 87 subjects were of Swedish origin and the diagnosis was based on histopathologic examination. The BPH subjects aged from 72 to 89 (mean 79 years) and the prostate cancer patients aged from 52 to 86 (mean 67 years). Leukocyte DNA was used for PCR and microsatellite analysis to determine the CAG alleles.

In total, we detected 13 different CAG alleles. The size increases from A1 to A13. Assuming the variance as continuous, Wilcoxon rank test showed that prostate cancer patients had CAG alleles significantly shorter than controls (p<0.01). The most frequent CAG allele in prostate cancer patients was A4 (25%), in contrast to low frequency (3%) in the controls (p=0.0142, Fisher's exact test).

As the BPH group was well controlled with age and histopathology to substantially exclude latent prostate cancer, the data clearly demonstrate frequent occurrence of short CAG alleles in prostate cancer patients and may indicate constitutionally short CAG alleles to be of importance in the development of clinical prostate cancer.

O 3.13

Prostate specific antigen (PSA) in prostatic tissue Ake Pousette et al.

Background: In order to explore the use of intracellular PSA concentration a method was earlier developed for measurement of PSA in aspirations biopsies from prostatic tissues (t-PSA).
Material and methods: Aliquots ot the asprira-tion biopsy material from patients with sus-pected prostatic carcinoma were frozen to burst the cells and after thawing 10.000xg supernatants and pellets were prepared. PSA was quantitaded by immunoassay in the super-natants and correlated to the DNA concentra-tions in the pellets and expressed as µg PSA/µg DNA.
Results: The present paper summerizes the re-sults from over 200 patients and a 5 to 10 years followup. A significant correlation was found between t-PSA and grade of differenti-ation of the prostate tumor, showing lower t-PSA values in highly malignant tumors. Low t-PSA values were correlated with increased malignancy grade, tumor stage and a shift from diploid to aneuploid carcinoma. Decreasing t-PSA values corresponded to increasing serum PSA values. The prognostic value of t-PSA was compared with other markers in patients with newly detected prostatic carcinoma. In a Cox multivariatc analysis, cytology and tissue PSA content were the most important prognostic in-dicators for progression in hormonally treated patients.
Conclusion: We conclude that t-PSA provides additional information about the prognosis of a prostate tumor.

O 3.14

QUALITY OF LIFE AFTER RADICAL PROSTATECTOMY IN OUTCOME RESEARCH.

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INTRODUCTION AND OBJECTIVES: One remaining question in the future concerns the impact of curative radical surgery on the individual`s quality of life and life perspective. In recent studies tumor specific moduls were developed according to the guidelines of the EORTC. These tumor modules were used together with the QLQ C-30 core questionaire of the EORTC. Our group developed a prostate specific module for radical prostatectomy which was tested in a retrospective study.
METHODS: In a retrospective study 130 patients with localized prostate cancer were interviewed with questionaires between 1 and 3 years after radical prostatectomy. They received the EORTC QLQ C-30, the new developed tumor specific module and the IPSS score. The acceptance of this quality of life study was high. Nearly 90 % of the patients sent their questionair back to our institution. The quality of life data were analyzed together with the clinical data of the patient. Statistical analysies was done with SPSS programm (DOS-System).
RESULTS: Regarding to the problems concerning sexuality and incontinence after radical prostatectomy more detailed information was possible due to the new developed tumor specific module. All patients answered the questions dealing with sexuality, partnership and incontinence. 58 % of the patients admitted severe limitation of their sexuality with consequtive high problems in partnership. Details of the multidimensional analysis of quality of life data are presented.
CONCLUSION: The present retrospective study help to increase the reliability and validity of the new instruments. The study will give more detailed look at all aspects of quality of life after radical prostatectomy. In the future it is necessary to test this instrument in a prospective trial with baseline quality of life data.

Cell interaction & growth factors

P 1.1

MUTANT EGFR IS OVER-EXPRESSED IN PROSTATE CANCER

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Introduction: Recent reports suggests a decrease in expression of EGFR(wild-type) in CAP when compared to BPH, although EGFR mRNA is increased. This is contrary to findings in other epithelial malignancies in which over-expression of both EGFR and its mRNA is well documented. To investigate this disparity we evaluated the presence of a mutant EGFR (EGFRvIII, an activated tyrosine kinase) in both BPH and CAP. We also assessed the presence of androgen receptor(AR) in the stromal and epithelial nuclei of these tissues.

Methods: Peroxidase immunostaining with EGFRvIII was performed on 20 BPH and 30 CAP archival specimens. 10 of the BPH and 20 of the CAP specimens were also stained with AR.

Results: EGFRvIII expression was confirmed in both BPH and CAP, but staining was stronger and more widespread in CA with the luminal cells staining strongest. AR staining was heterogenous in all BPH and CAP epithelial nuclei, and in all BPH stromal nuclei. However AR was absent in the stromal nuclei of 16 of the 20 CAP specimens stained.

Conclusions: The decrease in wild-type EGFR expression in CAP may be due to presence of the EGFRvIII. This mutated form of the receptor may play a role in the autonomous growth of CAP.

P 1.2

DIAGNOSTIC VALUE OF GROWTH FACTORS AND THEIR RECEPTORS IN HUMAN PROSTATE CANCER

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Cancer of the prostate (PCa) is the most frequent cancer in men, with an estimate of about 30,000 new cases and 15,000 deaths in Germany p.a.. Observations of a decreasing respon-se of prostate cancer to androgen withdrawal and the involve-ment of growth factors in the control of tumoral growth led to a hypothesis of a common tumor growth. The purpose of our stu-dy was to measure the expression of growth factors and their respective receptors as additional marker for diagnosis and follow up studies.
PCa tissue with and without androgen ablation was characteri-zed immunocytochemically with antibodies against EGF, NGF, bFGF, TGFß1/2 and their receptors. Semiquantitave RT-PCR was performed using primers directed against the respective cDNAs.
Growth factors were mainly localized in the stromal parts of the tissue, the respective receptors in the epithelial cells. The in-tensity of immunocytochemical staining was lower in cancer tissue compared to controls and correlated with the RT-PCR results. Only TGFα, TGFß1 and TGFß2-R content was in-creased in Gn-RH treated specimens and in tissue of ⊇ pT3 graded tumors. In follow up observation these patients develo-ped tumor recidives.
An addition to standard parameters growth factors and their receptors are helpful tools for diagnosis and follow up studies especially of less differentiated aggressive prostate cancer.

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P 1.3

DIFFERENT BEHAVIOURS OF HUMAN BLADDER CANCER IN CELL CULTURE IDENTIFY DIFFERENT HISTOLOGIC AND ANGIOGENIC FEATURES

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To better understand the biological pathways that distinguish superficial papillary from "in situ" or invasive solid bladder cancers,thirty surgical biopsies of human bladder tumours were cultured "in vitro" using the explant culture technique. The behaviour of tumour cells in culture was thus evaluated according to cell morphology, growth pattern and culture survival, and five different proliferative classes were identified. In tumour biopsies was then investigated the expression of epidermal growth factor receptor (EGF-r) and of vascular endothelial growth factor (VEGF) using immunohistochemical reactions. The results showed that primary papillary superficial tumours which express both EGF-r and VEGF belong to class 1A, whereas primary papillary superficial tumours not expressing both EGF-r and VEGF belong to class 1B. To classes 2A, 2B and 3 belong primary invasive tumours or recurrences which do not express EGF-r but express VEGF if they have papillary aspects. Solid tumours, either "in situ" or invasive, do never express VEGF independently from the class which they belong to.

This work was supported from AIRC (Italy)

P 1.4

EXPRESSION AND FUNCTIONS OF GROWTH FACTORS AND THEIR RECEPTORS IN INVASIVE HUMAN TRANSITIONAL CELL CARCINOMA CELLS.

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INTRODUCTION: Studies on Epidermal Growth Factors (EGF), Fibroblast Growth Factors (FGFs), and Transforming Growth Factor β_1 ($TGF\beta_1$) suggested their implication in transitional cell carcinogenesis. This induces effects on cellular processes like proliferation and migration. Expression studies on human transitional cell carcinomas (TCCs) demonstrated a modulated expression of these growth factors and their receptors in association with a more aggressive phenotype. However, little is known about functions of growth factors in invasive TCCs.
METHODS: In this study, we established and characterized a new invasive human TCC cell line designated 1207. Expression patterns of FGFs, $TGF\beta_1$ and EGF-like growth factors and their receptors in 1207 cells and the original tissue were analyzed by immunocytochemistry and the reverse transcriptase polymerase chain reaction. These data were correlated with functional proliferation and migration studies with 1207 cells.
RESULTS: We observed no significant difference in expression patterns between the original TCC tissue and the deriving cell line indicating the resemblance of the cell line to the original tumor. The proliferation response to EGF, TGFα, amphiregulin, heregulin-α, FGF-1, and FGF-7 correlated with the presence of EGF receptors (c-erbB1, c-erbB2, cerbB3, and c-erbB4); and FGF receptors (FGFR1 and FGFR2) respectively. A distinct expression pattern of these growth factors was also found in corresponding tissue sections, indicating their function in vivo. The highest proliferation stimulating responses were noticed for amphiregulin and heregulin-α. In addition, TGFα induced migration of 1207 cells. Despite the presence of TGFβ receptors, only a small inhibiting response to $TGF\beta_1$ was noted.
CONCLUSION: These data support the notion that notably EGF-like proteins mediate TCC growth and invasion through autocrine pathways.

P 1.5

APOPTOSIS AND EXPRESSION OF BCL-2 PROTEIN FAMILY, VEGF, AND PCNA IN TRANSITIONAL EPITHELIUM.

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Apoptosis (APO) plays an important role in normal tissue homeostasis, where it helps to mantain appropriate cell numbers. Dysregulation of APO producing reduced rates of cell turnover can lead to pathological accumulation of cells and contribute to the development of neoplasia. A group of genes with sequence homologies with Bcl-2 regulates APO by both positive (Bax, Bcl-XS, Bak and Bad) and negative signals (Bcl-2, MCL-1 and Bcl-XL). Moreover, tumor cell growth are regulated by the expression of the angiogenetic protein VEGF. In this paper we report the immunohistochemical analysis of the patterns of expression of Bcl-2 protein family,VEGF, APO and PCNA, a marker of cell proliferation, in 10 normal (N) and 40 neoplastic (T) vescical transitional epithelia (VTE).

	N-VTE	T-VTE (G1-G2, Pt0)	T-VTE(G3,Pt1-3)	T-VTE(in-situ)
Bcl-2	+[a]	-	-	-
MCL-1	+[b]	+[c]	+++[c]	+++[c]
Bcl-X	+[b]	-	+++[c]	++[c]
Bax	+[b]	+[c]	+++[c]	+++[c]
APO	-	-	+/++[c]	±
PCNA	-	±	+++[c]	++[c]
VEGF	-	-	+++[c]	+

a)basal layer; b)apical layer; c)diffuse.

We have delineated for the first time the *in vivo* patterns of Bcl-2 protein family and VEGF together with APO and proliferative index in N-VTE and T-VTE. The results obtained show the absence of Bcl-2 and an increased expression of other members of Bcl-2 protein family in high-grade and in *in situ* T-VTE. This was accompanied more by an increased proliferation than by APO. VEGF appeared more expressed in high-grade T-VTE than in *in situ* T-VTE suggesting that its expression become important in invasive stage.

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P 1.6

THE ROLE OF APOPTOSIS IN TRANSITIONAL CELL CARCINOMA OF THE BLADDER

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INTRODUCTION AND OBJECTIVES: Apoptosis is the mode of genetically controlled cell death that accompanies a variety of biological processes such as homeostasis in tissue populations. In oncology, the propensity of tumor cells to respond by apoptosis has been described and may be a prognostic marker for cancer treatment. In the present study the incidence of apoptosis in specimens of superficial and invasive transitional cell carcinoma of the bladder (TCC) was quantitativly evaluated.

METHODS: Apoptosis was determined by a non radioactive, immunhistochemical, enzymatic in-situ end-labeling of apoptosis induced DNA strand breaks (TUNEL-reaction). Analysis of stained cells was carried out by light microscopy. 35 bladder cancer and 8 normal bladder specimens were examined. For exact quantification, the „apoptotic index" was defined as the number of apoptotic cells per mm^2 neoplastic epithelium.

RESULTS: Histological classification revealed 75% superficial and 25% invasive TCC. The apoptotic index in all bladder cancer specimens was significantly higher than in normal transitional epithelium ($p<0.0001$): 25.93 vs. 1.01. The apoptotic index grew with increasing grade and stage, though only the correlation to tumor grade reached statistical significance: grade I-carcinomas had an average index of 12.97, grade III-carcinomas of 40.98. There was no significant difference in the index between superficial and invasive bladder cancer: 20.17 vs. 36.32.

CONCLUSIONS: The occurence of apoptosis in bladder cancer is significantly higher than in normal transitional epithelium. Moreover, the apoptotic index correlates significantly with increasing tumor grade. Further evaluations are ongoing to determine whether apoptosis may represent an independent prognostic marker for TCC.

P 1.7

CELL ADHESION MOLECULES: CORRELATION OF SERUM LEVELS WITH TUMOR STAGE IN PATIENTS WITH BLADDER CANCER

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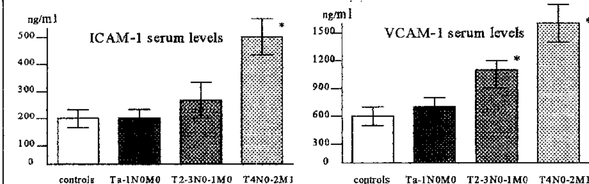
Introduction: Recent studies in human malignancies suggest that cellular adhesion molecules (CAM) play an important role in tumor progression and metastasis. To evaluate the role of CAMs in patients with bladder cancer, we examined the serum levels of intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1) and E-Selectin and correlated the results to tumor stage.

Material and Method: Serum levels of 74 patients with bladder cancer and 65 controls were examined for ICAM-1, VCAM-1 and E-Selectin by specific ELISA tests. Patients with bladder cancer were divided into 3 groups: (group 1) Ta-1, CIS, N0, M0; n=37; (group 2) T2-3, N0-1, M0; n=22 and (group 3) T4, N0-2, M1; n=15.

