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ABSTRACTS

1st World Congress on Urological Research organized by the European Society for Urological Oncology and Endocrinology (ESUOE) and the American Society for Basic Urological Research (SBUR)

September 1–3, 1995, Rotterdam, The Netherlands

Congress chairman: Dr. J. A. Schalken, Nijmegen, The Netherlands

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EDITORIAL

The present issue of Urological Research includes the papers presented at the 1st World Congress on Urological Research, a meeting organized by the European Society for Urological Oncology and Endocrinology (ESUOE) and the American Society for Basic Urological Research (SBUR), held in the World Trade Center, Rotterdam, The Netherlands, September 1–3, 1995.

The great interest in the 1st World Congress on Urological Research is reflected by the submission of over 220 abstracts. The program committee was able to select 180 high quality abstracts, covering areas of interest in contemporary urological research such as the genetic basis of cancer, molecular prognostic factors, mechanisms of endocrine growth control and molecular approaches to biological and gene therapy.

The organizing societies, ESUOE and SBUR, strongly support the interaction between the clinical and basic researcher. The large number of excellent submissions for the 1st World Congress on Urological Research demonstrates the effort and dedication of researchers in urology to effectively attack clinical questions, especially in a time when many challenging and imminent problems are faced by the urologist. Hopefully, this joint meeting will stimulate intercontinental collaboration and future progress in urological research.

J. A. Schalken, Nijmegen

1A Molecular and cellular basis of BPH

P 1

THE FREE/TOTAL PSA RATIO FOR DETECTION OF PROSTATE CANCER IN A COMMUNITY BASED POPULATION

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Goal: The goal of this study was to analyse the application of PSA assays, which can measure total serum PSA and free serum PSA, in a community based population. The free/total ratio has been used in selected series to differentiate between the prostate carcinoma (PCa) and benign prostate hypertrophy (BPH). The relation between age and the free and total serum PSA was also established.

Material: In 1726 men between 55 and 74 years total serum PSA, DRE, and TRUS were performed to detect PCa. 823 men participated in the feasibility studies, and 903 in the European Randomized Study for Screening on Prostate Carcinoma. 67 carcinomas were detected (3.9 %). In addition 188 asymptomatic blood donors (age < 43 years) served as a control group. The comparative data between the DELFIA assays and of the Abbott IMx and Hybritech Tandem E assays are included.

The DELFIA PROSTATUS-TM PSA Free/Total assay provides simultaneous dual label measurement of free and total PSA by using time-resolved fluorimetry of europium (free PSA) and samarium chelates (total PSA). The PROSTATUS-TM PSA EQM provides a highly sensitive single label (europium) assay of total PSA. Both assays measure total PSA with the same reagent combination to give equimolar detection of PSA in free and complexed form.

Results: The coefficient of variation of both DELFIA assays was between 2.6 and 4.4 % over 17 runs with 6 control samples. Age references ranges including 95 % of the serum values (ng/ml):

age	40-44	45-49	50-54	54-59	60-64	65-69	70-74	>75
Total PSA	3.1	3.3	3.8	4.3	4.9	5.5	6.3	6.7
Free PSA	0.72	0.79	0.87	0.96	1.1	1.2	1.3	1.4

ROC curves illustrated the relative potential of F/T ratio to increase the specificity of PSA to detect PCa in various ranges.

Conclusions: Both DELFIA PROSTATUS-TM PSA Free/Total and PROSTATUS-TM PSA EQM assays show low variations between batches and runs, and a very close agreement between these two assays. The F/T ratio differs significantly between PCa and normal men. The F/T ratio may improve specificity of total PSA for detection of PCA in a community based population, but larger numbers are needed to reach significance in the PSA range of 4-10 ng/ml.

P 2

IMPROVEMENT OF PROSTATE CANCER SCREENING BY DETERMINATION OF THE RATIO FREE/TOTAL PSA IN ADDITION TO PSA LEVELS

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The serum PSA level is widely used in screening programs for the early detection of prostate cancer. However the specificity for cancer detection is limited because serum PSA is also elevated in patients with BPH and/or prostatitis. The main portion of PSA in the circulation is complexed to the protease inhibitor α1-antichymotrypsin, a smaller portion is present as free PSA. It has been shown recently, that the ratio of free/total PSA in serum is lower in malignant prostatic disorders than in non-malignant ones. (Christensson et al., 1993) and therefore might be a parameter to improve screening specificity. In a retrospective study we tested whether free PSA helps to distinguish between BPH and prostatic carcinoma in men with elevated serum PSA.

A total of 266 men have been identified with elevated serum levels using a conventional PSA determination kit (Abbott IMX). Subsequently their disease was proven by biopsies. Sera samples of these patients were stored frozen at -80°C for further measurements. Free and total PSA levels in these serum samples were measured using the DELFIA PSA dual label F/T PSA kit (Wallac Oy Turku, Finland). This kit uses two differently labelled antibodies, one specific for free PSA the other one for complexed PSA, and allows simultaneous equimolar measurement of free PSA and α1-antichymotrypsin-complexed PSA. The assay was performed according to the recommendations of the manufacturer. The mean Free/Total PSA of 64 patients with prostate cancer was 0,10 and was significantly (p=0,001) different from the mean of 202 men who were histologically free of cancer which was 0,17. ROC-curve analysis showed that using a free/total PSA <18 % as a criteria for biopsy in men with an elevated PSA (age-specific reference ranges), 37% of the negative biopsies could be eliminated while still detecting 94 % of all carcinomas. These results show that free/total PSA is a useful determination and can improve specificity of prostate cancer screening.

P 3

CELL KINETIC IN EPITHELIUM AND STROMA OF BENIGN PROSTATIC HYPERPLASIA

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The induction of benign prostatic hyperplasia (BPH) from normal prostate is obviously associated with a distinct increase in epithelial and stromal proliferation. We have shown previously that the further increase of BPH volume in aging men is not associated with a further increase in proliferation. For this it was studied whether an imbalance between programmed cell death (apoptosis) and cell proliferation may explain continuing growth in aging men.

In prostates of 17 men with BPH removed by open prostatectomy (43-94 years old; mean age 70 years) with a mean weight of 77 gm (range 30-124 gm) proliferating cells were localized immunohistochemically with the Ki-67 antibody. Apoptotic cells were detected using the terminal transferase assay. Counting approximately 8000 nuclei per prostate proliferation and apoptotic index was calculated using a computer assisted image analysis system.

Mean proliferation index ± standard deviation in epithelium (0.142 ± 0.097) and stroma (0.121 ± 0.082) was nearly identical. Mean apoptotic index in epithelium (0.172 ± 0.156) was slightly higher than corresponding proliferation index. In stroma, however, no apoptotic cells were detectable. Proliferation index and apoptotic index in epithelium and proliferation index in stroma showed no correlation neither to patient age nor to prostate volume.

In epithelium of BPH obviously cell kinetic is balanced. On the other hand our results indicate stromal growth, due to cell proliferation in the absence of cell death. This may explain the continuous increase of BPH volume in aging men.

P 4

IN VITRO STUDIES ON THE EFFECT OF FINASTERIDE ON THE 5α-REDUCTION OF ANDROSTENEDIONE TO ANDROSTANEDIONE IN EPITHELIUM AND STROMA OF HUMAN BENIGN PROSTATIC HYPERPLASIA

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Previously we have shown that finasteride inhibits the conversion of testosterone to dihydrotestosterone in epithelium and stroma of human benign prostatic hyperplasia (BPH), the inhibition being much stronger in epithelium (1). However, besides testosterone androstenedione, the second main circulating androgen in men, is also a potential substrate of prostatic 5α-reductase. The aim of the present study was to describe the effect of finasteride on the 5α-reduction of androstenedione to androstanedione in epithelium and stroma of human BPH.

BPH tissue (n = 5) was separated mechanically in epithelium and stroma. 5α-reductase activity was determined using five different androstenedione concentrations (50 - 5200 nM) alone or in the presence of varying concentrations of finasteride (10 - 125 nM). The androgen metabolites were separated by HPLC. The inhibition constants Ki were derived from Lineweaver-Burk plots. The significance of the differences between the means was calculated by students' t-test. $p < 0.05$ was considered significant.

The main results were: (1) Using a finasteride concentration of 75 nM and an androstenedione concentration of 200 nM, the mean inhibition [% ± SEM] of 5α-reductase activity was significantly ($p = 0.005$) higher in epithelium (69 ± 2) than in stroma (52 ± 4). (2) 5α-reductase activity in epithelium and in stroma was dose-dependently inhibited by finasteride. (3) Both in epithelium and stroma finasteride acts as a competitive inhibitor. The mean inhibition constant Ki [nM ± SEM] for that competitive inhibition was significantly ($p < 0.01$) lower in epithelium (10 ± 2) than in stroma (21 ± 3).

The present study demonstrates that in human BPH finasteride inhibits not only the 5α-reduction of testosterone but also of androstenedione. As indicated by the mean Ki values the inhibition of androstenedione reduction is comparable to the inhibition of testosterone reduction. In further studies it has to be clarified whether the inhibition of the 5α-reduction of androstenedione gives rise to a shift towards the reduction of androstenedione to testosterone by the enzyme 17β-HSOR$_{red}$.

(1) H. Weisser et al. (1994), Steroids 59: 616-620.

P 5

Tenascin Expression in Human Prostate: Is Tenascin Involved in Tissue Homeostasis?

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Recent results indicate that tenascin, a large extracellular matrix (ECM) glycoprotein, is involved in tissue remodelling processes, that are associated with morphogenesis and oncogenesis. Tenascin was also demonstrated to be associated with stromal-epithelial interactions. However, at present little is known about the function of tenascin, also in human prostatic tissue homeostasis.

In order to gain further insight in the role of tenascin in prostatic neoplasms, we performed an immunohistochemical approach, using monoclonal antibodies against tenascin, and laminin. Dual immunofluorescence (D.I.F.) staining was also performed on selected tissues to correlate tenascin expression and basement membrane condition, as was assessed by laminin expression. Pre-existing morphologically normal glands, histological processes in the adenoma, associated with benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma (PCa) lesions were included.

Tenascin was exclusively expressed in the ECM, mainly at the periphery of the acini, tumor foci and blood vessels. Where the basement membrane was intact, no difference was observed in tenascin expression between BPH and pre-existing "normal" tissue. No correlation between tenascin and proliferation was found. Well- and moderately differentiated tumors showed enhanced tenascin expression compared to non-malignant lesions, together with weak or occasionally disrupted pattern of laminin expression, which are presumably indicative for a disturbed basement membrane. Tenascin staining was very faint or even absent in high grade tumors in which a progressive loss of basement membrane and a significant decrease in stromal content were clearly evident.

Our data indicated that tenascin is probably critically important associated with the tissue homeostasis of prostate.

P 6

EXPRESSION OF INTERFERON-γ IN THE HUMAN PROSTATE

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As previously demonstrated the pathogenesis of benign prostatic hyperplasia (BPH) is associated with lymphocytic infiltration by activated memory T-helper cells. Stimulation of these BPH-tissue derived T-lymphocytes *in vitro* revealed that the majority of $CD4^+$ T cells belong to the group of T-helper 1 cells, characterized by massive interferon-γ (IFN-γ) and interleukin-2 (IL-2) production. Furthermore, whereas normal prostatic stromal cell lines (PSC) revealed no proliferative response to IL-2 and only a slight response to IFN-γ, BPH-PSC lines and 7 of 11 BPH-PSC clones were found to show consistently hyperresponsiveness when stimulated with IFN-γ and IL-2.