Results: Median levels of ICAM-1 and VCAM-1 were found to be significantly higher in metastatic tumor disease ($p<0.05$) (group 3). Significant elevated levels of VCAM-1, but not of ICAM-1 and E-Selectin were found in patients with lymphatic spread (group 2).



There was no statistically significant difference between controls and patients with superficial bladder cancer (group 1) in the evaluated CAMs and no difference between controls and patients with metastatic disease (group 3) in E-Selectin.

Conclusion: These data demonstrate that adhesion molecules ICAM-1 and VCAM-1 play an important role in bladder cancer metastasis. The serum level of VCAM-1 is tumor stage dependent elevated in patients with lymph node and distant metastasis and could serve as a prognostic marker.

P 1.8

THE ROLE OF CATENINS IN CADHERIN MEDIATED INTERACTIONS Marianne Linkels, Marion J. Bussemakers, Jack A. Schalken, Urological Research Laboratory, Academic Hospital Nijmegen, The Netherlands.

The Ca^{++}-dependent cell adhesion molecule E-cadherin plays an important role in the maintenance of epithelial integrity. Cadherins are linked to the cytoskeleton through catenins (α, ß, γ) what is essential for the intercellular adhesive function of E-cadherin. Beta- and gamma-catenin can bind directly to the cytoplasmic part of E-cadherin whereas alpha-catenin connects this E-cadherin-ß/γ-catenin complex to the cytoskeleton. Recently it has become clear that for carcinomas the loss of E-cadherin mediated adhesion is an important step in the progression of the disease. One of the mechanisms leading to defective cadherin function, is impaired association with the cytoskeleton because of the absence or dysfunction of α-catenin (demonstrated by transfecting α-catenin into PC3 cells). In order to gain more insight in the role of catenins, yeast two hybrid studies have been initiated to identify cytoskeletal proteins that interact with alpha-catenin and which may play a role in cadherin mediated signal transduction pathways. Using this system, we want to identify the domain(s) of α-catenin that binds to ß- and/or γ-catenin and we want to investigate to which cytoskeletal proteins α-catenin can bind besides its association with actin. For use in the interaction trap we recently isolated full length human α-catenin cDNA and a cDNA expression library from a cervix carcinoma cell line (A431) is constructed. At this moment we are performing an interaction trap. The results of this will be investigated further in the near future, where interactions in relation to the progression of prostatic carcinomas and metastasis will receive special attention.

P 1.9

HETEROGENEOUS EXPRESSION OF E-CADHERIN AND p53 IN PROSTATE CANCER: clinical implications

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Abnormal E-cadherin and p53 expression assessed by immunohistochemistry correlates with poor survival of prostate cancer patients. To determine the value of presurgical assessment of these molecular markers, a comparitive analysis of prostate biopsies and prostatectomy specimens from 46 patients was performed. Overall, the prevalence of abnormal p53 immunoreactivity in extensively sampled prostatectomy specimens was low (9%), and was virtually restricted to cases that were already identified to have a poor prognosis based on prostatectomy tumorvolume and grade. Therefore, it can be expected that this marker is not useful as a prognostic marker in prostatic specimens. In 26% of the cases, abnormal immunoreactivity for E-cadherin was found, and these revealed considerable intra- and intertumor heterogeneity. This finding explains, at least in part, the low sensitivity (18%) for detecting abnormal E-cadherin expression with biopsies. Thus, the value of presurgical assessment of E-cadherin expression by biopsy would depend on a broad and systematic scrutiny of all tumors and all tumor areas in a given case. Hence, more accurate and objective imaging techniques are urgently needed to decrease the problem of sampling error from prostatic adenocarcinomas. Until then, abnormal E-cadherin expression is clinically most useful to advise in post-prostatectomy treatment recommendations.

P 1.10

E-CADHERIN EXPRESSION FAILS TO PREDICT TUMOR RECURRENCE AND LONGTERM SURVIVAL FOLLOWING RADICAL PROSTATECTOMY

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Introduction : For bladder cancer decreased expression of E - cadherin, mapped to chromosome 16q21, has been suggested to result in a higher tendency towards infiltrative behaviour and a decreased longterm survival of patients. For prostate cancer, the prognostic value of decreased E - cadherin expression for local recurrence and the longterm survival of patients following radical prostatectomy is discussed controversally.

Material and Methods : With a median follow - up of 64 months 67 radical prostatectomy specimens of different stage and histological grade were immunohistochemically investigated for E - cadherin expression using a monoclonal antibody (Transduction laboratories). According to the relative amount of positively stained tumor cells reaction was classified into sex groups. Decrease of E - cadherin staining as a prognostic factor for tumor recurrence and longterm survival of patients was calculated by univariate (Log rank) and multivariate (Cox regression) analysis for the whole group and for each subgroup alone and was correlated with further biological variables such as age, tumor stage and histological grading.

Results : During the follow - up period tumor recurrence was observed in 17 of 67 patients (25 %) and 12 patients (18 %) died from tumor progression. Neither for the whole group nor for each subgroup alone a decreased E - cadherin expression was identified as a prognostic parameter for tumor recurrence ($p = 0.42$) or death from prostate cancer ($p = 0.17$) during univariate or multivariate statistical analysis.

Conclusion : For patients with localized prostate cancer detection of a decreased E - cadherin expression does not seem to reveal any prognostic value in addition to classical prognostic parameters like tumor stage or histological grading.

(This investigation was supported by a grant from the „Deutsche Forschungsgemeinschaft"(DFG) : Ku 877/3-1)

P 1.11

TUMOR SUPPRESSOR PROTEIN MASPIN IDENTIFIES PROSTATE CANCER SPECIMENS AT LOW RISK FOR TUMOR PROGRESSION

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Introduction : Maspin, a recently identified protein, was primarily discussed as a proteinase inhibitor related to the serpin family. Recombinant maspin is capable to block the growth, motility and invasiveness of breast cancer cell lines. Aim of the present study was to investigate maspin expression in prostate cancer specimens and to correlate the result of the immunohistochemical staining reaction with tumor progression and the longterm survival of patients following radical prostatectomy.
Material and Methods : With a median follow - up of 62 months maspin expression was immunohistochemically investigated in 42 radical prostatectomy specimens of different stage and grade (T1-4) and correlated with age, stage, histological grading, E - cadherin expression (Transduction Laboratories) as well as the tendency towards tumor progression and longterm survival of patients by univariate (Log rank test) and multivariate (Cox regression analysis) statistical analysis.
Results : None of the patients exhibiting maspin expression with a relative amount of $\geq$ 80 % of tumor cells stained positively developed tumor progression resp. died from prostate cancer during the follow - up period. In contrast, in all patients dying from prostate cancer $\leq$ 40 % of tumor cells exhibited a positive staining reaction. Surprisingly, maspin expression was negatively correlated with E - cadherin expression ($p = 0.014$) (Spearman - test).
Conclusion : Immunohistochemical staining for maspin seems to identify patients revealing a favorable clinical prognosis resp. a low risk for tumor progression following radical prostatectomy for the treatment of localized prostate cancer.
(This study was supported by a grant from the „Deutsche Forschungsgemeinschaft", DFG : Ku 877/3-1)

P 1.12

INDUCTION OF HAPTOTACTIC MIGRATION OF MAT-LYLU CELLS BY DUNNING PROSTATE-DERIVED MOTILITY FACTOR

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In the past decades, a number of Dunning R3327 rat prostatic tumor sub-lines have been established that differ in androgen sensitivity, differentiation state, and metastatic potential. We use the sub-lines G (well-differentiated, non-metastatic), AT2.1 (anaplastic, non-metastatic), and MAT-LyLu (anaplastic, metastatic), as a cell panel to investigate cellular changes that lead to metastatic behavior of cancer cells.
Previously, we have shown that conditioned media from AT2.1 and MAT-LyLu cells induce migration of MAT-LyLu cells in a modified Boyden chamber migration assay, whereas G cell conditioned medium does not contain this Dunning prostate-derived motility factor (DPMF). Now we report that DPMF is a trypsin-sensitive protein factor that induces haptotactic migration of MAT-LyLu cells. Furthermore, RGD-containing peptides specifically inhibit DPMF-induced migration. These results suggest that DPMF is an extracellular matrix component-like factor. Bioactive, partially purified fractions, obtained by Concanavalin A and anion exchange chromatography, showed several bands on SDS-PAGE with molecular weights ranging from 200 to 250 kD. Further purification and subsequent protein sequencing will eventually reveal the nature of DPMF.

P 1.13

INTERFERENCE OF PENTOSANPOLYSULFATE (PPS) WITH CELL ADHESION, MOTILITY AND PROLIFERATION OF HUMAN PROSTATIC CELL LINES *IN VITRO*. Denis H. Schamhart, Bonnie Molenaar, Alex C. Westerhof, Elizabeth C. de Boer and Karl-Heinz Kurth. Dept. Urology, Amsterdam, The Netherlands.
Administration of (semisynthetic) glycosaminoglycans, like heparin and PPS, has been noted to inhibit progression of a variety of neoplasms at levels, suggested to affect growth, angiogenesis and/or metastasis. Currently, the mechanism of action is unknown. Hypothesizing cell-extracellular matrix adhesion/interaction is a common factor in all these processes, this study focussed on the effects of PPS on the cell adhesion-dependent phenomena, motility and proliferation, of a series of human prostatic cell lines *in vitro*.
Cell lines, LNCaP, DU145 and PC-3 were cultured in standard RPMI medium with 10% fetal calf serum. Cell proliferation (total cells) was determined with the XTT assay. Cell adhesion was quantitated as cells remained attached after washing the cultures twice. Cell migration was determined with a Boyden chamber assay with Matrigel as attractant.
The results indicated that, in contrast to natural GAG's (heparan-, dermatan- and chondroitinsulfate), PPS (0-300 μg/ml) added *during* the process of cell adhesion to the substrata plastic, fibronectin or collagen IV inhibited adhesion (A) and subsequent proliferation (P) of LNCaP [$IC(A)_{50}$ and $IC(P)_{50}$ < 1 μg PPS/ml) and DU145 ($IC(A)_{50}$ = 20 μg PPS/ml; $IC(P)_{50}$ = 50 μg PPS/ml on plastic], but not PC-3 cells. A linear relationship was observed between the degree of PPS-inhibited adhesion and rate of cell proliferation. Cell migration of DU145, but not of PC-3 cells appeared to be inhibited in a PPS concentration (10-300 μg/ml) dependent manner.
In conclusion, interference of a nonnatural, GAG-like structure, PPS, with cell adhesion may affect cell proliferation and motility. These data may explain the inhibitory effect of GAG-'s, like heparin and PPS, at various levels of tumor growth progression, i.e. proliferation, angiogenesis and metastasis.

P 1.14

EFFECTS OF CONDITIONED MEDIUM FROM ACTIVATED MONOCYTES ON PROLIFERATION AND SECRETION IN PROSTATE CARCINOMA CELLS. Zoran Culig[1], Alfred Hobisch[1], Anton Hittmair[2], Francoise Geisen[3], Marcus V.Cronauer[1], Christian Radmayr[1], Georg Bartsch[1], Helmut Klocker[1] and Günther Konwalinka[3], Departments of Urology[1], Pathology[2], and Hematology and Oncology[3], University of Innsbruck, Austria.
It has been demonstrated that the immune cells secrete substances which decrease proliferation in a number of hematopoietic tumors. In this study we have investigated possible interactions between activated monocytes and human prostate cancer cells. For this purpose, peripheral blood monocytes were stimulated by phytohemagglutinin (PHA) for 48 hours. LNCaP cells were supplemented with increasing concentrations of conditioned medium (CM) from activated monocytes. Controls included unsupplemented LNCaP cells and LNCaP cells treated with PHA alone. CM from activated monocytes decreased proliferation of LNCaP in the absence and in the presence of androgen. Maximal inhibition was about 35% after treatment with 40% CM. PHA itself showed no effect on proliferation. The cells became elongated with dendritic-like processes. However, changes in the expression of luminal cytokeratins 8 and 18 were not observed in CM-treated cells on immunohistochemistry. Treatment with CM from PHA-activated monocytes decreased androgen receptor levels in LNCaP cells up to 40%. Prostate-specific antigen (PSA) was downregulated by CM in a concentration-dependent manner. In the absence of androgen, the PSA level was reduced by about 50%. CM antagonized stimulatory effects of the synthetic androgen methyltrienolone on PSA secretion by reducing the PSA amount by 70%. Our results demonstrate that PHA-activated monocytes secrete substances which can slow proliferation of prostatic cancer cells and downregulate PSA. These findings may have implications on therapeutic regimens for human prostate cancer.

P 1.15

SODIUM-PHENYLBUTYRATE: EFFECTS ON CELL CYCLE AND APOPTOSIS IN PROSTATE CANCER CELL LINES

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Sodium-Phenylbutyrate (NaPB) is a potent differentiating agent and currently under investigation for the treatment of prostate cancer (CaP). We have studied the impact of NaPB on proliferation and apoptosis of LNCaP cells *in vitro* and *in vivo* and furthermore the recently developed xenograft model of advanced CaP, LuCaP 23. *In vitro*, LNCaP cells were treated with NaPB at concentrations of 2.5 and 5 mM. Cell cycle analysis was carried out using two parameter flow cytometry (Ki-67, DAPI). Induction of apoptosis was examined using the TUNEL procedure. *In vivo*, 10 athymic mice/group (LNCaP, LuCaP 23) were treated with NaPB (1200 mg/ kg body weight/ day as intraperitoneal injections for 21 days), castration alone, castration plus NaPB or normal saline (control). Tumor volume and serum PSA secretion were measured weekly. *In vitro*, PSA-secretion/cell was increased by NaPB. Cell proliferation was inhibited in a time and dose dependent manner resulting in a cell cycle arrest in G0/G1 phase. Induction of apoptosis was noted beginning at concentrations of 2.5 mM. *In vivo*, tumor growth stabilized or regressed in treated animals as compared to controls. Effects on serum PSA secretion were variable implying that changes in serum PSA levels are not necessarily correlated with changes in tumor growth during therapy with a differentiating agent. The significant cytostatic and differentiating activities of NaPB provide further rationale for clinical trials of this agent inCaP.

P 1.16

EXPRESSION OF MATRIX METALLOPROTEINASE 2 AND 9 IN RENAL CELL CARCINOMA CELL LINES

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Introduction: Degradation of the extracellular matrix around tumor cells is an essential step in the process of tumor invasion and metastasis. The family of structurally related metalloproteinases (MMPs) plays an important role in matrix degradation by tumor cells.

The gene expression of MMP-2 and MMP-9 was studied in 8 cell lines derived from renal cell carcinoma (RCC). The overexpression of MMPs was correlated to tumor stage at the time of surgery.

Material and methods: 8 cell lines derived from renal cell carcinoma were analyzed for MMP-2 and MMP-9 expression using RT-PCR. 4 cell lines were derived from patients with locally confined (pT2) RCC, the other ones were derived from patients with locally progressed or metastasized tumors (≥pT3a and /or M1).

Results: 5 of the 8 cell lines showed an overexpression of MMP-2. 3 of the cell lines were positive for MMP-9. All cell lines derived from patients with advanced carcinoma had an overexpression of MMP-2 whereas only one of the cell lines derived from locally confined tumors had an MMP-2 overexpression. The 3 cell lines positive for MMP-9 were derived from patients with advanced RCC.

Conclusion: MMP-2 and MMP-9 are involved in degradation of extracellular matrix which is an essential step in tumor invasion and development of metastasis in renal cell carcinoma.

Molecular biology & genetic

P 2.1

CISPLATIN RESISTANT UROTHELIAL CANCER: THE POSSIBLE ROLE OF FERRITIN

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INTRODUCTION: Cisplatin is one of the most active drugs in cancer chemotherapy. However, its efficacy is hampered by the development of cisplatin resistance. To find new genes associated with cisplatin resistance we isolated differentialy expressed cDNAs from drug sensitive RT 112 cells and their drug resistant sublines CP3 and CPF.

METHODS: RNA from all cell lines has been extracted and converted to cDNA. Analysis for differentially expressed nucleotide sequences has been performed by the Differential Display (DD) method.The three different sublines have been screened with 24 primer combinations and 37 differentially expressed cDNAs have been excised from the DD-gel. All fragments have been reamplified and cloned.

RESULTS: By Northern blotting we found two differentially expressed cDNAs. One was overexpressed in CPF cells and and was homologous to a so far uncharacterized cDNA sequence from human aorta. The other showed complete homology to human ferritin.

CONCLUSIONS: The differential display is a powerful method to find genes associated with drug resistance.Ferritin overexpression may be a new resistance mechanism since this protein sequesters free iron which is a strong catalysator for the production of hydroxyl radicals resulting in oxidative cell damage.

P 2.2

STRUCTURAL ABERRATIONS OF CHROMOSOMES 8P AND 16Q ARE RELATED TO DNA PLOIDY AND NUMERICAL CHROMOSOME ALTERATIONS IN HUMAN PROSTATE CARCINOMA: .

Yi Pan, Hideyasu Matsuyama, Naining Wang, Satoru Yoshihiro, Chunde Li, Bernhard Tribukait, Peter Ekman and Ulf S. R. Bergerheim, Stockholm, Sweden (Presented by Dr. Pan).

Deletions of parts of chromosomes 8p, 10q and 16q are some of the most frequent alterations seen in human prostate carcinoma and have been suggested to be major genetic alterations involved in the multistep process of the tumorigenesis of prostate carcinoma. Our previous fluorescence *in situ* hybridization (FISH) studies show that the deletions of chromosomes 8p and 16q are associated with increasing malignancy of prostate tumors. To furthermore investigate the role of these chromosomal aberrations, deletions of 8p, 10q and 16q were compared with DNA ploidy, chromosomal numerical aberration and tumor metastases.

Touch biopsies from 32 primary and 22 metastatic specimens were examined by interphase cytogenetics FISH technique for both centromere and cosmid probes for chromosomes 8, 10, 16. Flow cytometric DNA analysis was applied for the same tissue specimens.

In twenty-two metastatic tumor, we found 8p deletions and 16q deletions in 20 (91%) and 15 (68%), respectively. It is significantly higher than in primary tumors with 59% and 41%, respectively. There was no difference in 10q deletion between primary and metastasic tumors. Deletion of chromosomes 8p and 16q were highly related to chromosomal centromeric alterations. Among 23 diploid primary tumors, deletions on 8p were observed in 12 tumors but 16q deletions were found only in 7 tumors. DNA aneuploidy was related to numerical aberration of chromosome 16, but not to chromosome 8.

Both deletions of 8p and 16q are related to increasing malignancy. Deletion of 16q is related to genetic instability (DNA aneuploidy) whereas 8 and 8p are not.

P 2.3

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF TWO STEROID 5α-REDUCTASE ISOZYMES IN THE CANINE PROSTATE.

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The dog has been extensively used as an *in vivo* model to test the pharmaco-kinetics and effects on pathological prostatic growth of 5α-reductase inhibitors. However, no information is available on the existence or characteristics of canine 5α-reductase isozymes. Here, two pH-optima of 5α-reductase activity in dog prostatic homogenates are described, comparable to the pH-optima of rat and human 5α-reductase isozymes. Kinetic analysis of 5α-reductase enzymatic activity at pH 7.0 revealed a high affinity (K_m = 2.67 nM, V_{max} = 4.75 fmol/min/mg protein) and a low affinity isozyme (K_m = 1.23 μM, V_{max} = 0.674 pmol/min/mg protein). The apparent affinity constants compare favourably to the human and rat isozymes type II and I respectively. The human type II specific inhibitor finasteride selectively inhibited the high affinity isozyme, whereas the human type I specific inhibitor MK386 preferentially inhibited the low affinity isozyme. The human type I specific inhibitor LY306089 was non-specific for the dog isozymes. We postulate that the low and high affinity isozymes described in this poster represent the dog type I and type II 5α-reductase isozymes respectively. The dog thus proves to be a valid model for the treatment of pathological prostatic growth with selective 5α-reductase inhibitors.

P 2.4

LOSS OF 5 ALPHA REDUCTASE EXPRESSION IN DISTANT METASTASIS FROM PROSTATE CANCER.

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To gain some insight into the action of 5 alpha reductase isoenzymes in prostate cancer and in metastatic lesions, we have examined the mRNA expression of 5 alpha reductase type I (R-I) and type II (R-II) in primary tumours of different grade and also in secondaries obtained from patients with lymph node and boney metastasis. In all these studies localisation of the mRNA was visualised by in situ hybridisation employing the digoxigenin labelled RNA probe technique. In well differentiated prostate cancer, the results demonstrated that the expression of both isoenzymes was confined to the epithelium and the nucleus of the stroma. However as the tumour progressed and became less differentiated, we observed a decline in smooth muscle staining for both the R-I and R-II. Furthermore, many tumours with grade 8-10 showed no mRNA expression for either isoenzyme in the stroma. Significantly, none of the prostate tumour metastasis expressed mRNA for 5 alpha reductase whether these had metastasised to the lymph node or bone. Interestingly all tumour metastasis displayed PSA positivity indicating infiltration by the prostate cancer cell. We are now examining the possibility that the loss of hormone sensitivity in prostate cancer may be linked to the availability of 5 alpha reductase in these cells.

P 2.5

MOLECULAR STAGING OF THE CLINICALLY LOCALIZED PROSTATE CANCER BY RT-PCR : THE IMPORTANCE OF THE CHOICE OF THE PRIMERS

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INTRODUCTION AND OBJECTIVES : Preoperative staging of prostate cancer is one of the most important problem that we have to resolve. Today, the combination of all available tests is not enough ccurate to determine the final stage of prostate cancer : 35-40% of the operated patients have a locally invasive disease on pathology. Many authors have proposed the use of detection of circulating cells by RT-PCR, but there is no study that pointed out the rule and the importance of the primers. We have chosen primers with two essential characteristics : 1) they are 100% specific of PSA m RNA (hKLK3), not able to amplify hKLK2, and 2) our primers are able to amplify all the different splices of PSA m RNA (PA 75, PA 525, PA 424).

METHODS : From June 95, to June 96, 44 men ages 50 to 76 years (mean 64.5 years) underwent radical prostatectomy for prostate cancer. We collected for each patient a blood sample before surgery to detect circulating cells by RT-PCR.

RESULTS : Of the 44 patients, 28 were pT2 (63%) and 16 were pT3 (37%). We detected circulating cells in 3 pT2 and in 6 pT3. Statistically, there was a significant difference between these two groups ($p = 0.03$). The PCR was also able to differentiate patients with seminal vesicles invasion ($p = 0.02$), capsular penetration ($p = 0.03$) and positive margins ($p = 0.02$).

CONCLUSIONS : It seems that the RT-PCR is able, prior to surgery to distinguish between pT2 and pT3 and is also able to differentiate some anatomopathological criteria. Our strategy improved the staging as compared to the previous published data. Nethertheless, the presence of false positives (14% positive pT2) is not compatible with the use RT-PCR in routine clinical practice.

P 2.6

THE DETECTION OF HEMATOGENOUS MICROMETASTASES BY RT-PCR OF „PROSTATE ONCOGENE PTI-1" FOR THE DIAGNOSIS OF PROSTATE CANCER

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INTRODUCTION AND OBJECTIVES: RT-PCR for the detection of hematogenous micrometastases promises new possibilities in the diagnosis and staging of prostate cancer (CaP). We investigated whether the detection of prostate cancer cells in blood by reverse transcriptase PCR (RT-PCR) with primer against the „prostate oncogene PTI-1" (Shen et al., PNAS, 1995) could be used in such an assay.

METHODS: We analysed blood-cells of 44 patients with untreated CaP (mean PSA 20.3 $\pm$ 3.35 ng/ml) and 51 patients with benign prostate hyperplasia (mean PSA 9.01 $\pm$ 1.88 ng/ml, Abbott AxSYM-PSA assay). The buffycoat fraction was prepared with a ficoll gradient centrifugation and the RNA was extracted. DNA was digested by using Dnase I. 4 µg RNA were reversely transcribed using a cDNA Synthesis Kit from Pharmacia by random priming. Integrity of the RNA was checked by RT-PCR with primer against G3PDH for 23 cycles. The first RT-PCR was performed using 3 µl of the cDNA reaction and PTI-1 primer for 40 cycles. One tenth of this PCR was used to perform the second (nested) PCR using PTI-1 specific primer. Agarose gelelectrophoresis was used to detect the amplified PTI-1 fragment.

RESULTS: PTI-1 could be detected in 20 of 44 patients with CaP. In 10 of 51 patients with BPH a band was observed at the expected molecular weight of PTI-1. Therefore, a sensitivity of 45% and a specificity of 80% was obtained. Using the cut-off level of 4 ng/ml for PSA a sensitivity of 84% and a specificity of 55% was calculated. Detection of PTI-1 in the patients with CaP could not be correlated with the PSA value or tumor stage.

CONCLUSIONS: The nested RT-PCR with PTI-1 primer offers a new possibility for the diagnosis of prostate cancer. Furthermore it seems to offer the advantage of detecting malignant cells rather than only prostate cells in the bloodstream. It is however unclear wether the 10 of 51 patients with BPH may have also a latent cancer which lead to $PTI1^+$-cells in blood.

P 2.7

G PROTEIN-COUPLED RECEPTOR-INDUCED MIGRATION OF HUMAN TRANSITIONAL CELL CARCINOMA CELLS.

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OBJECTIVES: Migration is a crucial step for malignant tumor cells in developing metastasis. Aim of the present study was to examine the influence of G protein-coupled receptors on motility of the human transitional cell carcinoma cell line J82.

METHODS: The cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) was determined with the fluorescent calcium indicator dye Fura-2 in a spectrofluorometer. Cell motiliy was assayed in a 48-well microchemotaxis chamber.

RESULTS: Activation of heterotrimeric Guanine-nucleotid-binding protein (G protein)-coupled receptors by lysophosphatidic acid (LPA), thrombin and histamine resulted in rapid and transient increases in $[Ca^{2+}]_i$. Pretreatment with pertussis toxin (PTX), that specifically ADP-ribosylates and uncouples G_i-type G proteins, inhibited the LPA-induced increases in $[Ca^{2+}]_i$ by about 50 %. PTX had no effect on the increases in $[Ca^{2+}]_i$ induced by thrombin or histamine. Motility of J82 cells was strongly stimulated by LPA and thrombin, whereas histamine was without any effect. Pretreatment with PTX completely inhibited the LPA-induced motility of J82 cells in a highly sensitive manner (half-maximal inhibition at about 10 pg/ml PTX), but had no effect on the thrombin-induced cell motility.

CONCLUSIONS: Motility of J82 cells can be induced by LPA and thrombin, whereas activation of motility by these receptors obviously involves different subtypes of G-proteins. As PTX is a potent inhibitor of the LPA-induced cell motilitiy, G_i-type G protein targeting may provide new therapeutical strategies in cancer therapy. PTX could be a possible candidate.

P 2.8

KAI1/CD82 EXPRESSION IN TRANSITIONAL CELL CARCINOMA

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Recent investigations have suggested that KAI1/CD82 is a metastasis suppressor gene for human prostate cancer. KAI1 is located on chromosome 11p, a region of frequent allele loss in advanced transitional cell carcinomas. In this study expression of KAI1 in urothelial carcinoma cells was examined.

Six urothelial cancer cell lines and 16 freshly frozen tumor samples from patients undergoing surgery for transitional cell carcinoma were studied. Total cellular RNA was purified by the Trizol method. A semi-quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was carried out to amplify a 575 bp KAI1- and a 350 bp GAPDH-fragment. The digoxigenin labelled PCR-samples were run on a 1,5 % agarose gel, blotted to a nitrocellulose membrane and visualized using a luminescence conjugated anti-digoxigenin antibody. The KAI1 expression ratio was defined as the value obtained by densitometry of the band for KAI1 divided by that of GAPDH for each specimen.

Two of six cell lines (T24 and 639V) showed no KAI1-expression. Four of 10 muscle invasive high grade transitional carcinomas showed complete loss of and an additional one barely detectable KAI1 expression. In contrast, in all 6 papillary low grade tumors investigated at least low KAI1-expression was detectable.