Therefore normal (n=2), benign hyperplastic (n=5) and malign (n=5) prostatic tissues were screened for IL-2 and IFN-γ protein and m-RNA expression. Screening for anti-IL-2 and anti-IFN-γ reactivity by immunhistolgy revealed no staining for IL-2 and intense reactivity with 2 different monoclonal antibodies (mAb) directed against IFN-γ. The specificity of anti-IFN-γ reactivity was confirmed by using nonsense isotype control mAb as well as dose-dependent inhibition with rIFN-γ. Although all prostatic tissue specimens analyzed revealed anti-IFN-γ reactivity, BPH tissues exhibit the most intense staining and in contrast to normal and malign prostatic epithelial cells (PEC) BPH-PEC showed membrane reactivity. To confirm if the demonstrated reactivity is due to IFN-γ and to analyze if IFN-γ is produced intra-prostatically, total RNA was prepared from all tissues using guanidine thiocynate. Total RNA was then reverse transcribed into c-DNA and PCR amplification was performed using IL-2 and IFN-γ specific primers. The specificity of PCR reaction was confirmed by Southern blotting and hybridization with internal, labeled either IFN-γ or IL-2 specific oligos. Similar to the immunhistologic results both normal and the 5 malign prostatic tissues, although clearly positive, exhibit much less IFN-γ message when compared to the amount expressed in BPH while IL-2 was completely negative.

P 7

EXPRESSION OF STEROID SULFATASE mRNA IN HUMAN NORMAL, BENIGN HYPERPLASTIC AND CANCER PROSTATE TISSUES BY REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION.

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An accumelating body of evidence strongly suggests that estrogens appear to play an important role in the pathogenesis of human benign prostate hyperplasia (BPH) and estrogen deprivation has been shown to reduce prostatic volume and to improve BPH-related symptoms. Since conversion of estrone sulfate to estrone by the action of steroid sulfatase (estrone sulfatase) might be an important source of estrogen production in prostate tissues, the expression of this enzyme in human prostate was investigated. Total RNA was isolated from frozen prostate tissues (9 BPH, 5 cancer and 1 normal). Human steroid sulfatase cDNA was synthesized from RNA by reverse transcription using specific primers followed by polymerase chain reaction (PCR). Analysis of PCR products revealed the presence of a 482 bp fragment specific for steroid sulfatase in all prostate tissues investigated. Our results clearly demonstrate, for the first time, that human normal, BPH and prostate cancer tissues express steroid sulfatase. The implications of this enzyme in estrogen production and in the regulation of its activity might provide an additional target to control local estrogen production in human BPH.

P 8

PROLIFERATIVE EFFECT OF bFGF/FGF2 ON HUMAN PROSTATIC FIBROBLASTIC AND EPITHELIAL CELLS *IN VITRO*

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Stromal enlargement is known to play a key role in the development of begnin prostatic hyperlasia (BPH) in humans. Growth factors such as FGF2 may be involved in this mechanism. We have tested FGF2 stimulatory effects on proliferation of cultured fibroblasts derived from human hyperplastic and cancerous prostatic tissues, and on two immortalized human prostatic epithelial cell lines, PNT1A and PNT2. Cells were cultured in serum-free medium supplemented with a serum substitute (MEDIASER I, I.J.B., Reims, France) devoided of growth factors. Cell proliferation was assayed by the MTT test (Boehringer, Mannheim, Germany) and statistical comparisons performed by one-way ANOVA. Fibroblasts derived from either hyperplastic or cancerous prostate grew as well in the serum-free medium as in medium supplemented with fetal calf serum (no statistical difference) indicating that this serum-free medium was consistent with the study of growth factor effects. Human recombinant bFGF induced a significant proliferative effect on both fibroblast cultures ($p < 0.001$) with a maximal effect after five days and for concentrations ranging from 1 to 10 ng/ml (60 to 600 pM). No difference was found on the proliferative effect of bFGF between the two types of fibroblasts. Similarly, FGF2 stimulated significantly the growth of the two prostatic epithelial cell lines tested ($p< 0.01$ for PNT1A and $p< 0.001$ for PNT2). Maximal effect was also obtained after five days and for a concentration of 5 ng/ml (300 pM). The effect was more pronounced on the most differentiated epithelial cells PNT2 than on the less differentiated PNT1A cells. This *in vitro* study provides evidence that FGF2 may stimulate prostatic fibroblasts and epithelial cells, thus implicating this growth factor in an autocrine/paracrine loop which may be of importance in the development of prostatic hyperplasia.

P 9

Differential expression of alpha1-adrenergic receptor subtypes in BPH determined by reverse transcriptase PCR (Polymerase Chain Reaction)

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Introduction In the treatment of symptomatic BPH, the use of alpha1 adrenergic antagonists has received much attention. In the prostate three alpha1 adrenergic receptor (AR) subtypes are molecularly cloned among which the alpha 1a, 1b, and 1d AR (new nomenclature) and it was shown that the alpha 1a AR is the most predominant subtype (Price DT; J Urol 150:546). In order to gain more insight into the relative expression of alpha1 AR subtypes in the prostate we have developed a semi-quantitative PCR assay which can be used to determine the expression of alpha AR subtypes in immunologically defined TURP and biopsy material.

Material and Methods BPH and normal prostatic tissue obtained by TURP and trans rectal fine needle biopsies was step sectioned. 20 μm sections were used for RNA extraction and adjacent sections were taken for immunohistochemical analysis using tissue type specific antibodies against the IF proteins keratin (epithelial cells), vimentin (fibroblasts) and desmin (smooth muscle cells). First strand cDNA was synthesized and used as a template for PCR analysis using primers derived from the cloned alpha 1a, 1b and 1d cDNA's. Alpha1a and alpha1d expression was determined quantitatively by spiking parallel reactions with increasing amounts of a truncated fragment of the target DNA. Using available software systems the amount of the AR's was quantified.

Results Using the cloned fragment as an external standard, a semi quantitative insight into the relative expression levels was obtained. In the 25 BPH samples, analyzed, the alpha1a/1d ratio varied from 5-50. Comparative analysis with immunohistochemical determination of tissue type revealed a correlation of alpha1a AR expression and the amount of stromal cells ($r=0.9$, $p<0.001$). The quantitative PCR analysis of the alpha1a and 1d expression showed that there is a two to fivefold difference between the BPH samples analyzed. When multiple biopsies of one patient were analyzed, differential expression of the AR's was found indicating a heterogeneous pattern.

Conclusions The PCR analysis confirms earlier findings that in human prostate the relative expression of alpha1a AR is highest. There is a difference in the relative expression of the alpha1a and alpha1d AR in the BPH specimens that were analyzed. The alpha1a AR expression is correlated with the stromal content of the tissue. The semi-quantitative PCR analysis can be used in a comparative study of alpha1 AR expression and treatment efficacy using well defined biopsy material.

P 10

BENIGN PROSTATIC HYPERPLASIA: A CORRELATION BETWEEN ALPHA-1 ADRENOCEPTORS DENSITY, THE AUA SYMPTOM INDEX, AND OTHER PARAMETERS.

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A group of 25 men with BPH where a-blockade therapy failed to relieve symptoms, underwent open prostatectomy. Patients were investigated whether a relationship could be established between the binding affinity (Kd) or receptor density (Bmax) of prostate a1-adrenoceptors and: the American Urological Association (AUA) symptom index, age, prostate weight, PSA, flowmetry. **Patients and methods**: in all patients, fragments were dissected during surgery from inside the hyperplasia and the peri-urethral region corresponding to the bladder neck. In all patients a-blockers were discontinued from at least 3 months. Tissues were frozen and tested with [^{3}H]-prazosin. The apparent dissociation constant (Kd) and the maximal number of binding sites (Bmax) were estimated. A non parametric rank correlation coefficient was computed. **Results**: total AUA scores appeared to be correlated to Bmax values measured inside the adenoma (BMAX) and in the cervical portion (BMAXCERV). BMAX and BMAXCERV showed furthermore good correlation and both were correlated with the same single AUA score parameters: total AUA score (AUATOT), AUA symptom number 2 (AUA2), number 3 (AUA3), and number 6 (AUA6). Some degree of intercorrelation has been showed by AUATOT with AUA2, AUA3, AUA6 and by AUA2 withAUA6. No significant relationship between Kd and Bmax, and AUA scores, urinary flow, PSA, prostate weight, and age was found. **Conclusions**: the development of bladder outlet obstruction in the group of men examinated, evaluated by the AUA score, appear to be associated with a reduced number of a1-adrenergic binding sites in the prostate adenoma: for higher values of the AUA score we observed reduced values of a1-adrenoceptors density. There was no observed correlation between a1-adrenergic receptor density and age of patients or prostate weight. The reduced number of adrenergic receptor sites in patients with symptomatic BPH may explain the limited clinical efficacy of a-adrenergic blockers observed in some patients.

P 11

α1-ADRENERGIC RECEPTOR SUBTYPE-SELECTIVITY OF TAMSULOSIN

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We have compared the α1- adrenergic receptor subtype selectivity of tamsulosin with that of alfuzosin, doxazosin, naftopidil and terazosin in rat tissues and human prostate.

Alfuzosin, doxazosin, naftopidil and terazosin did not discrimate α1- adrenergic receptor subtypes in rat spleen, cerebral cortex and kidney. These drugs also had similar affinities for cloned α1-adrenergic receptor subtypes. In contrast tamsulosin recognized transiently or stable expressed α1- adrenergic receptor subtypes with a rank order of potency $\alpha_{1c} \geq \alpha_{1a/d} > \alpha_{1b}$. In saturation binding studies [^{3}H] tamsulosin displayed a qualitatively similar rank order of potency but the quantitative differences were less pronounced. In human prostate a rank order of potency of tamsulosin > terazosin ≥ alfuzosin > naftopidil was observed. We conclude that tamsulosin displays some selectivity for α_{1c}-adrenergic receptors which appear to dominate in the human prostate.

P 12

EXPRESSION OF ALPHA-1 ADRENERGIC RECEPTORS IN HUMAN PROSTATE TISSUE AND IN CULTURED PROSTATE CELLS

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Smooth muscle cell (SMC) tonus plays an important role in the pathogenesis of benign prostatic hyperplasia (BPH), the most common non-malignant tumor of males. One of the major modulators of prostatic smooth muscle cell tension are the alpha-1 adrenergic receptors. In prostatic tissue mainly α1C (new nomenclature α1A) and α1A (new nomenclature α1D) adrenergic receptor subtypes have been identified. In order to characterize the regulation of these receptors in the prostate we cultured human prostatic SMCs, myofibroblasts and epithelial cells and measured expression of α1C and α1A m-RNAs in different prostate cell types and in tissue specimens.

Prostatic SMCs were cultured in MCDB-131 medium supplemented with horse serum, non-essential amino acids, insulin, transferrin, selenide, estradiol and dexamethasone and conditioned medium of a prostatic carcinoma cell line (CRL 5813). After 3-4 passages in cell culture about two thirds of these cells stained positive for SMC-specific myosin or for desmin. Prostatic myofibroblasts were obtained from tissue specimens in DMEM supplemented with 10 % FCS, and primary epithelial cells in keratinocyte serum free medium (KSFM) supplemented with bovine pituitary extract, EGF, transferrin and selenide.

Using RT-PCR we measured α1C and α1A mRNAs in relation to the house keeping ß-actin mRNA which was used as an internal control. In tissue specimens both m-RNAs were detected. When tumor and BPH specimens from the same patient were compared, tumor tissue consistently contained less α1C and α1A mRNA than BPH tissue. In cultured primary epithelial cells a very small amount of α1A m-RNA was present which may be due to some contaminating fibroblasts. In myofibroblast as well as in SMC cultures α1A m-RNA but no α1C mRNA was detected. Consitant with the tissue specimen results stroma cells cultured from BPH lesions expressed more α1A m-RNA than stroma cells cultured from tumor lesions. Our results show that the transfer of stromal prostatic cells into cell culture induces a rapid down regulation of α1C transcription but does not affect α1A transcription. It will be interesting to elucidate the mechanism of this differential regulation.

P 13

EVIDENCE FOR THE PRESENCE OF REGIONAL DIFFERENCES IN THE PROPERTIES OF ENDOTHELIN RECEPTORS IN RABBIT LOWER URINARY TRACT. Jamshid Latifpour, Yuji Fukumoto, Motoaki Saito, Kazuhiko Nishi, and Robert M. Weiss. Section of Urology, Yale University School of Medicine, New Haven CT, USA.

As regional distributions of adrenergic and cholinergic receptors in the lower urinary tract are well correlated with physiological functions, we examined the binding properties of endothelin (ET) receptors in rabbit bladder dome, bladder base and urethra using [^{125}I]ET-1. The data demonstrated the presence of a single class of specific, saturable, high affinity [^{125}I]ET-1 binding sites in all of the regions of the lower urinary tract studied. The densities of [^{125}I]ET-1 binding sites in bladder dome, bladder base, and urethra were, 190 ± 5, 109 ± 9, and 128 ± 11 fmol/mg of protein, respectively. Unlabelled ET-1 and ET-2 inhibited [^{125}I]ET-1 binding to the membrane particulates from the various regions of the lower urinary tract with single high affinity constants. A selective ET_A receptor antagonist, BQ 123, and selective ET_B agonists, ET-3 and sarafotoxin S6c (STXc), inhibited [^{125}I]ET-1 binding to bladder dome, bladder base and urethra with high and low affinity constants indicating the presence of both ET_A and ET_B receptor subtypes in these tissues. The proportions of high affinity binding sites for ET-3, representing ET_B receptors, were approximately 25%, 27% and 46% in bladder dome, bladder base and urethra, respectively. Corresponding values for STXc were approximately 17%, 28% and 43% in bladder dome, bladder base and urethra, respectively. In contrast to the findings for ET-3 and STXc, the proportions of high affinity binding sites for BQ 123, representing ET_A receptors, in bladder dome, bladder base and urethra were approximately 84%, 74% and 60%, respectively. These regional differences in the density and subtype specificity of ET receptors in the rabbit lower urinary tract may affect the regulatory functions which are specific to each region of the urinary tract. (supported in part by NIH grants DK 38311 and DK 42530, and an Edwin Beer Grant from New York Acad. of Med.).

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ENDOTHELIN (ET) RECEPTOR (Rc) IN NORMAL PROSTATE AND BENIGN PROSTATIC HYPERTROPHY (BPH)

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Our aim is to 1) characterize endothelin receptors (Rc) in normal and BPH human prostate tissue, 2) identify their histomorphological distribution and 3) show the possible proliferative effects of ET1. We demonstrate the existence of specific and high affinity ET1 binding sites in stromal and epithelial prostate tissue by autoradiography combined with histomorphology and by binding assays with ^{125}I ET1. By saturation analysis with 125I ET1: for 5 normal prostate Kd= 0.11 nM ± 0.02, Bmax= 1086 fmol/mg protein ± 399; for 5 BPH Kd= 0.17 nM ± 0.04, Bmax= 964 fmol/mg protein ± 445. The displacement of ^{125}I ET1 was shown by ET1, ET2, ET3 and by BQ123 a specific competitor of ET1 for ETA Rc and by IRL1620 and Sarafotoxin (S6c) specific competitors of ET1 for ETB Rc. For normal prostates Kd= 0.15 nM ± 0.03, Ki ET2= 0.36 nM ± 0.15, Ki ET3= 53.4 nM ± 21, Ki BQ123= 2.25 nM ± 0.91, Ki IRL1630 and S6c unmeasurable. For BPH Kd= 0.13 nM ± 0.04, Ki ET2= 1.39 nM ± 0.50, Ki ET3= 104 nM ± 37, Ki BQ123= 2.98 nM ± 1.20, Ki IRL1620 and S6c unmeasurable. ^{125}I ET1 binding with an excess of BQ123 were 13% of the total ET1 binding for normal prostate and 14% for BPH. ^{125}I ET1 binding with an excess of IRL1620 or S6c were 89% of the total ET1 binding for normal prostate and for BPH. In conclusion, the two types of ET1 Rc, ETA and ETB, are present in normal tissue and in BPH tissue, however ETA is predominant. A proliferative effect of ET1 was observed by increase of tritiated thymidine uptake in a prostatic fibroblastic cell line, PF1SV1. Further studies are warranted to determine the role of endothelin in the etiology and physiopathology of BPH.

P 15

Expression of Endothelin1 and the ET_A and ET_B Endothelin Receptors in Cultured Prostatic Cells.

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Endothelin1 (ET1), in performing its role as a potent constrictor of vascular smooth muscle, is postulated to be involved in the pathogenesis of benign prostatic hyperplasia (BPH). Indeed, previous studies have demonstrated ET1 immunoreactivity in glandular epithelium in addition to concentration-dependent responses of prostatic tissues to ET1 during *in vitro* isometric tension studies (1). Samples of total RNA prepared from the androgen-insensitive prostate cancer cell lines DU145 and PC3 were analysed for ET1 expression by RT/PCR and were seen to elicit a signal on 2% agarose gels (1097bp fragment). In contrast, the 1097bp ET1 fragment is almost undetectable in the androgen-responsive prostate cancer cell line LNCaP. Primary epithelial and fibroblast cells derived from BPH tissues exhibit sufficient ET1 gene expression to produce a signal in RT/PCR. Measurement of the ET1 content of conditioned media (72h) by radioimmunoassay (RIA) demonstrated basal secretion from both LNCaP (0.54pM) and primary fibroblasts (0.58pM and 0.71pM). The DU145, PC3 and primary epithelia conditioned media had ET1 contents equivalent to 6.25pM, 2.1pM and 4.58pM respectively. Using primer pairs specific to ET_A and ET_B receptor cDNA, RT/PCR has shown strong signals corresponding to both receptor subtypes in primary epithelial and fibroblast cells. LNCaP, DU145 and PC3 display markedly less receptor expression than the primary cultures and indeed the ET_B subtype is undetectable in all three cell lines.

1. Langenstroer P, Tang R, Shapiro E, Divish B, Opgenorth T, Lepor H (1993). Endothelin-1 in the Human Prostate : tissue levels, source of production and isometric tension studies. The Journal of Urology 149, 495-499.

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1B Approaches to biological and gene therapy

P 16

EFFECT OF GLYCOSAMINOGLYCANS ON ATTACHMENT AND PROLIFERATION OF PROSTATIC CELL LINES *IN VITRO*

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Introduction. Signals mediated by intercellular or cell-matrix adhesion are critical for the progression of solid tumors and metastasis. Major compounds affecting cell proliferation, attachment and motility appear to be proteoglycans and their constituent glycosaminoglycans (GAGs). This study focusses on the effects of glycosaminoglycans, such as the semi-synthetic pentosanpolysulfate (PPS), on the proliferation and attachment of prostate carcinoma (PCa) cell lines, grown *in vitro*.

Materials & methods. The human PCa cell lines, DU145 and PC-3 were cultured in 96-wells containing RPMI medium supplemented with 10% fetal calf serum. Cell proliferation (total cells) was determined with the XTT assay (OD490). Attached cells were defined as the cells quantitated after washing twice with PBS prior to the XTT assay.

Results. With both cell lines a steady increase of the ratio "attached/total cells" ($R_{A/T}$) was observed over a culturing period of 6 days. The $R_{A/T}$ was 0.1 ± 0.0, 0.4 ± 0.1, 0.5 ± 0.1 and 0.8 ± 0.1 for DU145 cells and 0.1 ± 0.0, 0.3 ± 0.0, 0.5 ± 0.1 and 0.7 ± 0.1 for PC-3 cells at day 1, 2, 3 and 6 after inoculation, respectively. Addition of PPS *at time of inoculation* resulted in a PPS concentration-dependent inhibition of proliferation and attachment of DU-145 cells. At 1, 5, 10, 50, 100 and 300 μg/ml of PPS the rate of proliferation (OD490/d) decreased from 0.35 ± 0.03, 0.37 ± 0.00, 0.27 ± 0.03, 0.16 ± 0.00, 0.12 ± 0.01, 0.12 ± 0.01, respectively, compared to untreated DU-145 cells (0.33 ± 0.02). After 6 days of culturing the $R_{A/T}$ was 0.8 ± 0.1, 0.6 ± 0.2, 0.5 ± 0.0, 0.7 ± 0.1, 0.4 ± 0.0, 0.1 ± 0.0, 0.1 ± 0.0 at 0, 1, 5, 10, 50, 100 and 300 μg/ml of PPS, respectively. In contrast, no significant PPS-associated effects were noted when PPS was added after 1 day of pre-culturing of DU-145 cells in the absence of PPS. PC3 cells appeared to be insensitive to PPS, at all concentrations tested (1-300 μg/ml), indepent of time of addition.

Conclusion. The present data suggest a profound, but cell line dependent, effect of the GAG, PPS (10-300 μg/ml) on cell attachment resulting in a decline of cell proliferation. These findings warrant further investigations on glycosaminoglycans as a class of chemotherapeutic agents possibly interfering with cell-extracellular matrix interactions.

P 17

TARGETING GENE THERAPY TO PROSTATE CANCER

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No prostate cancer treatment has proven to be efficient as soon as the tumour become invasive. It is therefore of some importance to design a a new therapeutical approach using cellular and molecular biology tools. A major breakthrough may come from the development of a tissue specific transfer strategy of «suicide» genes as a gene therapy. This approach may allow the treatment of primary cancer and distant metastasis. Indeed, the specific targeting of prostate cells should render metastases accessible to therapy for the first time in prostate cancer.

Three stages are involved in development of human gene therapy, (i) the transduction by viral vectors or transfection by DNA-mediated systems of appropriate target cells, (ii) enhancement of prostate-specific gene expression, and (iii) the feasibility assessment of different effector genes to direct «suicide» or proliferation control of the targeted prostate cancer cells. To test these alternatives, we have standardised *in vitro* and *in vivo* models: human prostatic epithelial and fibroblatic cells lines (Int. J; Oncol, 6: 333-343, 1995), human-rodent xenografts and non human primates.

By using recombinant adenovirus expressing the gene reporter β-galactosidase (rAd-RSV- β-gal) and the suicide gene thymidine kinase (rAd-HSV-tk) we have tested in vitro the efficiency of transfection as well as the toxicity of the tk/ganciclovir treatment. In primary cultures of human prostate cancer epithelial and fibroblastic cells, we observed a transduction of the reporter gene in 100% of the epithelial cells with 100 virus particles per cell, while the fibroblastic cells dispayed scarse staining. This epithelial selectivity to rAd was further confirmed on the immortalised human prostatic cells lines PNT2 and PNT1A, known to be extremly insensitive to transfection. Confirmation of this selectivity has been carried out in human prostate cancer xenograft on nude mice while tk toxicity was assayed in vitro and in vivo.

As a result, we expect to improve the efficiency of the cellular and molecular targeting to prostate cancer. By providing a new approach to treat prostate cancer, it should assist in a significant improvement for both the patient and the social budget

P 18

POLYAMINE METABOLISM AND PROGRAMMED CELL DEATH IN PROSTATIC CANCER CELLS

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The standard therapy used for the treatment of metastatic prostatic cancer, androgen ablation, fails to induce programmed cell death in androgen-independent prostatic cancer cells. However, *in vitro*, androgen-independent cells can be triggered (by calcium ionophores) to undergo apoptosis, i.e. the pathway needed for this programmed cell death is retained. Recent evidence indicates that polyamines are associated with programmed cell death. Based on these considerations we studied, using an *in vitro* system, whether polyamine biosynthesis (ODC activity) and intracellular levels of polyamines are changed during calcium-ionophore induced apoptosis of androgen independent prostatic cancer cells. In addition, we investigated whether compounds known to interfere with polyamine metabolism (DFMO, BENSpm, MDL72527) effected the process of programmed cell death. Studies were performed with the highly metastatic, androgen-independent AT-3 tumour subline. To detect apoptotic cells the ISNT (*in situ* nick translation) technique was used to visualize fragmented DNA in cultured AT-3 cells . In AT-3 cells treated with 10 μM ionomycin a pronounced increase of cellular DNA fragmentation and fragmented cells (apoptotic bodies) were observed. ODC activity was augmented under these conditions. Putrescine levels increased whilst spermidine and spermine levels decreased. Subsequently, we investigated whether apoptosis can be initiated or prevented by manipulating polyamine metabolism. Cells were treated with DFMO, BENSpm or MDL72527 in combination with 10 μm ionomycin to induce apoptosis. These studies showed that if putrescine biosynthesis is blocked (by DFMO) and/or polyamines are depleted (by BENSpm) cells cease to proliferate but fail to undergo apoptosis after treatment with ionomycin. These results suggest a role for ODC activity and/or polyamines in the induction and/or progression of apoptosis in prostatic cancer cells. Inhibition of polyamine oxidase by MDL72527 did not interfere with apoptosis which suggest that oxidation products produced from polyamine oxidation are not involved in apoptosis of prostatic cancer cells.