Decreased expression of KAI1 was frequently observed in muscle invasive high grade transitional cell carcinomas. Large scale studies including follow-up data are needed to establish the role and possible prognostic value of KAI1 in transitional cell carcinomas. The cell lines T24 and 639V, which lack KAI1 expression, can be used for further functional analysis in vitro.

P 2.9

P-GLYCOPROTEIN AND TOPOISOMERASE-IIα EXPRESSION IN SPECIMENS FROM HUMAN KIDNEYS AND MATCHED KIDNEY CARCINOMAS.

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Introduction: Chemotherapeutic treatment of disseminated renal cell carcinoma (RCC) is greatly impaired by the expression of multidrug resistance mechanisms. Several factors have been shown to be responsible for the insensitivity of tumor cells to cytotoxic drugs. Elevated P-glycoprotein (Pgp) and decreased Topoisomerase II α (TopoIIα) expression, a DNA-replication related nuclear enzyme, have been implicated in experimental drug resistance of RCC. We now analyzed these tumor resistance markers in primary human RCC specimen.

Methods: We prospectively assembled tumor and matched normal kidney tissue-samples, distant from the tumor, from 25 RCC patients of the clear-cell type. Expression of Pgp and TopoIIα was determined by immunohistochemistry using MoAbs JSB1 and anti-TopoII and by quantitative RT-PCR. Results were correlated to grade of the tumors, as a major prognosticator, and to normal kidney tissue.

Results: Considerably decreased expression of TopoIIα was seen in all tumor samples, compared to normal kidney tissue. Grade II and III tumors expressed slightly more TopoIIα than grade I tumors. Pgp expressions strongly fluctuated. All 15 grade I tumors expressed Pgp; 2 tumors showed increased Pgp, 13 had decreased Pgp compared to normal tissue. The higher grade tumors all showed decreased expression of Pgp.

Conclusion: Apparently, the Pgp expression pattern follows the dedifferentiation from normal tissue to different tumor grades. Thus, Pgp itself is not associated with poor prognosis of RCC. However, decreased TopoIIα expression, which may cause cytotoxic drug resistance, correlates with malignant transformation

P 2.10

EXPLANT CULTURES DERIVED FROM NORMAL AND BPH PROSTATES

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In the past we have studied primary stromal cell cultures from human prostate specimens. This study will focus on prostate epithelial cell cultures established from explants cultures. This enzyme-free method permits the establishment and expansion of pure epithelial cell cultures in a short amount of time. Depending on the patients or donors age, we observed the first outgrowth of epithelial cells after 3-5 days. After 10 days cells became 60% confluent and the tissue piece was removed. The cells were grown on tissue culture plastic and maintained in WAJC 404 supplemented with 10% FBS. Under these culture conditions, epithelial cells proliferated rapidly and grew to confluence in a monolayer. Depending on the donors or patients age doubling time varied between 32.8 and 87.5 hours. In order to further characterize the phenotype of the primary cultures we performed immunocytochemical staining for cytokeratins. We found that cells stained positively for cytokeratins 5 and 15, which are specific for basal cells. The majority of the epithelial cells (up to 90%) stained positively for those cytokeratins. However, utilizing an antibody that can detect cytokeratins 8 and 18, 10-15% of the cells stained positively for these particular luminal cell markers. Not one single cell stained positively for smooth muscle myosin heavy chain (SMHC). Recent studies utilizing collected human spermatocele fluid (SCF) demonstrated that it is mitogenic to human prostatic stromal cells in culture. However, SCF was not mitogenic to prostate epithelial cells in vitro. In order to study the effect of SCF stimulated stromal cell conditioned media (SCF-CM) on epithelial cells, we performed a six day proliferation assay. We found that SCF-CM was able to stimulate epithelial cells growth significantly compared to the diluent (WAJC) and compared to the control CM. The control CM was obtained from unstimulated CM derived from the same stromal cell culture.

Taken together, we found that our explant cultures were composed of 90% basal cells and 10% luminal cells. Stromal cell contamination has not been observed. Epithelial cells were not able to respond to pure SCF but to SCF stimulated stromal cell CM. These data will provide us with new insights into stromal and epithelial cell interaction in the human prostate.

P 2.11

AN IN VITRO MODEL FOR VIDEOIMAGING OF PROSTATIC SMOOTH MUSCLE CELL CONTRACTIONS - EFFECT OF α1-ADRENOCEPTOR ANTAGONISTS

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Introduction and Objectives: Stromal tissue has been shown to be the most prominent structure in the development of benign prostate hyperplasia (BPH). SMC tension is known to play a major role in the induction of bladder outlet obstruction caused by BPH. We established an in vitro model for video visualization of single cell contractions and used it to examine the effects of α1-adrenoceptor antagonists on prostatic SMCs.

Methods: SMCs were isolated from human prostate specimens. Cells were cultured in a selective medium supplemented with growth factors and steroid hormons. Culture flasks were coated to allow cell contraction. SMC contractions were visualized with a cell culture microscope equipped with a time-lapse video system.

Results: Coating of the culture flasks with viscous substances such as extracellular matrix or a cell adhesion substrate allowed SMCs to contract. Adrenergic stimulation with 10µM of noradrenalin or phenylephrine resulted in contraction of about 40% of the cells. Control cultures of SMCs or phenylephrine-stimulated foreskin fibroblasts showed only sporadic contractions (up to 5% of the cells). The number of SMCs contracting after stimulation with noradrenalin or phenylephrine was reduced to control levels by preincubation with α1-adrenoceptor antagonists.

Conclusions: Videomicroscopy of cultured SMC is an applicable method to investigate human prostate SMC contractions. This model offers the possibility to study drug-effects on prostatic SMC with a simple in vitro model.

P 2.12

EFFECT OF PERMIXON ™ IN A NOVEL IN-VITRO CO-CULTURE MODEL FOR BPH.

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Introduction and objectives: There has recently been an increasing interest in drugs which inhibit 5 alpha reductase isoenzymes in BPH. Amongst the established inhibitors of 5 alpha reductase type I (R-I) and type II (R-II) is the lipidosterolic extract of the dwarf palm serenoa repens (Permixon™). In this study we set out to demonstrate its effects in a novel co-culture model of BPH.

Methods: Epithelial and stromal cells cultured from BPH tissue were subsequently grown in co-cultures. These co-cultured cells were treated with 10µg.ml Permixon™ following which the impact of the drug on PSA production, 5 alpha reductase activity and cell growth and structure were investigated.

Results: Epithelial cells from co-culture demonstrated positive immunostaining for PSA before and after treatment with Permixon™. Likewise, Permixon™ did not affect the concentrations of PSA in the conditioned media but these levels were elevated following supplementation with testosterone. We also measured the 5 alpha R activity (moles/cell/30min) in the epithelial cells before (R-I: $3.29 \pm 0.39 \times 10^{-17}$; R-II; $4.05 \pm 0.55 \times 10^{-17}$) and after (R-I: $1.75 \pm 0.541 \times 10^{-17}$; R-II: $1.59 \pm 0.449 \times 10^{-17}$) exposure to Permixon™. The enzyme activity of the stromal cells was also inhibited by the drug (R-I before: $1.6 \pm 0.169 \times 10^{-18}$; R-II before: $9.08 \pm 0.50 \times 10^{-19}$; R-I after: $1.22 \pm 0.24 \times 10^{-18}$; R-II after: $6.77 \pm 0.59 \times 10^{-19}$). Electronmicroscopy studies demonstrated a dissruption of cellular membranes, an accumulation of fatty globules within the cytoplasm and polarisation of the chromatin for both co-cultured cells after treatment.

Conclusions: Permixon™ inhibits both isoenzymes of 5 alpha reductase in this model of BPH without affecting PSA production. This makes it useful in the treatment of BPH.

P 2.13

ROLE OF CYTOCHROME P450 4B1 IN INDUCTION OF BLADDER CARCINOMA

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Arylamines such as benzidine were amongst the first pure chemicals to be identified as human carcinogens. Metabolism studies with many arylamines have shown cytochrome P450 catalysed N-hydroxylation to be a critical step in the activation of these compounds leading to the formation of toxic and carcinogenic metabolites.

In this study, we investigated mutagenic activation of these aromatic amines by multiple forms of P450 and also investigated the presense of P450 forms in bladder by immunological study. We have purified Cyp4b1 from renal microsomes of male mouse. Cyp4b1 had high mutagenic activity towards 3,3'-dichlorobenzidine(DCB) by umu gene expression system, which can detect DNA damage. Antibody against Cyp4b1 efficiently inhibited this activity. DCB is established as inducer of bladder cancer in the rat. Ten forms of purified rat P450s including CYP4B1 were used in umu test for DCB. CYP4B1 had extensively higher mutagenic activation towards DCB than other forms. In immunoblotting, rat bladder microsomes gave a single band of CYP4B1. Furthermore, rat bladder epithelium was specifically stained with this antibody.These findings suggested that CYP4B1 has an important role in induction of bladder carcinoma by carcinogenic aromatic amines such as benzidine. The presence of CYP4B1 in human bladder was also confirmed by immunoblotting and RT-PCR. The expressionof CYP4B1 mRNA in bladder cancer patients was higher than that in non bladder cancer patients.

P 2.14

INDUCIBLE P53 STABLY TRANSFECTED IN UROTHELIAL CARCINOMA CELL LINES: A USEFUL MODEL FOR THE INVESTIGATION OF CELL CYCLE DEPENDENT GENES.

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Several genes participate in cell cycle regulation in a p53-dependent manner. In order to examine the relationship between these genes in the cell cycle regulation of urothelial carcinoma cells an in vitro model with an inducible p53 was developed.

Stable transfection of either wild type or mutant (273Arg→His) p53-cDNA driven by an inducible metallothionein promotor into urothelial carcinoma cells with mutated endogenous p53 was carried out by the calcium phosphate method. Stably transfected clones were selected for a neomycin resistance gene and identified by Southern blot. P53- and P21 expression after 0, 16 and 40 h exposure to $ZnCl_2$ was investigated by Western blot using the monoclonal antibody PAb 1801 and WAF1 PAb1, respectively.

Southern blot analysis identified mutant p53 clones for T24 and VM Cub1 but only wild type clones for VM Cub1. In contrast to untransfected cell lines, stable clones showed a time-dependent increase of P53 expression after exposure to $ZnCl_2$ up to 40 hours. The increase in P53 expression was parallelled by an induction of P21 in wild-type clones.

In order to study P53 action in urothelial carcinoma cells an in vitro model with an inducible p53 was developed. The validity of this model is demonstrated by the induction of p21 following activation of the metallothionein promoter by $ZnCl_2$. This model system appears promising to analyze further p53-dependent candidate genes (e. g. bax, bcl-2, mdm-2) and their interaction in cell cycle regulation.

P 2.15

LECTIN-INDUCED ALTERATIONS ON THE PROLIFERATION OF PROSTATIC CANCER CELL LINES.

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INTRODUCTION AND OBJECTIVES: Lectins are carbohydrate-binding proteins other than enzymes or antibodies which are present in a wide range of dietary constituents and are highly resistant to digestion. While lectins are known to influence the cell growth of several types of normal and neoplastic tissues, their roles in the case of prostatic cancer cells remain relatively unexplored. In the current study we report the *in vitro* influence of five lectins on the cell proliferation of the androgen-sensitive LNCaP and the androgen-insensitive DU145 and PC3 human prostatic cancer cell lines.

MATERIALS AND METHODS: The prostatic cells lines were cultured with five lectins, namely peanut (PNA), wheat germ (WGA), Concanavalin A (Con A), *Griffonia simplicifolia* (GSA-IA4) and *Phaseolus vulgaris* (PHA-L) agglutinins. The cell proliferation was assessed by means of the colorimetric MTT assay. Four lectin concentrations were tested i.e. 0.1, 1, 10 and 100 µg/ml at 5 experimental states, i.e. 2, 3, 5, 7 and 9 days following the addition of each lectin to the culture media.

RESULTS: The 5 lectins under study had a globally significant dose-dependent toxic effect on prostatic cancer cell proliferation. Nevertheless, low doses of GSA-IA4 and PHA-L significantly ($p<0.05$ to $p<0.001$) increased the cell proliferation of PC3 cells.

CONCLUSIONS: The present data strongly suggest that some lectins might influence the proliferation of prostatic carcinoma cells. In addition, because lectins are present in the diet, and are able to pass into the systemic circulation, the present results suggest that some lectins might exert a modulatory role on prostate cancer growth under clinical conditions.

P 2.16

INTRATUMORAL INTERLEUKIN 2 DEPOTS INHIBIT GROWTH OF DUNNING PROSTATE ADENOCARCINOMA EFFECTIVELY

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OBJECTIVES: Local continuous interleukin 2 (IL-2) application is not toxic. Immunotherapy may be a treatment option for inoperable, recurrent or hormone refractory advanced prostatic carcinoma. Toxicity will be a critical issue in these patients. We studied the effect of local continuous interleukin 2 therapy on the Dunning prostatic adenocarcinoma in the rat.

METHODS: 60 juvenile male Copenhagen rats with Dunning prostatic adenocarcinoma implanted subcutaneously into the flank and only after proven tumor growth were treated with either IL-2 depot preparations (n=30) or with albumin depots (=controls). Tumor doubling time was 4.5 days. Depots were implanted 4 days after tumor transplantation directly to the tumor site. Serum cytokine levels were determined day 6 and 20 for human IL-2 and rat interferon g.

RESULTS: Depots were tested to release biologically active IL-2 for at least 8 days. No toxicity was observed. Recovery from implantation was quick and animals with local IL-2 release seemed unimpaired compared to control rats. Tumor growth in the IL-2 depot treated rats was significantly reduced ($p< 0,001$) compared to controls. No complete tumor remission was achieved. Cytokine serum analysis did not show significant differences compared to controls, indicating local antitumor mechanisms.

CONCLUSIONS: Local continuous interleukin 2 application is useful to control growth of prostate carcinoma in the rat. Low toxicity of intratumor application is a very important issue for this treatment option.

Immunology & preclinical research

P 3.1

DENDRITIC CELLS CULTURED FROM PERIPHERAL BLOOD AS ADJUVANT FOR THE ACTIVE IMMUNOTHERAPY OF UROLOGIC TUMORS.