P 19

GENERATION OF ANTI-IDIOTYPE MONOCLONAL ANTIBODIES RELATED TO PSA.

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As a consequence of the idiotype network theory it has been suggested that internal image anti-idiotype antibodies must be able to substitute for the nominal antigen. Therefore, the development of monoclonal anti-idiotype antibodies (Ab2) bearing the internal image of a tumor-associated antigen (TAA) is of great interest.

Prostate specific antigen (PSA) has proven to be useful as an indicator for disease progression and response to treatment in prostate cancer (PCa). Since tumors derived from other organs do not show PSA expression, PSA has been successfully used as a maker in PCa patients. However because of PSA production from normal prostate cells, a number of false positive has been obserbed in patients with BPH. Moreover, recent investigations revealed that several molecular forms, i.e., free-PSA, complexed-PSA, exist in the serum and the seminal fluid. However biological activities of these have not been clarified, i.e., the regulation of PSA activity. These characteristics make this antigen a good candidate for anti-idiotype approach.

For generation of Ab2, anti-PSA mouse MAb IgG1-kappa(Ab1) was cross-linked to Keyhole Limpet Hemocyanin and used as immunogen. BALB/c mice were immunized with Ab1-KLH , which specifically reacts with PSA and their splenocytes were fused with SP2/0 mouse myeloma cells. In 3 fusions, 6 anti-idiotype antibodies specifically reacting with anti-PSA MAb were isolated from more than 3000 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAb binding pocket and not for the C_H2 (outside pocket) of IgG1. Furthermore, these Ab2 were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAb IgG. All Ab2 showed clear binding to anti-PSA MAb in non-reduced gels, wheares no reactivity was observed in reduced gels, indicating that Ab2 recognize a conformational epitope compromized of heavy and light chain, and not a structual epitope outside anti-PSA MAb binding site. We have also initiated the functional characterization of these Ab2, i.e., whether they are able to induce antibodies capable to compete with the parental anti-PSA MAb. For the generation of anti-anti-idiotype antibodies (Ab3) resembling Ab1 (Ab1'), NZW rabbits were immunized with purified Ab2. Sera from rabbits immunized with Ab2 showed reactivity with PSA but not with antigen-negative control cell lysates, indicating Ab1' induction.

In summary, we have isolated 2 anti-idiotype MAbs that appear to bear the internal image of PSA. Such Ab2 can be tested for PSA enzymatic activities and therapeutic potential. We are currently performing cross-blocking RIA to determine whether these Ab2 recognize different epitopes in anti-PSA MAb binding pocket.

P 20

PROSTATE TUMOR GROWTH ENHANCEMENT AND MODULATION OF NATURAL KILLER CELL ACTIVITY BY TRANSFORMING GROWTH FACTOR BETA 1 (TGF ß1).

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Engineered TGF-ß1 overexpression in prostate cancer cells (Dunning subline R3327 MATLyLu) causes significantly larger tumors than parental MATLyLu (MLL) tumors. Importantly, TGF-ß1 overproducing (TGF-OP) and control tumors both have the same doubling time. Flow cytometry revealed that both tumor types also had the same percentage of cells in S-phase. Therefore TGF-ß1 overexpression affects tumor size without affecting the rate of proliferation or growth. However, in T-cell deficient animals like nude mice or nude rats no size differences between TGF-OP and MLL tumors after inoculation of $5x10^5$ tumorcells was seen, from which we assumed that an inhibition of T-lymphocytes in TGF-OP tumors is responsible for larger tumors. The same effect of eliminating size differences between TGF-OP and MLL tumors was observed in Copenhagen rats which Natural killer (NK)-cell activity had been either stimulated by daily intraperitoneal (i.p.) injection of 1.2 mg Poly I:C, a potent stimulator of interferon production, or inhibted by injection of 200µl $ASGM_1$-antibody (Wako, Richmond VA) i.p. 3 days before cell inoculation and at additional intervals (days 2,6,10,14,17) afterwards, implying a role of NK-cells in prostate tumor growth modulation by TGF-ß1 (Contractor / Breul / Barrack, J.Urol. 151:366A, 1994). These data represented a paradox because both T- and NK-cells seemed responsible for tumor growth enhancement in TGF-OP tumors. Assuming an influence of inoculated cell number on prostate tumor growth in nude mice, tumorcell inocula with increasing numbers of TGF-OP or MLL tumor cells were examined for their ability to form tumors. Interestingly, after inoculation of $2x10^6$, $1x10^6$ or $5x10^5$ tumorcells no difference in tumor volume was observed between MLL and TGF-OP tumors ($2x10^6$: 6.99±0.93 ver. 7.32±0.46; $1x10^6$: 5.91±1.07 ver. 7.21±0.88; $5x10^5$: 4.84±0.81 ver. 7.28±0.85) (mean±SE). In contrast a significant difference between TGF-OP and MLL tumor volumes in nude mice was observed after inoculation of $5x10^4$ tumor cells (5x104 : 1.89±0.30 ver. 3.42±0.35 ($p<0.005$)). The growth promoting effect of TGF-ß1 is also apparent in nude mice but only in low number cell inocula. Therefore effects of TGF-ß1 on T-lymphocytes are not responsible for the observed growth promotion in TGF-OP tumors. Thus, it appears that in ratsTGF ß1 overexpression may enhance tumor growth at least in part by inhibiting NK-cell function and thus enabling more tumor cells to survive immune surveillance and produce larger tumors. (Supported by CA 16924 and DFG Co 183/1).

P 21

EVALUATION OF CELLULAR TUMOR REJECTION MECHANISMS IN PERI-TUMORAL BLADDER WALL AFTER BCG TREATMENT.

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The evaluation of tumor rejection mechanisms in bladder and their modulation are a central point in the understanding of the antitumoral effect of BCG on urothelial tumors. In a preliminary study we have shown that normal bladder was an immunocompetent site (lymphocyte subsets, signaling molecules and antigen presentation). The objective of this study was to compare immunologic events in peritumoral bladder before and after BCG to the basal situation of normal bladder. Bladder biopsies were taken from 10 patients before BCG, after completion of treatment (3 weeks) and three months later and compared to 11 biopsies of normal bladder. Immunohistochemistry was performed on frozen specimen or specimen embedded in a low melting paraffin after acetone fixation (Modamex) using a bridge technique and alkaline phosphatase conjugated antibodies (APAAP) for the following monoclonal antibodies: T cell markers (CD3, CD4, CD8), adhesion molecules (ICAM-1, B7) and antigen presenting cells (APC) (B lymphocytes, macrophages, langerhans and dendritic cells), MHC class I and class II expression.
In peritumoral wall the level of T cell expression was clearly depressed compared to normal bladder with a CD4 phenotype predominance. At 3 weeks after treatment T cells were recruited with a tendency to reach the level of normal bladder. CD3 cells were much more expressed suggesting the recrutment of other immunocompetent cells (NK, γδ cells). At 3 months T lymphocytes decreased to reach the level of pre-BCG expression. Costimulation or adhesion molecules were decreased (ICAM-1) or absent (B7) in pre-BCG specimen. After BCG stimulation (3 weeks) B7 and ICAM were restored. At 3 months B7 disapeared and ICAM decreased again.
Similarly in peritumoral bladder before immunostimulation there was a deficiency of APC compared to the normal bladder (B lymphocytes, macrophages, MHC class I and II). Intravesical BCG temporarly re-established the normal immunocompetent situation.
These datas suggest that the mechanisms of tumor rejection in peritumoral bladder are impaired. BCG treatment can stimulate T cell recruitment, activate cell adhesion molecules and antigen presentation. This immunostimulation is brief and has disappeared at 3 months. These results support the concept of an immune suppression associated with bladder cancer and the use of maintenance therapy.

P 22

IL8 AND ONSET OF BCG-INDUCED IMMUNE RESPONSE

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Introduction. In superficial bladder cancer patients intravesically treated with BCG influx of neutrophilic granulocytes into the urine is observed, already after the first instillation. This suggests a role for IL8 in the onset of the BCG-induced immune response, since IL8 is a potent chemoattractant for neutrophils. In this study we investigated whether IL8, produced by urothelial cells in response to BCG, is the first to appear in the urine after BCG instillation. In addition the stability of IL8 in urine and association between early urinary IL8 and subsequent immune response (urinary IL2 and IL6) was studied.
Methods. Induction of IL8 by urothelial cells was investigated *in vitro* by adding BCG (Connaught; 5E6 CFU) to the T24 bladder carcinoma cell line (5E5 cells) and measuring IL8 in the medium after 6 hours. Urine samples, obtained at 2 h intervals after the first 3 weekly BCG instillations of IL2 and IL6 positive $(IL2/IL6)^{\oplus}$ patients (n=13) and non-$(IL2/IL6)^{\oplus}$ patients $(IL2/IL6)^{\ominus}$ (n=6), conditions previously shown to be significantly correlated with clinical response, were tested for IL8 by ELISA (CLB, Amsterdam).
Results. In the presence of BCG T24 cells produced 2.1 ± 0.6 ng IL8/5E5 cells, constitutive production was 1.3 ± 0.0 ng/5E5 cells.
IL8 titers prior to instillations were 0.06 ± 0.14 ng/μmol creat (n=53). Maximum IL8 titers occurred between 2-6 h (87%, n=39) after inst. Max. BCG-induced IL8 titers in $(IL2/IL6)^{\oplus}$ pts were 0.5 ± 0.6 (median 0.3), 1.2 ± 1.6 (median 0.6) and 1.2 ± 1.6 (median 0.5) ng/μmol creat after inst. 1, 2, and 3, respectively. This increase in IL8 was observed at an earlier week than IL6 and IL2. Using the maximum IL8 values measured within 6 h after the first inst., significantly higher IL8 concentrations were found in $(IL2/IL6)^{\oplus}$ pts (0.5 ± 0.6; median 0.3 ng/μmol creat) compared to $(IL2/IL6)^{\ominus}$ pts (0.1 ± 0.1; median 0.1 ng/μmol creat) (P=0.05; Wilcoxon). Furthermore, in contrast to other cytokines, IL8 was stable in urine for at least 24 h at 4, 20 and 37 °C.
Conclusions: IL8 may play a role in the onset of the BCG-induced immune response. The presence of *detectable* IL8 very *early* during the BCG therapy course, and its stability in urine at 37 °C, justify evaluation of IL8 as a prognostic factor of responsiveness to BCG therapy.

P 23

EVOLUTION OF THE CELLULAR IMMUNE RESPONSE, INTERLEUKIN-2, AND INTERFERON GAMMA PRODUCTION AGAINST TUBERCULIN AND THE MAJOR SECRETED BCG ANTIGEN (AG 85 COMPLEX) DURING BCG TREATMENT OF SUPERFICIAL BLADDER TUMORS.

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The antitumor effect of the Bacillus Calmette-Guerin (BCG) in superficial bladder cancer seems to be largely related to cellular immunological mechanisms, although its precise mode of action is unknown.
Antitumor response of BCG seems to be initiated by the attachment of BCG to bladder wall via Fibronectin (FN). The major secreted BCG antigen, AG 85 complex (AG 85), binds specifically to Fibronectin. The evolutions of the cellular immune response, interleukin-2 (IL-2) and interferon gamma (IFN-γ) production against Tuberculin (PPD) and AG 85 have been tested in a control group of 20 untreated bladder tumor patients and before and after 6 weekly intravesical BCG instillations in a group of 31 superficial bladder patients.
A major increase (> 2 SD) in the lymphoproliferative response against PPD and AG 85 was observed respectively in 70 % and 58 % of the treated patients while no increase was found in the control group. An increase in the production of IL-2 against PPD and AG 85 was noted respectively in 75 % and 63 % of the treated patients. An increase in the IFN-γ production against PPD and AG 85 was observed respectively in 50 % and 63 % of the treated patients.
This study demonstrates a specific immune activation against AG 85 Fibronectin-binding complex during BCG treatment of superficial bladder tumors.