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INTRODUCTION AND OBJECTIVES: Dendritic cells are potent antigen-presenting cells specifically adapted to initiate immune responses. Based on recently developed cell culture protocols experimental prove has been obtained that dendritic cells also elicit antitumor immune responses. However, the original methods were based on media supplemented with fetal calf serum which is clearly not suitable for clinical use. We describe here the generation of "clinical grade" dendritic cells.

METHODS AND RESULTS: To this end, peripheral blood monocytes were prepared by a short adherence and cultured with granulocyte /macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) in commercially available, clinicalla approved media (AIM-V and X-VIVO20) further supplemented with 1% autologous plasma. Day-6 dendritic cells were characterized by flow cytometry and a panel of monoclonal antibodies. In addition, fluoresceinated Dextran and bovine serum albumin (BSA) were used to demonstrate the endocytic capacity of these cells which can be exploited for tumor antigen loading. Treatment of dendritic cells with tumor-necrosis factor-alpha for 2 to 3 days downmodulated endocytosis and upregulated a number of surface antigens including CD83 and CD86 (B7-2) in dendritic cells.

CONCLUSIONS: Tumor-antigen-pulsed, mature CD83-positive dendritic cells with high levels of co-stimulatory molecules should be strong stimulators of T lymphocytes with tumor reactivity.

P 3.2

CYTOKINE RESPONSE PATTERNS IN LEUKOCYTE CULTURES OF PATIENTS WITH URINARY BLADDER; RENAL CELL AND PROSTATE CARCINOMAS

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The events that result in a protective immune response are coordinated to a large extent by cytokines produced by T helper 1 (Th1) and T helper 2 (Th2), T-cell subsets, which are two arms of the immune system. Th1 cells preferentially produce IL-2, IFN-γ and TNF, resulting in a cellular response, whereas Th2 cells produce IL-4, IL-5, IL-6 and IL-10 and stimulate a humoral response. Disregulated expansion of one or the other subset may be associated with pathological conditions.

In order to investigate whether there is a change in the cytokine response pattern in patients with growing malignant tumors we determined the production of IL-2, IFN-γ, IL-4, IL-6 and IL-10 in the mitogen-stimulated leukocyte cultures of 47 patients with urinary bladder cancers, 62 patients with renal cell carcinomas and 111 patients with prostate carcinomas at different clinical stages as compared to 122 healthy controls.

Production of the Th1 cytokines IL-2 and IFN-γ was significantly lower in the cell cultures of the patients of all three tumor groups compared to the controls and there was a clear decline of the Th1 cytokines with increasing tumor stage. The levels of the Th2 cytokines IL-4 and IL-10 were comparable to the controls or somewhat lower. However, IL-6 production was significantly increased in the leukocyte cultures of all tumor patients and the levels of this cytokine, produced by Th2 cells and monocytes, increased with increasing tumor mass.

The data of this study suggest that there may be a progressive loss of a Th1, i.e. cellular immunological activity and simultaneously an elevation of IL-6 production in the peripheral leukocytes during tumor progression. Since Il-6 is a growth factor for urinary bladder, renal cell and prostate carcinoma cells this modulation of the immune reactivity may be beneficial for the tumor but fatal for the host.

P 3.3

MONOCYTE/MACROPHAGE INFILTRATION IN RENAL CELL CARCINOMA

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Aims: Monocytes/macrophages are a major component of the cellular immune response in tumour disease, and were shown to be associated with the stage of tumour disease. Cytotoxicity and extracellular matrix remodelling are properties discussed for tumour associated macrophages (TAMs). In this study the expression of formalin-resistent monocyte macrophage antigens in tumour nephrectomy specimens was analyzed.

Methods: Renal cell carcinomas were graded according to Thoenes. Paraffin-embedded specimens of normal kidney, the tumour-host interface (THI), and central tumour areas were investigated immunohistochemically using the monclonal anti-bodies (mab) Ki-M1P, 25F9, MRP8, 27E10, MRP14 and Mac 387 comprising late and early stages of the inflammatory response. Infiltration by macrophages was correlated with the tumour grade, the stage of disease, localization, and morphology.

Results: Grade 1-carcinomas contained a less dense infiltrate of Ki-M1P+ and Mac387+ TAMs than grade 2 and 3 tumours. Central tumour areas and the THI showed a more dense infiltrate of Ki-M1P+ TAMs and subtypes (except 27E10+ cells) in comparision with tumour-free tissue. Carcinomas in progress were characterized by a higher content of 25F9+ TAMs. In tumours with spontaneous necrosis especially 27E10+ and Mac387+ TAMs were detected in higher amounts.

Conclusions: a. The mab Ki-M1P detects a broad range of TAMs. The late stage inflammatory antigens 25F9 and MRP8, the acute stage markers 27E10 and MRP14, and Mac387 detect minor subpopulations of TAMs. b. Except 27E10+ cells all TAMs show a more dense infiltration within the tumour and THI compared with tumour-free tissue. c. The mab 25F9 detects a TAM subtype associated with disease progress. d. 27E10 and Mac387 expression in TAMs may reflect cytotoxic properties ot TAMs.

P 3.4

PLASMA LEVELS OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) IN PATIENTS WITH METASTATIC UROLOGICAL CANCERS

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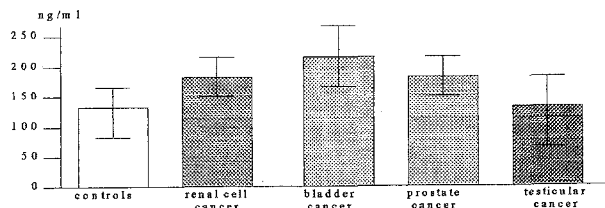
Introduction: MCP-1 has mainly been implicated as an important chemotactic mediator of monocyte and T-lymphocyte infiltration of tissues in a wide variety of inflammatory diseases. In addition, a function in immunological tumor response in human malignancies has been suggested. The aim of this study was to evaluate the relevance of MCP-1 in systemic tumor response in patients with metastatic urological cancers.

Material and Method: Plasma levels of 56 patients with metastatic urological cancers (renal cell cancer, n=15; bladder cancer, n=14; prostatic cancer, n=22 and testicular cancer, n=5) and 40 controls were examined by specific ELISA test for MCP-1. Metastatic location included lung, bone, brain, liver and skin.

Results: Average levels of MCP-1 were not found to be significantly different between controls and any of the examined groups.



There was no significant difference in comparison of MCP-1 plasma levels between patients with renal cell carcinoma, bladder cancer, prostatic cancer or testicular cancer.

Conclusion: In advanced urological cancers, the average plasma level of MCP-1 is not significantly elevated. Nevertheless, some patients show distinct elevated MCP-1 plasma levels.This implicates a limitated MCP-1 involvement in certain metastatic urological cancers. In general, circulating MCP-1 does not represent a possible link between systemic tumor disease and immunological response.

P 3.5

HUMAN PROSTATE-SPECIFIC TRANSGLUTAMINASE IS ASSOCIATED WITH THE PARTICULATE, PROSTASOME CONTAINING FRACTION IN PROSTATIC EXCRETIONS.

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Human prostate-specific transglutaminase (hTG_P) is a cross-linking enzyme that might function in the suppression of sperm antigenicity and in semen coagulation. To investigate the physiological function of hTG_P we developed a polyclonal antiserum against the C-terminal of hTG_P using a GST-hTG_P fusion protein and studied the presence of hTG_P in prostatic fluids (PFs) and seminal plasmas (SPs). Also the presence of hTG_P in prostasomes, multilamellar vesicles of prostatic origin occurring in semen, was studied.

We examined six PFs from radical prostatectomy specimens and over fifteen human SPs from men visiting the andrology clinic. Prostasome enriched fractions from SPs were obtained by differential ultracentrifugation (200,000xg pellet). The hTG_P protein was detected by immunoblotting and antiserum specificity was determined by blocking experiments.

The antiserum specifically recognized two protein bands in the PFs: one major band of 77 kDa in agreement with its predicted molecular weigth and a minor band of approx. 70 kDa. The exact size of the smallest protein varied between individuals. All tested SPs contained the 77 kDa protein; its concentration was, however, highly variable. In addition, a smaller fragment of 33 kDa was present in a number of SPs. More than 90% of hTG_P was recovered in the prostasome containing pellet. The remainder was present in a free form in the fluid. In conclusion, we have demonstrated hTG_P in PFs as well as SPs. Both body fluids contain different molecular forms of hTG_P. Our data strongly suggest that hTG_P is associated with prostasomes, which supports an immunosuppressive role for this protein in semen rather than its involvement in semen clotting.

P 3.6

IN VITRO LYSIS OF MULTICELLULAR SPHEROIDS FROM TRANSITIONAL CARCINOMA CELL LINES BY BCG-ACTIVATED KILLER-CELLS (BAK-CELLS).

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INTRODUCTION: Previously, we described a distinct killer cell phenomenon by Bacillus-Calmette-Guérin activated mononuclear cells (BAK-cells) against monolayer urothelial tumor cells as part of the mode of action of Bacillus-Calmette Guérin- treatment. To further study these killer cells, their potency as to act on complex in-vitro tumors, multicellular three dimensional tumor spheroids (MCS) from urothelial carcinoma cells were chosen as targets.

METHODS: BAK-cells were generated by coincubation of peripheral blood mononuclear cells with viable BCG-strain Tice. Lymphokine-activated (IL-2) killer cells and natural killer cells were referred as to control. In parallel, MCS were generated from urothelial cell lines by „liquid overlay" technique in the presence of ^{3}H-methionine. Coincubation of effector cells and MCS was performed and release of radioactivity as we well as the morphological aspect of the speroid was determined.

RESULTS: The complex three dimensional structure of the spheroid tumors was disintegrated by coincubation with BAK- and LAK-cells but not by natural killer (NK-) cells. Determination of radioactivity release revealed that this phenomenon was due to lysis of carcinoma cells.

CONCLUSION: The effectiveness and the need of BAK-cells in killing residual tumor cells in patients following BCG- instillation therapy can be assumed by our in vitro findings on intact three dimensional MCS. Further research as to define it's exact role in vivo is ongoing.

P 3.7

IN VITRO TESTING OF DIFFERENT BCG PREPARATIONS TO REDUCE SIDE OF INTRAVESICAL BCG

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Intravesical instillations of BCG after surgical resection of superficial bladder tumors is accompanied by side effects. It was previously shown that PMNs infiltrate the bladder wall in the course of BCG therapy and shed mediators of inflammation. Therefore, we established an in vitro system to test both activation of PMNs and BCG induced cytotoxicity against bladder tumor cells.

We investigated different preparations of viable and non-viable BCG preparations for their potency to reduce activation of PMNs without compromising BCG induced cellular cytotoxicity by BAK cells (BCG-activated killer cells). Activation of PMNs was measured by phagocytosis of BCG and in a degranulation bioassay, while cytotoxicity was determined by a ^{3}H-L-methionine release assay with bladder tumor cells as target cells. We found small but apparent differences between preparations of commercially available lyophilisate (L), highly viable log-phase cultured BCG (log), autoclaved BCG (A) and culture supernatant (SN). Activation of PMNs was: L>log>A>SN. In contrast, we found the cytotoxicity of BAK cells to be: L=A>log>SN.

These data show that different BCG preparations only have a slightly diverse potential to activate PMNs and cytotoxic BAK cells, respectively. Therefore, the use of other than currently commercially available BCG preparations could possibly reduce side effects to a certain extent, but is by no means sufficient to eliminate undesired effects of PMNs. For this reason in a follow-up experiment we will test a large panel of antiinflammatory and antiphlogistic drugs in the same assays.

P 3.8

Th1 URINARY CYTOKINES ARE RELATED TO BLADDER TUMOR RECURRENCE RISK AFTER BCG TREATMENT

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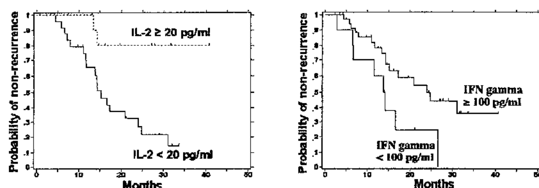
INTRODUCTION AND OBJECTIVES: The Th1 T helper cells release IL-2 and IFN-γ while Th2 cell release IL-4 and IL-10. The objective of this study was to evaluate the prognosis value of urinary cytokines in bladder cancer patients treated with BCG.

METHODS: IFN-γ, IL-2 and IL-10 urinary production after BCG (1 course of 6 weekly instillations with 150 mg of the BCG pasteur strain) was evaluated, by ELISA detection, in 47 patients with superficial bladder cancer (Ta, T1, CIS). Measurement were done at the 5th and the 6th instillations. Correlations between cytokine production and clinical outcome were evaluated by a Log Rank Test.

RESULTS: The median follow up was 22 months. Urinary cytokine production was not statistically correlated to tumor stage and grade. Patients with less than 20 pg/ml IL-2 or less than 100 pg/ml IFN-γ release, exhibited significantly more recurrences than those who were greater producers (Log Rank test p = 0.006 and 0.03 respectively). No predictive value was noted for progression risk. IL-10 had no impact on recurrence or progression.



CONCLUSIONS: The evaluation of the Th1 urinary cytokines could be a rationale tool for selection of patients who are at risk for recurrence after a single course of BCG treatment.

P 3.9

ON THE NUMBER OF BACILLUS CALMETTE-GUERIN (BCG) BACTERIA, INDUCING AN IMMUNE RESPONSE IN THE GUINEA PIG. Elizabeth C. de Boer, Arend H. Kolk, Sjoukje Kuijper, Karl-Heinz Kurth and Denis H. Schamhart. Dept. Urology, Amsterdam, The Netherlands.

Detailed knowledge about the number of BCG organisms binding to the bladder wall, leading to the immune response required for clinical efficacy, is not well established. Hypothesizing that the number of attached BCG organisms may determine clinical efficacy, this study focussed on the quantification of attached/incorporated BCG bacteria in the guinea pig bladder using PCR of specific BCG-DNA sequences.

Animals were intravesically treated with one instillation (1h) of saline or BCG (1E7 cfu). After 24h bladders were resected and homogenized with proteinase K. DNA purification was obtained by the guanidine isothiocyanate using diatoms for DNA binding. In the PCR, a 249-bp sequence of the insertion element *IS6110* (1,386 bp; 1 copy/BCG genome) was amplified. PCR products were detected by agarose gel electrophoresis.

After spiking of bladder specimen (prior to homogenization) with intact *M. tuberculosis* organisms a detection limit of 20 organisms/bladder was shown. PCR assays were performed in the presence of 50 fg DNA from *M. smegmatis* strain 1008 (with a modified *IS6110* element; 305-bp PCR product) to detect PCR assay inhibitors. After BCG instillation in the guinea pig bladder the number of BCG organisms appeared to be less than 20 bacteria/bladder (n=5). These data indicate a low number of BCG organisms binding to the bladder under conditions of BCG-induced local immune response and support our previous electronmicroscopical observations and a theoretical model, showing a high potential-energy barrier between BCG and bladder wall (≈ 100 x kinetic energy of 1E-20 J).

In conclusion, although in superficial TCC treatment, large numbers of BCG organisms are installed, the immune response seems to require a very low number of BCG bacteria.

P 3.10

HUMORAL RESPONSE AGAINST HEAT SHOCK PROTEINS AND MYCOBACTERIAL ANTIGENS AFTER INTRAVESICAL TREATMENT WITH BACILLUS CALMETTE-GUERIN IN PATIENTS WITH SUPERFICIAL BLADDER CANCER.

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INTRODUCTION AND OBJECTIVES: Few studies are available on antibody response during intravesical BCG immunotherapy for superficial bladder cancer. We analyzed the evolution in serum antibody response against the purified protein derivative (PPD) of M.*bovis* BCG and the heat shock proteins (hsp) recombinant HSP 65, P64 native purified protein, recombinant HSP 70 from M.*bovis* and recombinant GroEL from E.coli.

METHODS: 42 patients with a superficial TCC, 28 treated with six intravesical BCG instillations and 14 patients used as controls were part of this study. Antibody response was measured with a dot blot assay before BCG, and at six weeks and four months post BCG.

RESULTS: Antibody response data at four months post BCG were available in 17 patients. Against PPD a significant increase was already observed at six weeks follow-up. In contrast, IgG antibodies against hsps increased progressively from six weeks to four months post-BCG. A significant increase in IgG antibodies against PPD, HSP 65, P64, GroEL, HSP 70 at four month follow-up was observed in 10/17, 8/17, 10/17, 4/17 and 8/17 patients. IgG increase against P64 at four months follow-up as compared to baseline was significantly correlated with a tumor recurrence since 7 out of 8 patients with recurrent tumors had a very significant increase in antibody production whereas only 1 out of 9 patients without recurrence had a moderate increase (p<0.01).

CONCLUSIONS: The induction of an antibody response directed against a specific hsp seems to be a significant predictor of tumor recurrence after BCG therapy. Further studies are needed to confirm the place of a specific antimycobacterial antibody response as a clinical prognostic factor.

P 3.11

p53 OVEREXPRESSION, TUMOR CELL PROLIFERATION AND PROGNOSIS IN SUPERFICIAL BLADDER CANCER.

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Both, nuclear p53 overexpression and a high proliferative activity of tumors have been linked to poor prognosis in bladder cancer. To determine the prognostic significance of nuclear p53 overexpression and tumor cell proliferation in bladder cancer, 210 pTa and 99 pT1 tumors with a mean follow up 52 months were examined by immunohistochemistry (p53: DO7; Ki67 labeling index (LI): MIB1). Clinical endpoints were time to progression (to muscle-invasion; pT2 or higher) and time to recurrence. The risk of progression was clearly higher in pT1 carcinomas (26% progressed) than in pTa tumors (progression in 4%; $p<0.0001$). The histologic grade yielded no additional prognostic information over stage. p53 positivity was seen in 10% of pTa tumors but 48% of pT1 tumors ($p<0.0001$). This strong difference in p53 expression between pTa and pT1-tumors is consistent with a role of p53 for development of invasive tumor phenotype. p53 positivity was strongly related to tumor progression in pTa but not in pT1 tumors. A high Ki67 LI was not associated with tumor progression. However, pTa tumors with a high Ki67 LI had a significantly shorter time to recurrence than tumors with low proliferation rate ($p=0.0048$). CONCLUSIONS: The marked difference in p53 expression between pTa and pT1 tumors suggests a role of p53 alterations for progression from stage pTa to pT1. Accordingly, a prognostic significance of p53 alterations in pTa tumors - as found in this study - is expected. Further studies are needed to evaluate whether or not the longer time to recurrence found for low proliferative tumors justifies a longer interval between follow up cystoscopies in these patients.

P 3.12

INTRAVESICAL BCG THERAPY: SALICYLIC ACID REDUCES SIDE EFFECTS WITHOUT AFFECTING ANTI-TUMOR CAPACITY IN VITRO

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Intravesical Bacillus-Calmette-Guerin (BCG) therapy of superficial bladder cancer is an effective anti-tumor immunotherapy and this effect is at least in part based on the induction of cellular cytotoxicity. However, treatment with BCG also results in side effects and irritant symptoms, which might be caused by polymorphonuclear cells (PMN) infiltrating the bladder wall immediately after instillations.

In an attempt to reduce activation of PMNs without affecting BCG induced cellular cytotoxicity we tested a large panel of antiphlogistic and antiinflammatory drugs for their potency to activate PMNs and BCG activated killer cells (BAK cells) in vitro. Activation of PMNs was measured by phagocytosis of BCG and in a degranulation bioassay. Induced cytotoxicity was determined by a ^{3}H-L-methionine-release assay. Both glucocorticoids and non-steroidal-antiinflammatory drugs decreased PMN activation significantly. However, only salicylic acid showed no inhibitory effect on the desired BCG induced cellular cytotoxicity of mononuclear blood cells. When a concentration of 5 µg/ml salicylic acid or more was used activation of PMNs was no longer detectable. Concentrations of up to 100 µg/ml could be used without affecting cytotoxicity of BAK cells.

From our data we conclude that adjuvant salicylic acid could be a potent agent to reduce PMN caused side effects during BCG therapy, especially because killing of tumor cells by activated BAK cells will not be affected.

P 3.13

PROTOPORPHYRIN IX (PpIX) ACCUMULATION AND TURN-OVER IN PROSTATIC TUMOR CELLS DURING/AFTER δ-AMINOLEVULIC ACID (ALA) ADMINISTRATION. Denis H. Schamhart, Berry Meerten, Henricus Sterenborg, Theo M. de Reijke and Karl-Heinz Kurth. Dept. Urology, Amsterdam, The Netherlands.

Photodynamic therapy (PDT) has been developed for many neoplasms. Using ALA induced PpIX, prolonged photosensitivity can be reduced. Therapeutic efficacy depends on preferential PpIX accumulation in tumor cells. Here, we report on *cell line dependent* kinetics of PpIX synthesis from ALA *and* subsequent turnover of PpIX in human prostatic cells.

Kinetics of PpIX synthesis of LNCaP, DU145 and PC-3 (2.5E4 cells/96-well) cells were studied with increasing ALA concentrations (0-0.5 mg/ml). Intracellular PpIX ($PpIX_i$) decay was determined by a pulse (4h)/chase (20h) experiment. Fluorescence intensity (FI; peak at 636 nm by 400 nm excitation) was determined on monolayers to prevent perturbations.

Analyzed according to Michaelis-Menten kinetics, a low Km ($3\pm0E-5$ M) and Vmax (0.6 ± 0.0 FI/2h) of the well differentiated LNCaP cells was observed, compared to the poorly differentiated DU145 ($Km=25\pm4E-5$ M; $Vmax=2.1\pm0.3$ FI/2h) and PC-3 ($Km=18\pm5E-5$; $Vmax=2.1\pm0.2$ FI/2h) cells, suggesting a lower PpIX synthesis and $PpIX_i$ in LNCaP cells. $PpIX_i$ after a 4-h ALA incubation (0.33 mg/ml) was found to be 40, 67.5 and 62.5 ng/2.5E4 cells for LNCaP, DU145 and PC-3 cells, respectively. After 4-h ALA (0.33 mg/ml) preincubation an exponential decrease (conversion to haem, leakage) of $PpIX_i$ was found for LNCaP cells, resulting in an absence of detectable PpIX within 5-7h. No clearance from DU145 and PC-3 cells was observed over a 18-h period.

These data suggest a lower accumulation and rapid turnover of PpIX in well differentiated cells compared to poorly differentiated prostatic tumor cells. Although to be established for normal, highly differentiated cells, this difference may be used to develop an optimal treatment schedule varying the time period between ALA administration and 400-nm treatment.

P 3.14

PHOTODYNAMIC DIAGNOSTICS WITH 5-ALA IN BLADDER CANCER: BIOLOGY OF FALSE POSITVE LESIONS.

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Aim: The haem precursor 5-amino levulinic acid (5-ALA) is used for photodynamic diagnosis (PDD) in bladder cancer, and was shown to have a significantly higher sensitivity than conventional white light endoscopy. The question was raised whether the false positive lesions seen with this method differ biologically from non-fluorescent lesions.

Methods: In 140 biopsies with detectable surface urothelium comparative proliferation assessment was carried out using the antibody clone MiB-1 against Ki67, and measuring positive and negative nuclei with a true colour image analysis system. Fluorescing inflammatory lesions were investigated in parallel to assess fluorescence on sections and to immunohistochemically detect the composition of inflammatory cells (LCA and CD68). Further histology was reassessed carefully.

Results: A significant difference in proliferation was found between fluorescent lesions with benign histology (false positive) and those that were non-fluorescent and histologically negative (normal or inflammatory changes). Endoscopically diffuse and weak fluorescence of inflammatory lesions showed punctate staining in fluorescence of sect-ions and is related to a type or inflammatory lesion with high amount of macrophages. Reviewing the histology of false positive lesions revealed a high precentage of hyperplasias.

Conlcusions: While true false positive lesions in 5-ALA-PDD exist (inflammatory lesions and squamous metaplasia), part of the fluorescent lesions may be precancerous and will undergo mutational analysis. Also there is a chance to eliminate a high number of false positive fluorescence by defining cut-off values in on-line fluorescence measurements excluding a lot of the the dim fluorescence seen e.g. in inflammatory lesions.

P 3.15

PRODUCTION AND CHARACTERIZATION OF A BISPECIFIC ANTIBODY WITH SPECIFICITY FOR THE CD3 COMPLEX AND THE PROSTATE SPECIFIC ANTIGEN

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Numerous *in vitro* studies have shown that bispecific antibodies recognizing the CD3 receptor on T cells and an epitope on tumor cells can effect tumor cell lysis by activating cytotoxic T lymphocytes and by physically linking the effector and tumor cell, thereby providing MHC-unrestricted target specificity. Unfortunately, most tumor cell surface antigens are also found on other cells of various tissues. In contrast, prostate specific antigen (PSA) is expressed in males only on prostate cells (normal and cancer cells). Therefore, bispecific antibodies directed against this antigen and CD3 may be a tool for a highly specific tumor immune therapy affecting only cells of this organ.

We obtained bispecific antibody-secreting hybrid hybridomas by fusion of the anti-PSA Ab producing hybridoma RLSD-06 and a HAT-sensitive, geneticin-resistant transfectant of the mouse hybridoma OKT3 that secretes anti-hu CD3 Ab. Hybrid hybridomas were selected in HAT- and G418-containing medium. Clones were screened by an ELISA in which membrane-bound CD3 from the HUT 78 human T cell lymphoma cell line was used as solid phase and peroxidase-linked PSA for the detection of bound Ab. Several positive clones were found, however, only one (clone K3) was stable now for 10 months. Bispecific antibodies were purified from the culture supernatants of clone K3 by Protein G affinity chromatography and characterized by SDS electrophoresis and isoelectric focussing.

Binding of the antibody K3 both to cells of the CD3-expressing HUT 78 lymphoma cell line and to FITC-conjugated PSA was demonstrated by flow cytometry. Specific tumor cell lysis was tested with the PSA-expressing prostate carcinoma cell line LNCaP as target and human peripheral blood lymphocytes.

This bispecific antibody deserves further evaluation in a nude mouse system as a potential immunotherapeutic tool for prostate cancer.

P 3.16

IMMUNOHISTOCHEMICAL ANALYSIS OF HETEROGENEOUS ^{131}I-cG250 MONOCLONAL ANTIBODY UPTAKE IN RENAL CELL CARCINOMAS.

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Introduction: In a clinical phase I protein dose escalation study with ^{131}I radioiodinated chimeric monoclonal antibody (mAb) G250 (cG250) in patients with primary renal cell carcinoma tumor uptake of mAb ^{131}I-cG250 was highly heterogeneous. Tumor uptake between different tumors as well as within the same tumor varied as much as two orders of magnitude. In the current study we attempted to correlate tumor uptake and four histological parameters: antigen expression, vessel density, tumor viability and percentage of tumor cells.
Methods and Results: To investigate the heterogeneous uptake whole tumorslices (1 cm thick) of 5 different tumors were cut in cubicles of approximately 1 cm^3 according to a grid pattern. ^{131}I-cG250 uptake was determined in a gamma-counter and antigen expression (murine mAb G250), vessel density (mAb PAL-E), tumor viability (mAb RCK-102) and percentage of tumor cells (mAb RCK-102) were determined by immunohistochemistry. Sections were scored as <5 %, 5-25%, 25-50 %, 50-75 % or >75 % positive using a 10 x 10 ocular counter grid and a weighed mean was calculated. The correlation coefficient (r^2) between the uptake of ^{131}I-cG250 and each of these four parameters was determined. In only two of the five tumors analyzed a close correlation between ^{131}I-cG250 uptake and antigen expression was found (r^2 = 0.69 and 0.77) while the other three tumors did not show such a correlation. No correlation between any other parameter and mAb cG250 uptake was found. All tumor samples with high ^{131}I-cG250 uptake (> 0.1 % of the injected dose per gram tumor tissue) showed high antigen expression (>50 %). However, the reverse was not true: not all samples with high antigen expression showed high uptake.
Conclusion: These observations indicate that high antigen expression is a prerequisite for high antibody uptake. However, the mechanism governing antibody uptake cannot be explained by antigen expression alone nor by tumor vascularization, tumor viability or percentage of tumor cells. Future investigations on this phenomenon will focus on neovascularization as parameter and a multivariate analysis will be performed.