P 24

PHENOTYPICAL CHANGES IN SUBMESOTHELIAL MESENCHYMAL AND SMOOTH MUSCLE CELLS OF RABBIT BLADDER AFTER "FREEZING"

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We have evaluated the proliferative capacity and the change of cell phenotypes which occurred in the submesothelial region and in the smooth muscle (SM) compartment of rabbit bladder following a transient application of cold lesion ("freezing"). Bromo-deoxyuridine incorporation studies as well immunofluorecence assays performed with monoclonal antibodies specific for cytoskeletal (desmin, vimentin, cytokeratin (CK) 8 and 18, nonmuscle myosin) and cytocontractile (SM-type α-actin and myosin) proteins on cryosections from day 1 up to day 30 after surgery revealed that: (1) after an inflammatory reaction, a marked thickening of serosa develops, characterized by the transient presence of CK 8- and 18-containing myofibroblasts, (2) CK expression in this model is not a "reawakening" of a fetal-type phenotype, (3) an elastic membrane (similar to that observed in obstructed bladders) can appear in some areas and divides the thickened serosa in two regions with different levels of proliferating cells, (4) in areas devoid of this membrane, bundles of myofibroblasts arranged in long cables spread from the serosa and connect to the underlying SM, (5) SM cells display a limited tendency to proliferate and partially recapitulate an immature SM cell phenotype, and (6) there is no evidence for a maturation of submesothelial mesenchymal cells towards the SM cell phenotype 30 days after surgery, as happens in obstructed bladder.

These findings suggest that: (1) the inflammatory response and/or the mechanical activity of bladder are responsible for the observed phenotypic differences in the two experimental models, and (2) new SM cells are incorporated in regenerating SM from myofibroblasts of thickened serosa. Our results also suggest that some phenotypical modifications of submesothelial mesenchymal cells may have a profound impact on cellular therapy of some bladder dysfunctions.

P 25

P53-A POTENTIAL TARGET FOR GENE THERAPY IN UROTHELIAL CANCER?

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Inactivation of the p53 tumor suppressor gene is found in a subgroup of approximately 40 % of urothelial cancers. Therefore, reconstitution of wild type p53 function appears a promosing alternative for topical treatment of this subgroup. However, due to the variety of responses to the reexpression of wild type p53 observed in different cell types in vitro analysis of urothelial cancer cells is required. In this study five individual cell lines, previously shown to contain p53 mutations, were further examined in tranfection assays with wildtype (hup53), mutant (273His) and a p53 construct with a 539 base pair deletion (ΔII 755/1294) driven by the cytomegalievirus (CMV) promotor. Calcium phosphate mediated cotransfection of RSVneo and CMVp53 plasmids resulted in G418 resistant clones after 14 days of selection. Clones were either isolated and expanded for further analysis or Giemsa stained and counted.

All cell lines transfected with CMVhup53 showed drastically reduced clone numbers compared to CMVp53ΔII and RSVneo alone. CMVp53/273His transfections resulted in increased or reduced clone number depending on the cell line. DNA extracted from clones of cell lines T24, VM Cub 1 and 639V did not contain intact wild type or mutant p53 plasmids, respectively, as detected by Polymerase Chain Reaction (PCR) and Southern analysis. However, Southern analysis revealed intact RSVneo genes in transfectants of all cell lines examined. To further analyse the effect of p53 transient cotransfection of cell line VM Cub 1 with RSVßGal and CMVhup53, CMVp53/273His or CMVp53ΔII, respectively was carried out. ß-Gal staining after 24, 48, 72 and 96 h showed markedly reduced number of ß-Gal positive cells 48h after cotransfection with wild type p53. CMVp53ΔII and CMVp53/273His showed reduction of successfully transfected cells over time as expected.

The results presented here show that expression of wild type p53 is impossible in urothelial cancer cells with different p53 point mutations. The effect of wild type p53 occurs very rapidly within 48h after transfection as can be seen from the ß-Gal staining. Probably, reexpression of wild type p53 leads to cell death (maybe by triggering apoptosis as has been reported for other cell types). Therefore cells containing CMVhup53 and RSVneo or RSVßGal after transfection will die. This leads to a reduced number of clones exclusively containing RSVneo or a reduced number of ß-galactosidase expressing cells, respectively. Since wild type p53 rapidly kills urothelial cancer cells expressing mutant p53 it is a potential target for gene therapy of this subgroup.

To further analyse the mechanism of p53 induced death, metallothionin (MT) inducible p53 constructs have been developed. Stable transfection of MThup53 and MTp53/273His in urothelial cancer cell lines is in progress.

P 26

INHIBITION OF bFGF EXPRESSION BY HUMAN TRANSITIONAL CELL CARCINOMA (TCC) FOLLOWING EXPOSURE TO INTERFERON -α-2a.

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The invasive and angiogenic properties of human tumors are modulated by cytokines released into the organ microenvironment. One of these cytokines, interferon-α inhibits tumor induced angiogenesis. Our hypothesis to explain this phenomenon is that IFN-α downregulates the steady-state gene expression and protein production of the angiogenic molecule bFGF. Following in vitro exposure to low dose IFN-α-2a, we documented a decrease in bFGF mRNA and protein expression by human 253J B-V TCC cells at doses of IFN-α which were not cytostatic to the tumor cells. The level of bFGF in conditioned medium (CM) was reduced by IFN treatment, and stimulation of endothelial cell proliferation by CM following exposure to IFN was abrogated. Therapy of mice with orthotopic 253J B-V bladder tumors with low dose interferon-α-2a reduced tumor size. The tumors of treated mice were less vascular by factor VIII staining, and showed reduced expression of bFGF by immunohistochemistry. These experiments demonstrate that one mechanism to explain the antiangiogenic effect of IFN-α is through the downregulation of bFGF expression by tumor cells.

P 27

EFFECTS OF COLONY STIMULATING FACTORS (CSF) ON CELLULAR CYTOTOXICITY

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Problem: Clinically, CSF are used for the supportive treatment of chemotherapy-induced myelosuppression. As CSF also have pleotropic functions, we have analyzed the effects of these cytokines on cellular cytoxicity in vitro against bladder carcinoma cell-lines and short-term cultures of bladder tumors.

Methods: Cytotoxicity of mononuclear cells from peripheral blood was determined against several established bladder carcinoma cell-lines and against NK-sensitive K562 cells by means of a ^{3}H-L methionine assay, using different effector/target-cell ratios. Stimulatory effects of different concentrations of G-CSF, GM-CSF, IL3 and stem cell factor (SCF) on lymphokine-activated killer (LAK), BCG-activated killer (BAK) and natural killer (NK) cell cytotoxicity were analysed after pre- and co-incubation of effector and target cells with cytokines. Furthermore, the effect of CSF on proliferation of bladder tumor cells was determined.

Results: Bladder tumor short-term cultures and all bladder carcinoma cell lines exhibited resistance against NK-cytotoxicity, whereas susceptibility against LAK- and BAK-cytotoxicity was demonstrated. GM-CSF, but neither G-CSF, IL3 nor SCF were able to increase cellular cytotoxicity in a dose-dependent manner. No increase in cellular proliferation was evident under the conditions of the assay.

Conclusion: Stimulation by GM-CSF of LAK- and BAK-cytotoxicity and especially in NK-cytotoxicity against otherwise NK-resistant bladder tumor cells is a phenomenon which could be used for a therapeutic approach. CSF should be further analyzed as co-stimulatory agents in certain immunotherapies.

P 28

DENDRITIC CELLS FROM THE PERIPHERAL BLOOD OF RENAL CELL CARCINOMA PATIENTS

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Dendritic cells are considered to be the initiators of immune responses including those directed against tumors. The potential of dendritic cells for cancer immunotherapy has been recognized but clinical research was impossible due to the limited availability of these cells. We show in this study that substantial numbers of dendritic cells can be obtained from the peripheral blood of patients with renal cell carcinoma. Approximately 2-8 million cells with the characteristics of dendritic cells could be obtained from a 50-ml blood sample: phase contrast and electron microscopy revealed the typical cytoplasmic processes or veils; FACS analysis confirmed expression of dendritic cell associated molecules including MHC class I and II, CD1a, CD4, ICAM-1 (CD54), LFA-3 (CD58), B7-1 (CD80) and B7-2 (CD86), and absence of T-cell, B-cell and monocyte markers; in addition, these cells rapidly attached to and migrated on collagen-coated surfaces; moreover, cultured dendritic cells proved to be very potent costimulators of the phytohemagglutinin-induced proliferation of autologous tumor-infiltrating lymphocytes. The reproducible growth of functional dendritic cells from renal cell carcinoma patients is encouraging for the design of adoptive immuntherapy protocols.

P 29

TUMOR-ASSOCIATED INTERLEUKIN6 (IL6) SYNTHESIS OF RENAL CELL CARCINOMA (RCC): AN AUTOCRINE GROWTH FACTOR?

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Introduction. Evidence exists that IL6 is implicated in the process of neoplasia. *In vitro* studies and murine models suggest that IL6 may exert either an anti-proliferative or growth stimulating effect, depending on the nature of the malignancy. To examine the role of IL6 in RCC two human RCCs, grown in nude mice with a clinically distinct behaviour were used.

Results. *In vivo* the RC8 tumor line produces high amounts of human IL6, detectable in the circulation, expresses hIL6-mRNA and induces hypercalcemia. No such tumor-associated characteristics were noticed in the RC9 tumor line. After ip. injection of RC8 tumor bearing mice (tumor volume 100 mm^3) with neutralizing antibodies to hIL6 (CLB IL-6/8, 1 mg/animal), significant ($p<0.001$) inhibition of tumor growth was observed during 14 days (growth rate of 15 ± 2 mm^3/d versus 27 ± 2 mm^3/d). Furthermore, a significant ($p<0.001$) decline in serum calcium (2.8 ± 0.4 mmol/l) compared to untreated animals (4.2 ± 0.3 mmol/l) was perceived. Administration of anti-IL6 to RC9 bearing animals showed no effect on tumor growth or serum calcium. Infusion of hIL6 at a rate of 10 μg/d, using sc. implantated osmotic pumps, in RC8 (100 mm^3) bearing mice resulted in a significant ($p<0.001$) increase of tumor growth rate (54 ± 4 mm^3/d) compared to RC8 bearing animals treated with saline containing pumps (13 ± 4 mm^3/d). No effects were found in RC9 bearing animals. *In vitro*, RC8 as well as RC9 cells produced hIL6. No effect on cell proliferation was observed in the presence of anti-IL6 (100 ng/ml) or IL6 (20-2000 ng/ml) in both cell lines.

Conclusions. From these results it is tentatively concluded that IL6 may act as a growth stimulating factor in certain IL6 producing renal cell carcinomas. Observations made under optimal conditions for tumor cell growth as in culture do not reflect the *in vivo* situation.

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DECREASED EXPRESSION OF DNA-TOPOISOMERASE IIα IN MULTI-DRUG RESISTANT RENAL CARCINOMA CELL LINES.