Clinical research

P 4.1

Total and free-PSA and free/total PSA ratio as predictive tests in 84 patients with prostatic diseases

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Background: In order to explore the possible role of free PSA as predictive tests for patients with prostatic carcinoma, total and free PSA were retrospectively analyzed in serum from 84 patients with prostatic diseases and from 25 normal men.
Material and methods: Sera was stored at -20°C until analyzed by immunoassay methods from DPC (Immulite).
Results: We confirm the data shown by other groups, that the ratios free-to-total PSA for CaP patients are lower than for BPH patients and control subjects. So significant differen-ce was found between the total, free-PSA or free/total PSA when comparing the patients with differention grade G2 and G3. Neither could we detect any differences between the total or free-PSA or free/total PSA when comparing the patients with tumor stage T2 versus T3, T3 versus T4 or T2 versus T4, except for free-PSA, which shows lower values for T2 than for T4. Free-to-total Psa ratios does not enhance the efficacy of PSA for predicting time to death in prostate cancer.

P 4.2

INCREASED PEANUT AGGLUTININ LECTIN (PNA) ACCEPTOR SITE EXPRESSION IN HIGH-GRADE PIN AND PROSTATE CANCER.

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INTRODUCTION AND OBJECTIVES: In the normal and neoplastic epithelial cells some oligosaccharides may exhibit nonimmune, but selective binding to specific glycoproteins, some of which are extracted from plants and defined as lectins. The aim of the current study is to characterize the binding pattern of the peanut (*Arachis hypogaea*) lectin (PNA) in benign and malignant prostatic tumors. The relationship between the number of PNA acceptors and the PSA stain intensity has also been investigated.

MATERIALS AND METHODS: The PNA histochemical stain was measured by means of computer-assisted microscopy (image cytometry) in 28 benign prostatic hyperplasias (BPH), 15 prostatic intraepithelial neoplasias (PIN) (5 low-grade PIN1, 10 high-grade PIN2-3) and 119 prostatic adenocarcinomas (5 grade 1, 24 grade 2, 60 grade 3, 21 grade 4, 9 grade 5 according to the Gleason classification).

RESULTS: Neoplastic prostate tissues and high-grade PIN2-3 exhibit a significantly higher number of PNA acceptors than BPH and low-grade PIN1. No statistically significant correlation was observed between PNA histochemical stain intensity and prostate cancer grade. Although there were not the same epithelial cells which express PNA acceptors and PSA, a statistically significant correlation was observed between the number of histochemically related PNA acceptors and PSA immunostain intensity.

CONCLUSIONS: The significant relationship between the PNA acceptors and the PSA amounts in the prostatic tissues suggests that the expression of the two markers might be regulated by a common mechanism.
The determination of the amounts of PNA lectin acceptors, i.e., glycoproteins with β-D-galactosyl (1,3)-N-acetyl-D-galactosamine residues, in human prostate tissues enables a boundary to be established between benign and malignant biological potentialities. This may be especially important to determine the potential malignancy of PIN lesions.

P 4.3

Serum expression of different prostate specific antigen forms in different zones of the prostate

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To improve differentiation between benigne hyperplasia (BPH) from prostate cancer patients (P-Ca), the serum expression per gram tissue in BPH patients of total prostate specific antigen (t-PSA) and free PSA (f-PSA) in the transition zone (TZ) compared to the rest of the prostate (peripheral and central zone, PZ) was investigated. Additionally the occurrence of human glandular kallekrein (hK2), a close homologe to PSA, was determined in the different zones. 41 P-Ca (T1a/b-T3) were also included.

Methods: In 96 consecutive patients with histologically proven BPH t-PSA, f-PSA (Delfia Dual Label Assay, Wallac) and hK2 were measured before and 3 months after transurethral resection of the TZ. Difference of these parmeters were related to the resected weight. Prostate volume was evaluated by transrectal ultrasound.

Results: Total volume was 40.0+-18g, TZ 18+-14g, PZ 22+-11g.

	t-PSA(ng/ml/g) mean+-SD	f-PSA(ng/ml/g) mean +-SD	ratio f/t PSA	hK2(ng/ml/g) mean+-SD
Total Prostate	0.11+-0.05	0.02+-0.01	24%	0.0018+-0.0017
Transition Zone	0.19+-0.15	0.03+-0.02	21%	0.0011+-0.0021
Centr/Perip Zone	0.07+-0.06	0.02+-0.01	26%	0.0017+-0.0026

t-PSA, f-PSA: Total P.vs.TZ and TZ vs.PZ: $p<0.0001$

In T1a/b P-Ca, ratio f/t PSA was lower in TZ (7%) vs PZ (19%). In ROC curve analysis the ratio f/t PSA for PSA between 4-10ng/ml improved specificity compared to total PSA alone (A=0.78 vs. A=0.56).

Conclusions: TZ expresses 2.5 fold t-PSA compared to PZ. It has to be tested whether sonographic determination of zones can improve specificity for P-Ca. In BPH the ratio f/t is equal in all zones. The low ratio of TZ in T1a/b P-Ca supports the origin of incidental cancer in the periurethral TZ. hK2 concentration is marginal.

P 4.4

THE CLINICAL VALUE OF FPSA/PSA RATIO IN THE EARLY DETECTION OF PROSTATE CANCER

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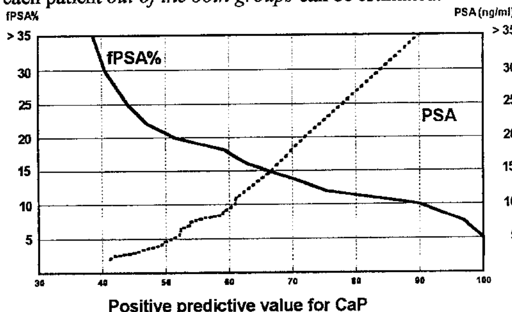
INTRODUCTION AND OBJECTIVES: Between patients with benign hyperplasia (BPH) and those with prostate cancer (CaP) there are significant differences in the ratio of total prostate specific antigen (tPSA) and noncomplexed (free) PSA (fPSA).

METHODS: Serum samples were obtained from 78 patients with untreated CaP and from 126 patients with histologically proven BPH and analyzed for the amount of total PSA and free PSA using the AxSym™ assay of Abbott.

RESULTS: Calculation of the free/total PSA ratio revealed a significant ($p<0.001$) difference in patients with CaP (11.25 %) versus patients with BPH (21.25 %). The positive predictive values were calculated for tPSA and fPSA ratio for different cut-off points. From this we developed a nomogram from which the risk of having cancer for each patient *out of the both groups* can be estimated.



Positive predictive value for CaP

CONCLUSIONS: These data indicate, that plotting the positive predictive values could be a better instrument for the diagnosis of prostate cancer than a static cut-off value for a patient cohort.

P 4.5

MULTIVARIATE ANALYSIS COMBINING PROSTATE SPECIFIC ANTIGEN (PSA) DENSITY OF THE TOTAL PROSTATE (PSAD) AND OF THE TRANSITION ZONE (PSA-TZ) AND FREE/TOTAL (F/T) PSA RATIO FOR PROSTATE CANCER PREDICTION.

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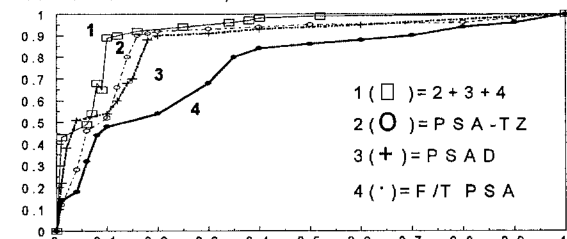
INTRODUCTION AND OBJECTIVES: PSAD, PSA-TZ and F/T PSA ratio have been proposed as methods for enhancement of prostate cancer prediction in men with a serum PSA less than 10 ng/ml. We have investigated the combination of these three markers in patients seen for early diagnosis.

METHODS: Measuring total prostate and transition zone volume by ultrasound using the prolate ellipsoid method and using F/T PSA ratio (Tandem, Hybritech, Inc), PSAD and PSA-TZ were calculated in 154 patients with a PSA <10 ng/ml, 94 with prostate cancer on biopsy and 60 with negative biopsies (sextant biopsies). Multivariate PROC logistic studies were done with the SAS program (Cary, NC, USA) in order to analyze separately or in combination the predictive power of each marker.

RESULTS: P75 calculated cut-offs were > 0.1590 and > 0.33 ng/ml/cc for PSAD and PSA-TZ. F/T PSA cut-off was < 0.248. Area under the ROC curve for the combination of PSA-TZ, PSAD and F/T PSA was 92.5%.



CONCLUSIONS: Combination of PSA-TZ, PSAD and F/T PSA ratio in patients seen for early diagnosis significantly enhances the prediction of prostate cancer as compared to the use of each marker separately.

P 4.6

PROSTATE SPECIFIC ANTIGEN IMMUNOREACTIVITY IN SPINAL FLUID

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OBJECTIVES: PSA is no longer believed to be tissue specific for prostate epithelial cells as it was documented in various human tissues. The physiological role of PSA in this context is currently unknown. Furthermore the distribution of PSA throughout the human body remains to be established. We therefore decided to look for PSA immunoreactivity in human spinal fluid.

METHODS: PSA concentration was measured in the spinal fluid of 34 men and 6 women. The spinal fluid was obtained prior to spinal anesthesia and was kept frozen at -20°C until analysis. We also examined the possibility for any correlation between spinal and serum PSA concentrations. PSA values were determined employing a super sensitive chemilumnescent enzyme immunoassay with a detection limit of 0.003 ng/ml (Immulite III. generation PSA, DPC Biermann).

RESULTS: PSA was detected in male spinal fluid. Median spinal fluid PSA was 0.03 ng/ml in the 34 men, whereas spinal fluid PSA below the detection limit in the 6 women. In men we could establish a positive correlation between spinal and serum PSA with a correlation coefficient of r= 0.85 ($p<0.001$). The highest spinal fluid PSA was 4.4 ng/ml in a patient with metastatic prostate cancer, whose serum PSA was 941 ng/ml.

CONCLUSION: These results confirm recent literature reports that PSA is detectable in various human tissues and fluids. However, the function of extraprostatic PSA has yet to be determined.

P 4.7

A NEW MODALITY OF TREATMENT OF LOCALIZED PROSTATE CANCER : INITIAL EXPERIENCE WITH RADIOFREQUENCY INTERSTITIAL TUMOR ABLATION (RITA) THROUGH A TRANS-PERINEAL ULTRASOUND-GUIDED APPROACH.

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INTRODUCTION AND OBJECTIVES: Radiofrequency energy (RF) creates very localized controlled necrotic lesions and has already been used therefore to ablate, among others, liver metastasis and benign prostatic tissue. This study was intended to determine the feasibility, safety and pathology of the lesions induced by RF energy delivered interstitially in patients with prostate cancer (PCa) scheduled for radical prostatectomy.
METHODS: RITA was performed in 16 patients with localized PCa prior to surgery. RF energy was delivered into the prostate by active needles (monopolar or bipolar) placed through a transperineal approach under transrectal ultrasound guidance. Needles are covered by retractable shields thus allowing to deliver the energy on a chosen and controlled area. In 15 patients, the procedure was performed just before radical prostatectomy. The last patient was treated by RITA under spinal anesthesia 1 week prior to surgery. Two lesions were performed per prostate. NADPH staining and Hematoxylin-Eosin (HE) was used to assess the extent of the necrotic lesions.
RESULTS: Average energy delivered ranged from 3000 to 11000 Joules with maximum central temperatures reaching 106°C for 12 minutes of ablation. No complications were encountered, especially no damage to the urethral sphincter or rectal wall. Macroscopic examination showed marked lesions including the prostate capsule of up to 2.2x1.5x4.5 cm. Microscopic examination showed clear delineated lesions both with NADPH (in prostates immediately removed after surgery) and HE (at 1 week after RITA). In 1 patient, no residual cancer was found on the specimen.
CONCLUSIONS: Transperineally delivered RF is capable of creating safely reproducible controlled necrotic lesions in PCa. The data presented provide basic information for the potential future application of RITA for localized prostate cancer.

P 4.8

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND INTERFERON-α 2B IN PATIENTS WITH RENAL CELL CARCINOMA

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OBJECTIVES: Recombinant human interferon-α 2B (IFN-α 2B) has well known clinical activity against renal cell carcinoma (RCC). Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) as an inducer of tumor necrosis factor (TNF), interleukin-6 (IL-6) and other cytokines has antitumorigenic effects both *in vitro* and *in vivo*. Therefore, a phase I trial of GM-CSF and IFN-α 2B was performed in patients with metastatic RCC.

METHODS: Groups of 3 patients received GM-CSF (µg) at dose levels I (15), II (30), III (60), IV (90), V (120), VI (150) or VII (300) sc. in combination with IFN-α 2B at a fixed dose of 10 x 10^6 IU sc. three times weekly for 12 weeks. 21 patients entered the study.

RESULTS: Toxicity has been mild to moderate in the first four groups with fever/chills and fatigue. One patient in group II developed a grade 3 somnolence, one patient in group V, VI, and VII developed grade 3 toxicity with vomiting and weight-loss and one of the patients in group VII had an anginal attack upon the first administration of treatment. These five patients (24 %) dropped out of treatment schedule. Two complete remissions (CR) have been observed, one in group IV and one in group VI, both with lung metastases. Increases in WBC, neutrophils, lymphocytes, and monocytes were noted but were not related to dose levels of GM-CSF.

CONCLUSIONS: Results demonstrate that simultaneous administration of GM-CSF and IFN-α 2B is tolerated up to doses of 120-150 µg GM-CSF three times weekly. The authors conclude that GM-CSF/IFN-α 2B have antitumorigenic effects in pulmonary metastases in renal cell carcinoma patients, and that this observation warrants further study of GM-CSF/IFN-α 2B in this set of patients.

P 4.9

FLUORESCENCE CONTROLLED TRANSURETHRAL RESECTION OF BLADDER CANCER FOLLOWING INTRAVESICAL APPLICATION OF 5-AMINOLEVULINIC ACID.