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Chemotherapeutic treatment of renal cell carcinoma (RCC) is still strongly impaired by the relative insensitivity of tumor cells to cytotoxic drugs. Since many new anticancer agents act as DNA-topoisomerase (Topo) inhibitors, a better understanding of the Topo-associated multidrug resistance (MDR) mechanism seems necessary to improve chemo-therapeutic treatment options of advanced RCC. We established MDR-RCC cell lines RC2E, RC21E and RC21A by in vitro culturing of non-chemoselected RCC cell lines (RC2, RC21) in the presence of stepwisely increased concentrations of the Topo II poison VP-16. Compared to their parental lines, RC2E, RC21E and RC21A had 100-fold, 100-fold and 10-fold increased resistance, respectively, as measured by their growth adjusted IC50 values. Furthermore, RC2E and RC21E were also resistant to Vinblastine, which is not a Topo II inhibitor, whereas RC21A was not. Topo IIα, Topo IIß, P-gp and MRP expression was measured by Western blotting and quantitative RT-PCR. Topo II activities were determined using the P4-DNA unknotting assay. Possible qualitative alterations in the Topo IIα-gene were assessed by single strand conformational polymorphism (SSCP) analysis of the ATP and DNA binding regions. Our results consistently demonstrated a classic MDR1/Pgp-mediated resistance phenotype for RC2E and RC21E. RC21E and RC21A showed decreased Topo IIα expression and consequently decreased Topo II activity. Thus, RC21A displays a pure attenuated MDR phenotype. It appears, that selection by VP-16 can induce either Pgp-associated or Topo II-dependent MDR or a combination of both, whereas MRP does not appear to play a significant role in this resistant phenotype. While resistance of RC21A is stable for more than one year without any further drug selection, Pgp overexpression in RC2E decreased after 10 months. Furthermore, down-regulation of Topo IIα gene expression affected cell cycle and growth pattern characteristics, resulting in an increased doubling time and multilayered growth. In addition, resistant cell lines of this tumor model were tumorigenic in nude mice, thus permitting in vivo analysis. These RCC lines appear to be suitable models for studying Pgp and Topo II-associated MDR in solid tumors.

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ENHANCEMENT OF INTERLEUKIN 2-INDUCED LYMPHOKINE-ACTIVATED KILLER CELLS AGAINST RENAL CANCER CELLS BY RECOMBINANT INTERLEUKIN 7

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Adoptive immunotherapy using lymphokine activated killer (LAK) cells and Interleukin 2 (IL2) has been attempted against advanced kidney cancer. However, the response rate is not as good as expected, and severe adverse effects by IL2 such as pulmonary edema have been recognized. How to induce LAK cells with greater antitumor potency and less adverse effects of IL2 is an important issue. For this purpose, we evaluated the possibility to induce LAK cells and to enhance the IL2-induced LAK activity with IL7 *in vitro* for the purpose of improving the efficacy of LAK therapy against renal cell carcinoma and reducing its adverse effects. We established the cytotoxicity assay system using autologous renal cancer cells. Peripheral blood lymphocytes derived from patients with renal cell carcinoma were stimulated with IL2 and/or IL7 for 7 days, and tested in a 4-hour ^{51}Chromium release cytotoxicity assay against autologous cultured renal tumor cells. In all 10 cases tested, IL7 alone induced LAK activity. Moreover, IL2-induced LAK activity was enhanced by the addition of IL7. In these cases, at least additive effect was observed. Single color flowcytometry revealed the increase of IL2 receptor-positive lymphocytes by incubation with IL7 alone . These results suggest that IL7 might become a useful agent for patient with renal cell carcinoma when stimulated together with IL2.

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TUMOR-INFILTRATING T LYMPHOCYTES FROM RENAL CELL CARCINOMA EXPRESS B7-1 (CD80): T CELL EXPANSION BY T-T CELL COSTIMULATION

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B7-1 (CD80) provides costimulation for T cell activation by interacting with CD28 or CTLA4. Here we demonstrate the expression of B7-1 in freshly isolated and cultured lymphocytes from renal cell carcinoma. In fresh preparations of lymphocytes infiltrating renal cell carcinoma tissue B7-1 mRNA could readily be detected by reverse transcription PCR and two-color flow cytometry analysis revealed substantial B7-1 expression on the T cells of these isolates. As expected, tumor-derived T cells also expressed CD28, the B7 receptor. While B7-1 expression of tumor-derived T cells was maintained during culture in interleukin-2 supplemented medium, CD28 expression was further enhanced. We also show that B7-1 is functionally involved in T cell expansion: anti-B7-1 mAb inhibited the PHA-induced proliferation of tumor-derived B7-1$^+$ T cells (35%) in the absence of exogenous antigen-presenting cells indicating that B7-1 mediates T-T cell costimulation (self-costimulation). Our data demonstrate that T cells infiltrating renal cell carcinoma express B7-1 and that mutual costimulation via the CD28/B7-1 pathway contributes to the interleukin-2 driven expansion of tumor-derived T cells in vitro. The frequency of B7-1$^+$ T cells in tumor lesions and the level of B7-1 on these cells may also determine the time course of T cell expansion in vivo. However, self-costimulation might eventually lead to the state of anergy characteristic of tumor-infiltrating lymphocytes.

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ANTI-RCC X ANTI-DTPA BISPECIFIC MONOCLONAL ANTIBODIES FOR TWO-PHASE TARGETING OF RENAL-CELL CARCINOMA

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We aim to use bispecific monoclonal antibodies (bsMAbs) for targeting radiolabeled diethylene triamine pentaacetic acid (DTPA) to renal-cell carcinoma (RCC). In these pretargeting protocols the anti-tumor bsMAb is administered and allowed to clear from the circulation whereafter the radionuclide is given as a small rapidly clearing ligand. A pretargeting protocol may optimize radioimmunodetection and radioimmunotherapy of tumors in vivo.

Five anti-DTPA MAbs were produced reactive with DTPA loaded with different metals. This broad reactivity of the MAbs make them suitable for radioimmunodetection (using ^{111}In-DTPA or ^{99m}Tc-DTPA) as well as radioimmunotherapy (with ^{90}Y-DTPA). The ability of the anti-DTPA MAb DTIn1 to bind ^{99m}Tc-DTPA in vivo was tested in rats with focal *S. aureus* infection. Antibodies are known to nonspecifically accumulate in infectious foci. Priming with DTIn1 followed by ^{99m}Tc-DTPA resulted in rapid visualization of the abscess within 4 hr demonstrating that ^{99m}Tc-DTPA was specifically trapped by DTIn1. Priming with an irrelevant control antibody didn't result in visualization of the abscess. The retention of ^{99m}Tc-DTPA in the circulation by DTIn1 could be blocked by injection of a 10-fold molar excess of BSA-DTPA-In 30 minutes prior to injection of ^{99m}Tc-DTPA. These experiments indicate the feasibility of two- as well as three-phase targeting protocols in vivo with this anti-DTPA MAb.

The anti-DTPA MAb producing hybridomas were used for somatic cellfusion with hybridoma G250 directed against RCC, resulting in three bsMAb producing cell lines (G250 x DTIn1, G250 x DTY4 and G250 x DTIn2). Individual purification protocols were developed using protein A affinity chromatography followed by hydroxylapatite chromatography and/or cation exchange chromatography resulting in highly purified functionally active bsMAbs. The affinity constants of the bsMAbs for binding ^{111}In-DTPA were similar to the affinity constants of the parental anti-DTPA MAbs indicating that they can be used in targeting protocols.

Currently the ability of these purified bsMAbs to target radiolabeled DTPA to RCC xenografts is tested in a nude mouse model. In these experiments the tumor is pretargeted with bsMAb, followed by the injection of ^{111}In-DTPA.

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GERM-LINE MUTATION OF VON HIPPEL-LINDAU TUMOR SUPPRESSOR GENE: GENOTYPES ASSOCIATED WITH RENAL CELL CARCINOMA PHENOTYPE.

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Von Hippel-Lindau disease is a familial cancer syndrom demonstrating autosomal dominant inheritance. Renal cell carcinoma is the leading cause of death in this disease. The reported VHL gene cDNA contains an open reading frame of 855 bp together with a 3´-untranslated region. The exact 5´ end and initial codon of the coding region remain unknown. Referring to both sequence data of human and mouse VHL genes, we used 6 pairs of primers to cover the reported ORF and splicing sites in the mutation study of our 10 Swedish VHL families. Direct genomic DNA sequencing was used to detect the mutation.

An additional G was found before the first bp of the reported ORF that made up a potential splicing site. We also made clear the consensus sequence flanking the splicing site of each exon. Six mutations(4 were novel) were mapped to exon 1 and 3 of which 2 were single bp deletions and 4 were single base substitutions. Two large germ-line deletions were detected by Southern blot of EcoRI or PstI digestion. Four mutations that caused significant protein changes all predisposed to VHL associated renal clear cell carcinomas. Overall 8 germ-line VHL alterations were detected in 10 unrelated VHL families and additional 5 disease gene carriers were also distinguished.

Our data suggests that the reported ORF have already contained the complete coding sequence of the VHL protein and an additional 5´-untranslated region might exist. Mutation studies of VHL families are of high importance in clinical detection of disease gene carriers. Analysis of genotype and phenotype relation might provide insight in the VHL gene function.

2A Genetic basis of cancer

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MOLECULAR CHARACTERIZATION OF THE RENAL CELL CARCINOMA-ASSOCIATED ANTIGEN G250. *Egbert Oosterwijk, Mirjam de Weijert, Adrie van Bokhoven, Ruud H Brakenhoff*, Wilhelmus P Peelen, Frans MJ Debruyne.* Urological Research Laboratory, Academic Hospital Nijmegen, 6500 HB Nijmegen, * Free University Hospital, Amsterdam, The Netherlands.

The Renal Cell Carcinoma (RCC) associated antigen G250 was initially identified by monoclonal antibody G250 (mAbG250). The antigen is homogeneously expressed in >75% of RCCs and is absent in normal kidney and other normal human tissues, with the exception of gastric mucosal cells and cells of the larger bile ducts. Clinical trials with ^{131}I-labeled mAbG250 have indicated that the antigen is a potential therapeutic target.

Thus far, the molecular characterization of G250 antigen has shown that the antigen has the characteristics of a protein. Further molecular characterization has been hampered by the apparent extreme sensitivity of the G250 antigen for non-native conditions.

For the molecular cloning of the cDNA encoding the G250 antigen, a eukaryotic expression cloning strategy was used. A cDNA library was constructed using mRNA from a human RCC cell line in the eukaryotic expression vector pCDM8. Immunohistochemical screening of progressively smaller pools of cDNA which were transfected into COS cells has lead to the isolation of a cDNA (pMW1) of approximately 1.5 kb containing an open reading frame and having a polyadenylation signal was isolated. Transfection of this cDNA into a G250- RCC cell line resulted in the expression of G250 immunodetectable protein, showing that this cDNA indeed encodes the G250 antigen. Northern analysis of mRNA derived from RCC cell lines (mAbG250$^+$ and mAbG250-), surgical specimens (RCC and normal kidney obtained from the same patient), and normal human organs, revealed a single 1.5 kb transcript in mAbG250$^+$ cell lines and RCC specimens. No transcript was observed in mAbG250- cell lines, normal kidney specimens, nor any normal human organ investigated. Sequence analysis and database searching revealed that the G250 antigen is homologous with MN, a recently cloned human tumor-associated protein of 54/58 kD. Screening of a Lambda-FIX library resulted in the identification of six clones (EO1-6) containing different inserts, spanning the entire G250 gene. Preliminary evidence indicates that the G250 gene is located on chromosome 9. Further molecular characterization of this RCC-associated antigen may result in a better understanding of the molecular basis of kidney carcinogenesis.

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IDENTIFICATION OF DD3: A NEW GENE OVEREXPRESSED IN PROSTATIC TUMORS. *Marion JG Bussemakers, Adrie van Bokhoven, Maud Effting, Marcel JW Janssen, Ning Ru*, William B Isaacs*.* Urology Research Laboratory, University Hospital Nijmegen, The Netherlands, *Brady Urological Institute, Johns Hopkins Hospital, Baltimore, USA

Identification of genes specifically expressed in tumor cells but not in normal cells, or *vice versa*, is important for the understanding of the molecular basis of carcinogenesis. Furthermore, such genes may provide us with markers for early tumor detection. Recently, advanced molecular genetic tools like comparative genomic hybridization (CGH) revealed that besides frequent loss also amplification of chromosomal loci occurs in prostatic tumors.