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Aim: Small papilllary tumors and flat urothelial lesions can be easily missed during transurethral resection (TUR). Intravesical instillation of 5-amino-levulinic acid (ALA) induces accumulation of fluorescing protoporphyrin IX in neoplastic urothelial lesions. A special diagnosis light (D-light, Storz) was deveolped for TUR of bladder cancer under fluorescence control.
Methods: 1.5g of 5-ALA-hydrocloride (medac) dissolved in 50 ml 1.4% NaHCO3 is instilled intravesically 2-3 h prior to endoscopy. TUR is carried out with conventional resectoscopes. For excitation of fluorescence the D-light provides blue light (375-440 nm). The fluorescence contrast is enhanced by means of a yellow barrier filter (455nm). We report on first clinical experience with 328 ALA induced fluorescence endoscopies (AFE) in a total of 208 patients. After a bladder washing fluorescing lesions were resected using a 24 F flow resectoscope. A total of 713 lesions were taken.
Results: 159 neoplastic lesions were found with AFE. 82 lesions (24 dysplasias grade II, 22 CIS, 4 pTa, 32 x pTa G2 and 3) were additional findings at AFE not seen at normal light. In 14 of 29 cases, with negative endoscopy and positive cytology tumors could be found by detection of fluorescing lesions. 4 CIS and 13 dysplasias were found in patients with negative cytology. A third of biopsies showed false positive lesions. Local or systemic side effects, especillay photosensitization were not observed.
Conclusion: Detection of ALA induced fluorescence using the D-light increases the radicality of transurethral resection of bladder cancer. In case of suspicious cytological findings, fluorescence endoscopy is useful for detecting the precise site of the malignancy.

P 4.10

AROMATIC AMINES IN CIGARETTE SMOKE HAVE TO BE CONSIDERED AS A FACTOR RESPONSIBLE FOR ELEVATED BLADDER CANCER RISK IN SMOKERS

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Elevated risks for bladder cancer in cigarette smokers have been demonstrated in several epidemiologic studies. The substance group of aromatic amines - which have already been identified as a potent bladder carcinogen - is one of the numerous possible carcinogens which are contained in cigarette smoke. The study aim was to determine the percentage of persons with a low detoxifying capacity for aromatic amines ("slow acetylators") in smoking and non-smoking bladder cancer patients.

179 bladder cancer patients were interviewed for smoking habits estimating the amount of cigarettes, cigars and tobacco pipes consumed during lifetime ("pack years"). In all of the patients, N-acetyltransferase 2 - an enzyme involved in the detoxification of aromatic amines by acetylation - was phenotyped by HPLC using molar ratios of caffeine metabolites.

Cigarette smokers were overrepresentated in our study group (n=95, 53%). 72% of the cigarette smoking bladder cancer patients (n=68), but 58% of the bladder cancer patients who had given up smoking for 10 years or longer (26 of 45) and only 54% of the non-smoking bladder cancer patients (21 of 39) were "slow" acetylators.

Although the concentration of bioavailable aromatic amines in cigarette smoke is low, the results of this study underline that the impact of aromatic amines has to be considered as one of the several possible carcinogenic mechanisms responsible for elevated bladder cancer risks in cigarette smokers.

P 4.11

GLUTATHIONE *S*-TRANSFERASE μ IN BLADDER CANCER PATIENTS IN A HIGHLY INDUSTRIALIZED AREA

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The absence of Glutathione *S*-transferase μ (GSTM1), an enzyme involved in detoxifying polycyclic aromatic hydrocarbons (PAH) by conjugation to glutathione, has already been identified as a genetically determined risk factor for lung cancer. This study was designed to assess occupational risk factors in patients with urothelial carcinoma in an area of former coal, iron and steel industry with special regard to former exposure to polycyclic aromatic hydrocarbons (PAH).

89 bladder cancer patients were interviewed for occupations ever done for more than 6 months. All patients were genotyped for GSTM1 from lymphocyte DNA by PCR.

GSTM1 gene was lacking in 70% (62 persons) of all bladder cancer patients. The percentage of GSTM1 negative persons in our study group differed significantly from that of newborn infants in the East Ruhr Area (54%, Kempkes et al., 1996) and an ordinary population in Berlin (50%, Brockmoeller et al., 1994). Age, sex, smoking habits and histopathological tumor staging and grading had no significant impact on the distribution of GSTM1 gene. There was an ordinary distribution of GSTM1 negative bladder cancer patients in a subgroup of 30 occupationally non-exposed businessmen and administrative officers (54%). All 4 genotyped coke oven workers, 16 of 19 underground coal miners (84%), 10 of 13 bladder cancer patients occupationally exposed against fumes (77%) and 5 of 8 bladder cancer patients exposed against tar (62%) were GSTM1 negative.

The results are consistent with the view that lack of GSTM1 is an individual risk factor for bladder cancer in persons occupationally exposed to PAH to a high extent.

P 4.12

IMMUNOCYTOCHEMICAL ANALYSIS OF CELLULAR PROLIFERATIVE FRACTION OF PROSTATE CANCER AND LYMPH NODE METASTASES AFTER NEOADJUVANT THERAPY.

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INTRODUCTION: The detection of cellular proliferative fraction has been measured most commonly in primary tumours and has been shown to be an important prognostic factor in predicting recurrence and progression. Only few studies have evaluated the proliferative activity of metastatic lymph nodes and biological significance of nodal involvement is still debated. Despite the regressive effects of Neoadjuvant Androgen Deprivation Therapy (NADT) on prostatic cancer tissue is well known, the effect of NADT on metastatic tumour tissue has not been investigated yet. The aim of this study was to analyse the histopathological effects and the proliferative activity of regressive neoplastic cells analysing Proliferative Nuclear Antigen (PCNA) on prostatic tissue and lymph node micrometastases.

METHODS: We evaluated retrospectively 87 surgical specimens of pts. who underwent radical prostatectomy for clinical T2-3N0 prostatic carcinoma. 82 pts. underwent NADT by LHRH agonists and Flutamide during the three months before prostatectomy. As control group 5 untreated pts with nodal involvement was evaluated In 16 treated and in 5 untreated pts. lymph nodes metastases at the pelvic lymphadenectomy (pN1-2) were found. Anti-PCNA antibodies (PC 10; Dako) were used according to the streptavidin-horseradish peroxidase complex method. The PCNA expression and location were evaluated on prostatic treated tissue in 19 cases and on lymph node metastases in 21 cases. Statistical analysis was performed by the Mann-Whitney test.

RESULTS: Serum PSA before NADT was 26.26±12.03 ng/ml, while after NADT was 1.25±1.08 ng/ml. The evaluation of PCNA on prostatic adenocarcinomas was performed in 19 treated patients, the percentage of positivity was 1.33%(SD 0.38). In 12 untreated patients (control group) was 12.2% (SD 0.45%), $p<0.05$.

The evaluation of PCNA on nodal metastases was performed in 16 patients treated with NADT. In the 16 patients 65 lymph nodes had metastases out of 379 lymph nodes dissected (a mean of 23 nodes per patient). The mean PCNA value was 4.50%(SD 3.1). The PCNA value obtained in lymph nodes metastases in 3 out of 4 untreated patients was 19.67% (SD 0.95), higher than in treated patients, ($P=0.014$).

The percentage of positivity in PCNA staining on lymph node metastases was 4.50%(SD 3.1) in the treated cases and 19.67% in the untreated ones. The difference between lymph node metastases and prostatic treated tumour is statistically significant $p<0.05$. The morphological evaluation of metastases allowed the observation of moderate regressive aspects due to NADT in 3 out of 16 cases. Nuclei with positive staining for PCNA showed a regular or granular pattern or both, with variable intensity. Nuclei with more intense staining were behind the marginal sinus of lymph nodes. It has been observed that PCNA stained nuclei increased from small and large acinar pattern through cribriform to solid and trabecular pattern; there was also a tendency for stronger staining at the invasive edge of the neoplastic growth process than in the central region.

CONCLUSION: The PCNA value obtained in lymph nodes metastases is preliminary because of the low number of metastases found in our patients and because of the difficulties encountered in applying this method to micrometastases. We believe that NADT can have a moderating biological effect on nodal metastases since we observed only small and partial cellular regressive aspects and a slight reduction of indexes of cellular proliferation, both almost likely due to the presence of a cellular phenotype non-responsive to hormonal therapy.

P 4.13

VALIDATION OF SILVER-STAINED NUCLEOLAR ORGANIZER REGIONS FOR EVALUATION OF INVASIVE CHARACTER OF URINARY BLADDER CARCINOMA

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A series of 8 rats and 16 mice invasive bladder carcinomas were investigated for the presence of silver-stained nucleolar organizer resions (AgNORs) to clarify whether this parameter is applicable to the estimation of their invasive character. With regard to number of AgNORs per cells, neither rat nor mouse carcinomas showed any differences between invasive and noninvasive site. However, frequency of cancer cells bearing bizarre dots, irregular in size and shape, was significantly higher at site of actual invasion. Quantitative data generated using an image analyzer revealed significantly lower values for NOR roundness larger NOR size in invasive site. Double staining for the proliferation marker, proliferating cell nuclear antigen (PCNA) and AgNORs was performed in 8 rats' carcinomas and a close correlation was conformed. The number of AgNORs in PCNA positive cells were greater than in PCNA negative cells. A particularly strong correlation was observed for incidence of PCNA positive cells and bizarre dots. The quantitative data also demonstrated significantly differences in size and shape of dots. It is concluded that AgNORs have diagnostic value for the invasive character of bladder carcinomas.

P 4.14

THE USE OF THE DECISION TREE TECHNIQUE AND IMAGE CYTOMETRY TO CHARACTERIZE AGGRESSIVENESS IN WHO GRADE II SUPERFICIAL TRANSITIONAL CELL CARCINOMAS OF THE BLADDER.

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According to the World Health Organization (WHO) classification of human bladder tumours, three levels of malignancy can be identified, corresponding to grades I (low), II (intermediate) and III (high) malignancy levels. This classification system operates satisfactorily for two of the three grades in forecasting of clinical progression. Indeed, most of the grade I tumors are associated with good prognoses, and most of the grade III ones with bad ones. In contrast, the grade II group of tumours represents a very heterogenous group, in which tumours behave very differently at clinical level. In the present study, we used two computer-assisted methods to investigate whether it is possible to sub-classify grade II tumours. The two computer-assisted methods involve the computer-assisted microscope analysis (image cytometry) of Feulgen-stained nuclei on the one hand and the Decision Tree Technique on the other. This technique belongs to the Supervised Learning Algorithm and enables an objective assessment of the diagnostic value associated with a given parameter to be carried out. The combined use of these two methods shows that it is possible to identify (in a series of 292 superficial transitional cell carcinomas) one sub-group of grade II tumours which behave clinically like grade I tumours, and a second sub-group of grade II tumours which behave clinically like grade III tumours. Of the 9 ploidy-related parameters computed by means of image cytometry, it was the percentage of hyperdiploid and hypertetraploid cells nuclei and not conventional parameters like the DI or the DHT which enabled identification to be carried out.

P 4.15

p53 ACCUMULATION AND RESPONSE TO CHEMOTHERAPY IN PATIENTS WITH UROTHELIAL CANCER.

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The impact of the p53 accumulation on the chemosensitivity of the urothelial cancer in response to cisplatin-based chemotherapy was evaluated in this study.

Archival formalin fixed paraffin embedded sections from 52 patients with urothelial tumors who underwent chemotherapy for locally advanced and/or metastatic transitional cell carcinoma were stained for p53 protein using mouse mAb AB-6, recognizing both wild-type and mutant p53 (Dianova, Hamburg, Germany). A cut-off value of 20% of stained nuclei was used to discriminate between p53-positive and p53-negative tumors. Study end points, clinical and/or pathological responses to M-VAC, MC or CISCA and tumor-free survival were correlated with the results of immunohistochemistry.

Of the 52 tumors studied, 34 (65%) showed p53 accumulation and 18 (35%) were p53-negative. Overall tumor response to inductive chemotherapy (n=31) were 13% complete remissions (CR) and 22% partial remissions (PR). 5 patients (29%) with p53-positive tumors and 6 patients (43%) with p53-negative tumors had CR or PR. Mean duration of tumor-free survival after adjuvant chemotherapy studied in 21 patient was found to be 4.5 months for p53-positive tumors compared with 35 months for p53-negative tumors.

Although we did not observe a significant correlation between nuclear p53 staining and tumor response to inductive chemotherapy, overexpression of p53 was found to be associated with a short tumor-free survival of patients after adjuvant chemotherapy for nodal disease (n=21). Whether response to adjuvant chemotherapy requires functionally intact p53 suppressor gene or relates to a difference in tumor biology is subject of further investigation.

P 4.16

EXPRESSION OF P53 AND DRUG RESISTANCE MARKERS IN WILMS' TUMOR.

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Wilms' tumor (WT). or nephroblastoma, is an embryonal malignancy of the kidney that affects about 1 in 10.000 children. Histologically, the tumor is composed of 3 major elements in variable proportions and various patterns of differentiation; blastemal, epithelial and stromal cells. The appearance of this triphasic pattern is regarded as a favourable histology. However, anaplasia being present in a WT changes this to a markedly worse prognosis. In order to characterize the biological properties of the individual components of WT, we studied various markers by means of immunohistochemistry on paraffin embedded tissues of 20 WT cases. Expression of the P53 gene product was analysed with the MoAb DO-1. We scored 6/20 of the tumors positive for p53. Immunopositivity in general was focal in the blastemal and epithelial parts of the tumors with differences in staining intensity seen in some of the tumors. Positivity in the stromal parts was restricted to single cells. Because of its important role in the apoptotic pathway, we also looked for bcl-2 expression which was expressed in only a minority of the tumors. In addition, we looked for factors with significance for drug responsiveness; glutathione S-transferase Π and DNA topoisomerase IIα. GST Π was expressed almost exclusively in the majority of epithelial regions and DNA topoisomerase IIα could be detected in most of the tumors predominantly in epithelial and stromal areas. Anaplastic regions, however, sometimes stained very intensely.

We conclude that p53 may be an important prognostic factor in WT, being present in higher frequency in the more advanced tumors. A direct relation between tumors presenting with anaplasia and p53 immunoreactivity was found. In these tumors also a differentiation in staining intensity was found in the blastemal and epithelial elements.