Amplification of chromosomal regions is often associated with activation of transcription units (e.g. overexpression). Therefore, we applied the technique of differential display analysis to identify genes overexpressed in prostatic tumors: this technique has been proven to be a powerful tool to identify and clone differentially expressed genes. In our study, mRNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients was extracted and used for differential display. We identified several apparently differentially expressed mRNAs, one of them (DD3) detecting two transcripts (2.2 and 4.0 kb) that are specifically expressed in human prostatic tumors whereas no expression of these transcripts was found in normal or benign hyperplastic prostate tissue. Also in normal human lung, bladder, colon, pancreas, skin, heart, smooth muscle and kidney tissue, no DD3 transcripts could be detected.

Nucleotide sequence analysis of DD3 (0.6 kb) did not reveal an open reading frame nor did we find homology with any known gene. Isolation of additional DD3-related cDNA clones allowed a further characterization of the transcription unit of DD3 and showed that alternative / cryptic splicing occurs, giving rise to the differently sized transcripts.

To assess whether DD3 may be located in an amplified chromosomal region, we isolated DD3-related genomic clones which were used as probes to hybridize to metaphase chromosomes of lymphocytes. In this way we were able to map DD3 to chromosome 9q21-22, a region which has been shown to be amplified in a number of prostatic tumors, suggesting that overexpression of the gene may be a result of gene amplification.

In conclusion, by using differential display we have identified a new gene, DD3 which is overexpressed in prostatic tumors. Further studies will be required to elucidate the function of DD3, its putative role in prostate tumorigenesis and its potential usefulness in prostate cancer diagnosis/prognosis.

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CASTRATION INDUCED APOPTOSIS IS MODIFIED IN P53 KNOCK-OUT MICE AND ASSOCIATED WITH AN INCREASE OF ANEUPLOID PROSTATE EPITHELIAL CELLS.

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Several in vitro studies have provided evidence that wild type p53 protein is involved in apoptosis. The recent development of p53 knock-out mice has enabled the demonstration that in some cases, apoptosis may be significantly altered in absence of p53. In this report, we present evidence that prostate apoptosis is modified in p53 knock-out mice. Ventral prostate glands were obtained from normal, one allele p53, and nullizygous p53 transgenic mice at daily intervals after castration. These tissues were stained for apoptotic bodies with the use of the *in situ gap* labelling method and apoptotic bodies were quantified by microscopy. Although labeled apoptotic bodies were observed in post castrated tissues from all the variant mice, the onset of apoptosis was delayed and the occurrence of apoptosis was significantly reduced in the p53 nullizygous mice when compared to normal controls. Heterozygous p53-null mice were intermediate for these criteria. Examination of the internucleosomal DNA fragmentation pattern at 2 days of castration supports a significant diminution of apoptosis in p53 nullizygous mice. Additionally, we detected large nucleated and multinucleated cells in the prostate epithelium of non castrated nullizygous mice, and these abnormal cells were increased after castration. Flow cytometric analysis of these tissues confirmed a high number of 4C and 8C DNA content cells in the p53 nullizygous prostates and their frequency was increase after castration. In concordance with earlier study, we conclude that functional p53 protein is not essential for prostate epithelial cells to undergo castration induced apoptosis. However, wild type does appear to enhance this process, especially in the early period following castration, and this protein may regulate an aberrant cell cycling process that follow castration in prostatic epithelial cells.

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P-GLYCOPROTEIN MEDIATED MULTDRUG RESISTANCE IN ANDROGEN INDEPENDENT PROSTATE CARCINOMA CELLS

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Hormone unresponsive prostate carcinoma cells also display an enhanced resistance against a variety of drugs. Up to now it has not been thoroughly evaluated, if specific resistance factors, like the expression of the *MDR*1 gene play a role in this multidrug resistance and if there might be a link between drug resistance and hormone independent growth.

We investigated the resistance patterns of hormone sensitive and hormone independent Dunning rat carcinoma sbulines against 5 drugs, which are substrates of P-glycoprotein (vinblastine, taxol, doxorubicin, etoposide and colchicine) and 2 agents (methotrexate and cis-platinum), which are not transported by the pump. The hormone insensitive sublines AT.1, AT. 3.1., MatLu and Mat LyLu showed a clearly enhanced resistance(3 to 26-fold) against the p-glycoprotein substrates, compared to the hormone sensitive subline G, whereas all cells expressed comparable resistance levels against methotrexate and cis-platinum. By addition of verapamil the resistance against vinblastine (9 to 10-fold) and taxol (6.7 to 26.7-fold) in the hormone insensitive cells could be almost totally reversed. Furthermore, the fluorescent P-glycoprotein substrate rhodamine 123 was effectively pumped out of the four tested hormone independent cell lines, whereas the hormone sensitive G cells were unable to extrude the dye. By RT-PCR with primers specific for the rat *mdr*1b gene, we could easily detect *mdr*1b expression in the androgen independent cell lines, but not in the G cells. Our results suggest, that expression of the rat *mdr*1b gene confers chemoresistance on androgen independent Dunning prostate carcinoma cells. For androgen receptors are not detecable in those lines ,but easily can be demostrated in the hormone sensitive G-subline, we hypothize, that *mdr* expression might be negatively regulated by the androgen receptor.

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MOLECULAR CLONING OF THE cDNA FOR THE ANDROGEN-REGULATED HUMAN PROSTATE SPECIFIC TRANSGLUTAMINASE.

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Transglutaminases (TGases) are calcium-dependent enzymes that catalyze the covalent cross-linking of proteins or of proteins to polyamines. Previous results from our laboratory suggested an inverse correlation between TGase activity and metastatic ability of selected panels of prostate tumor cell lines. However, like in the rat prostate, two different TGases may be expressed in the human prostate, tissue type TGase (TG_C) and a prostate specific TGase (TG_P). Although TG_C down regulation is likely to be the major cause of decreased TGase activity in metastatic cells, possible changes in TG_P expression cannot be excluded. Therefore, isolation of TG_P cDNA might help us to discriminate between the two TGases in prostatic tissue.

Our approach for the isolation of the TG_P cDNA was based on the high similarity of the various TGases in the vicinity of the active center. A human prostate cDNA library was screened with a probe from the TG_C active site region using low stringency conditions. This resulted in the isolation of nine cDNA clones with varying insert sizes. Eight of these clones were shown to encode TG_C, but one, clone 4.2, exhibited high similarity with rat TG_P. This clone had an insert of 2.8 kb and complete sequencing revealed that part of its 5' end was probably lacking. Rescreening under high stringency conditions of the same cDNA library with a 2.2 kb *Sac* I-*Hpa* I fragment from clone 4.2 resulted in the isolation of the remaining part. The complete TG_P cDNA consists of 2983 nucleotides and encodes a protein of 684 amino acids with a predicted molecular weight of 77 kD. Comparison of the predicted amino acid sequence with that of the rat TG_P showed a 53% identity, while the similarity with TG_C was only 32,7%. Northern blot analysis of total RNA from benign prostatic hyperplasia showed a single band of approximately 3.4 kb. Hybridization to a multiple tissue northern blot revealed that the expression of TG_P was prostate-restricted. No expression was detectable in the human prostate cancer cell lines LNCaP and PC3 by northern blotting. In contrast, the PC346C cell line, which was recently developed in our laboratory, showed expression of TG_P mRNA. In this hormone-responsive cell line exposure to 0.1 nM of the synthetic androgen R1881 increased the amount of TG_P mRNA 2-3 fold, demonstrating that TG_P expression is androgen-regulated.

Currently, antisera specific for the C-terminal region of either TG_P or TG_C are being generated, which should enable us to study expression of both TGases in prostatic cells also at the protein level. Such studies are aimed to reveal the possible function of TGases in the pathophysiology of the human prostate.

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DIFFERENT COMBINATIONS OF GENETIC ALTERATIONS IN TCC PATHOGENESIS.

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Human Papilloma Virus (e.g. HPV16) has been identified in approximately 40% of transitional cell carcinoma (TCC) of urinary bladder (LaRue *et al.* Clin. Can. Res. 1:435,1995). The HPV16 E6 and E7 oncoproteins bind to the TP53 and RB tumor suppressor gene products leading to p53 degradation and pRb alteration, respectively. Many studies indicate that loss of wildtype p53 and pRb functions disrupt genome stability and cell cycle regulation, respectively. Our laboratory has demonstrated that transformation of human uroepithelial cells (HUC) with HPV16 E6 or E7 extends *in vitro* lifespan, but rarely leads to immortalization (Reznikoff *et al.* Genes & Devel. **8**:2227,1994). It has therefore been hypothesized that additional genetic alterations are needed to block cellular senescence. In the present study, we identified nonrandom clonal chromosome alterations that specifically accompanied HPV16 E6, compared to E7, immortalization of human uroepithelial cells (HUC). We report that four of five independent clonal lines of E6-HUCs showed 3p14->pter deletion (p-value=7×10^{-6}), while three of three E7-HUCs showed 20q11->qter gain (p-value=1×10^{-4}). These data suggest that gain of a gene(s) on 20q complements pRb alteration, while deletion of a 3p gene(s) works in synergy with p53 loss in overcoming the senescence block in HUC. In addition, a highly significant association was found between E6 transformation of HUC and karyotypic instability both at early passage (p-value=2×10^{-19}), and after propagation in culture (p-value=2×10^{-17}),implicating p53 loss in generating chromosome instability. Finally, 3p loss was associated with chromosome 9 instability (p-value=3×10^{-5}), and with subsequent clonal selection for cells with 9p21->pter loss (p-value=3×10^{-6}).

These results provide the first example of how the initial genetic alteration may direct late genetic changes and how ***different combinations of genetic alterations work together to block cellular senescence, initiate tumorigenesis, and lead to tumor progression.*** Supported by NIH R01CA29529 and R01CA67158.

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CLONALITY AND DETECTION OF TRANSITIONAL CELL CARCINOMA OF THE BLADDER USING *p53* MUTATIONS

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To establish the clonality of recurrent tumours in patients with transitional cell carcinoma (TCC) of the bladder, we identified five patients with *p53* mutations in their primary tumours and a history of recurrence. Seventeen recurrent tumours from these patients, spread over seventy eight months, were then analysed for the presence of identical mutations. The recurrences arose at new sites compared to the primary lesion. Sixteen out of seventeen recurrences were found to be monoclonal with respect to the *p53* mutation observed in the primary tumour. These results indicate that recurrences of G2/G3 TCC are clonally derived from an initial population of neoplastic cells, and probably arise through an intraluminal seeding mechanism. The results also demonstrate that tumour cell clones can persist for extended periods of time within the bladder. We were also able to detect exfoliated tumour cells by PCR and direct sequencing from urine samples. In a total of twenty eight urine samples from eleven patients with G2/G3 TCC containing a *p53* mutation it was always possible to detect exfoliated tumour cells whenever there was a recurrent TCC within the bladder. In five urine samples it was also possible to detect exfoliated cells on occasions when no recurrent tumour was detectable by cystoscopy. On three of these occasions the patients subsequently developed a recurrence (within 3,6 and 10 months). In the other two cases the urine samples were obtained too recently to rule out the possibility of recurrence. We are able therefore to compare the standard methods of follow-up screening for TCC recurrence with molecular screening methods using tumour-specific biomarkers.

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GELSOLIN: A CANDIDATE FOR SUPPRESSOR OF HUMAN BLADDER CANCERS.

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Human transitional cell carcinomas of the bladder frequently reveal chromosomal abnormalities that span a range between chromosome 9q12 and 9qter, even at early stages of bladder carcinogenesis. We have previously demonstrated that gelsolin have a suppressive potential against the H-ras transformed HIH/ 3T3 fibroblast. Since the gene which encodes an actin-regulatory protein, gelsolin, is localized in chromosome 9q33, we examined the expression of gelsolin in a number of human bladder cancer cell lines (DAB-1, EJ, MGH-U1, T24, UMUC-2, UMUC-6) and 18 bladder cancer tissues, and tumor suppressive ability of gelsolin in bladder cancer. Gelsolin expression and its localization were evaluated with Northern blot, Western blot analysis and immunohistochemistry. Furthermore, we transfected the human or mouse authentic gelsolin cDNA into a human bladder cancer cell line, UMUC-2 and carried out the colony forming assay in soft agar and tumorigenecity test in nude mice to examine the tumor suppressor activity of gelsolin. In all 6 cell lines and in 12 of the 18 tumor tissues, gelsolin expression was undetectable or extremely low in comparison with its expression in normal bladder epitherial cells. In 2 tumor tissues, only stromal cells including the muscularis mucosa, the smooth muscle layer and the cells surrounding the vesseles in tumor nests responded positively gelsolin. These results suggest that gelsolin expression is reduced or undetectable in bladder cancer cells. All human- and mouse- gelsolin transfectants exhibited a lower colony-forming efficiency (about 11%) than the control clones (about 26%) did. Moreover, all of the gelsolin transfectants showed no tumorigenecity in nude mice, while control clones grew progressively.

These results suggest that gelsolin plays a key role as a tumor suppressor in human urinary bladder carcinogenesis.

2 B Miscellaneous

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PRIMARY NORMAL HUMAN UROTHELIUM: AN *IN VITRO* MODEL

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Several studies suggested the involvement of growth factors in wound healing or tumour progression of human urothelium. However, little is known about the functional effects of growth factors on human urothelium, in part because of the lack of a sufficient in vitro model.

In order to obtain an in vitro model for normal human urothelium, we applicated a previously described, organotypic mouse model. Explants of normal urothelium of 5 different patients were grown on collagen type IV-coated, porous membranes. The cultures were examined for different parameters including expansion during culture, proliferation by BrdU incorporation, differentiation by (ultrastructural) morphology, and expression patterns of cytokeratin 18 and 19, and E-cadherin, and urothelial barrier function by transport of dyes. Cultures grew to confluency within 20-30 days showing a differentiated stratification of up to 4 cell layers. Virtually all cells stained with chain-specific antibodies against cytokeratins. Like in vivo, both cytokeratin 18 and E-cadherin were specifically present in the superficial cell layer . BrdU was found only in the basal or intermediate cell layers. No transport was observed of trypan blue or phenol red during culture suggesting an intact barrier function. After growing to confluency, some cultures were treated with growth factors FGF-1, FGF-2, FGF-7, TGFα or TGFβ1 in serum-free medium. As with mouse urothelium, TGFβ inhibited the proliferation. For the other growth factors, no significant effects were observed on the indicated parameters compared with serum-free medium alone.

In conclusion, the data support that we established a model for the primary culture of normal human urothelium with in vivo-like characteristics. With this model, we are able to perform mechanistical studies on cytokines that are suggested to be important for urothelial wound healing immunotherapy or tumour progression.

§ Recipient from the 1995 EBU fellowship

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INDUCTION OF DIFFERENTIATION AND STRATIFICATION IN CULTURED NORMAL HUMAN UROTHELIAL CELLS: POTENTIAL USE FOR COMPOSITE ENTEROCYSTOPLASTY.

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The ability to culture normal human urothelial cells has major implications for basic urothelial cell biology and offers immediate applications for urological reconstructive surgery. Most problems of conventional enterocystoplasty are attributable to the absorptive nature of intestinal epithelium. We are developing a composite enterocystoplasty in which sheets of autologous urothelial cells are combined with de-epithelialised bowel stroma. We have previously shown that clinically-useful amounts of normal human urothelial cells can be generated *in vitro*. Although the cells grow rapidly in monolayer culture and can be induced to stratify in high Ca^{2+} media, they retain the phenotype of basal/intermediate cells, and do not display the full barrier functions of differentiated urothelium.

In order to investigate urothelial differentiation *in vitro*, we have taken established normal human urothelial cell cultures and seeded the cells onto an extracellular matrix support ("Matrigel"). In low-calcium conditions, the cells remained as non-stratified monolayers. With increasing Ca^{2+}, the cells became longtitudinally orientated and formed papilla-like structures. At high (>2.0mM) calcium concentrations, stratification was more uniform with papillae restricted to culture margins. These cells also expressed antigens associated with late/terminal urothelial cytodifferentiation *in situ*, including the urothelial membrane antigen (UMA), uroplakins and cytokeratin 20.

Thus, cultured normal urothelial cells have retained the ability to stratify and differentiate normally *in vitro*. We suggest that urothelial "organoids" will have application in composite enterocystoplasty procedures.

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GAP JUNCTIONAL COMMUNICATION AND PROTEIN EXPRESSION IN *IN VITRO* CULTURE OF HUMAN BLADDER CARCINOMA.

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Object: With the intent to relate connexin expression and functional state of gap junctions to the differentiation of normal urothelium and urothelial neo-plasm, cell lines with different urothelial differentiation were compared in different growth states.
Methods: Cloned cell lines of normal urothelium (HCV29), a low grade non-invasive (RT4), and a high grade invasive bladder carcinoma cell line (J82) were grown as monolayers (exponential and plateau), and as three-dimensio-nal multicellular spheroids (MCS). Antibodies against three connexins (Cx26, rabbit-anti-human, Dr. Traub, and Cx43 and Cx32, rabbit-anti-human, Dr. Dermietzel) were used for staining of sections in indirect immunfluorescence and Western blotting, and data were compared to functional results from mi-croinjections with Lucifer Yellow. Experiments were further extended to he-terologous cocultures with the human fibroblast cell line N1 as ML and MCS.
Results: In ML, normal HCV29 cells showed increasing coupling with the degree of confluence, while tumor cell lines decreased in coupling. Decrease in coupling was paralled by the decrease of Cx26 from HCV29 to RT4, showing absence in J82 cells. Cx32 was mostly coexpressed with Cx26. Cx 43 was only found in all cell lines and was not related to coupling, however showed interesting differences in phophorylation in Western blotting experiments. In three-dimensional cultures of J82 a reexpression of Cx 26 was found, and was maintained in cocultures with fibroblasts. In the latter, tumor cells coupled more intensively, however mainly homotypically.
Conclusion: Dynamic *in vitro* systems help us understand the relation be-tween electrophysiological coupling and connexin expression and thus help us identify interesting marker molecules of tumor progression. Preliminary patient data show loss of Cx 26 with increase in malignancy, and exclude sole detection of Cx 43 as associated with malignant growth in bladder cancer.

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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF EXPLANT CULTURES OF HUMAN PROSTATIC STROMAL CELLS

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The study of stromal-epithelial interactions greatly depends on the ability to culture both cell types separately, in order to permit analysis of their interactions under defined conditions in reconstitution experiments. Here we report the establishment of explant cultures of human prostatic stromal cells and their immunocytochemical characterization. As determined by antibodies to keratin and prostate specific acid phosphatase, only small numbers (<5%) of epithelial cells were present in primary cultures; subsequent passaging further reduced epithelial cell contamination. Antibodies against intermediate filament proteins (keratins, vimentin, and desmin) and smooth muscle actin microfilaments demonstrated that the population of stromal cells cultured from benign prostatic hyperplasia and prostatic carcinoma differed in regard to their differentiation markers. Two contrasting phenotypes were identified in cultures derived from these two different lesions: One, exhibiting fibroblastic features, was predominant in cultures derived from benign lesions and a second, showing varying degrees of smooth muscle differentiation, was more abundant in carcinoma-derived cultures: the average number of α-smooth muscle actin positive cells in carcinoma derived primary cultures (mean, 75%; median, 73.6; SD, 7.4) was significantly larger ($P \leq 0.01$, Wilcoxon rank test) than the number in BPH-derived stromal cell cultures (mean, 25.6%; median, 21.0; SD, 15.8). Although less pronounced, similar results were obtained with the desmin antibodies. These differences, however, were only manifest during early passages. At subculture (up to the 7th passage) , the percentage of α-SM actin positive cells in BPH-derived stromal cell cultures increased, whereas in carcinoma-derived cultures the number of positive cells generally declined. The findings suggest a remarkable divergence in the stromal-epithelial relationships associated with these pathological conditions, and may provide us with a potential tool for studying these processes.

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THE INTERACTION OF PRIMARY PROSTATIC EPITHELIA WITH BONE MARROW STROMA AND PROSTATIC STROMA

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The mechanisms governing the development of skeletal metastases from prostate have been studied using an *in vitro* model culturing prostatic epithelial cells on confluent bone marrow and prostatic fibroblast cultures. Immunohistochemically characterised prostatic epithelial cells harvested from men with benign prostatic hyperplasia and prostate cancer were seeded onto confluent cultures of human bone marrow or on to prostatic fibroblasts. The bone marrow cultures were haematopoietically active and harvested from normal males or from patients known to have skeletal metastases from prostate cancer. Human prostatic fibroblasts were isolated from benign and neoplastic prostates. Epithelial growth characteristics were then recorded by counting increases in colony size and number over time. Further experiments then studied the effects of conditioned media from bone marrow and prostatic fibroblasts on clonal growth of prostatic epithelia as assessed by MTT and thymidine incorporation assays.
Results showed that normal bone marrow (NBM) consistently stimulated benign and malignant epithelial growth by levels 200 to 2000% more than those induced by prostatic fibroblasts (PFB). It was also noted that benign PFB supported epithelial growth to a greater extent than malignant PFB. Conditioned media studies showed that both benign and malignant epithelial cell growth was stimulated by soluble factors produced by NBM cultures, stimulating growth levels 150-1800% greater than that in controls. Initial conclusions are that human bone marrow produces factors which facilitate the growth of human prostatic epithelial cells and that the stimulatory properties of prostatic fibroblasts vary according to whether or not they have a benign or malignant origin.

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SEVEN NEWLY ESTABLISHED XENOGRAFT LINES AS MODEL SYSTEMS FOR THE STUDY OF PROGRESSION OF HUMAN PROSTATIC CANCER.

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It is generally recognized that human prostate tumor xenograft models are very difficult to establish. Recently, however, we were able to develop 7 new human prostate tumor lines using athymic NMRI nude mice. Neither the host animals nor the tumor specimens were pretreated in any way. The hormonal status of the host animals seemed not to determine the development of tumors.

Tumor model	Origin	Androgen dependence	AR	Differentiation	PSA
PC-295	LN	+	+	+	+
PC-310	PC	+	+	+	+
PC-324	TURP	-	-	-	-
PC-329	PC	+	+	+	+
PC-339	TURP	-	-	-	-
PC-346	TURP	+/-	±	-	+
PC-374	SM	-	±	±	+

Tumors have proven to be serially transplantable over a range of 6 to 17 mouse passages. Tumor "take" rates are stable and varies between 60 and 90%. Each mouse passage the human origin of the tumor tissue was histologically confirmed by a bisbenzimid DNA staining. Tumor histology of the original patient material was highly preserved during serial passage in nude mice. Tumor histology varied from well differentiated adenocarcinoma to poorly differentiated tumors with a solid growth pattern. The PC-295, PC-310 and PC-329 tumor lines were androgen dependent: tumors did not develop in female mice and castration of tumor-bearing mice resulted in tumor regression. The PC-346 tumor is also androgen dependent since no tumors developed in female mice. However, after castration-induced tumor regression regrowth occurred, which has resulted in the establishment of an androgen independent subline (PC-346I).
This unique set of transplantable human prostate tumor models offers the opportunity to study various biological aspects of human prostatic cancer during tumor progression.

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BASAL CELL-ENRICHMENT OF PRIMARY CELL CULTURE OF RAT PROSTATIC EPITHELIAL CELLS. Kenneth Y. Ilio, Jeffrey A. Nemeth, Chung Lee and John T. Grayhack, Department of Urology, Northwestern University Medical School, Chicago Illinois 60611 USA

The objective of this study was to identify the cells from rat prostatic epithelium which attach and proliferate in primary culture. Minced ventral prostate was dissociated by DNAse/collagenase digestion and suspended in RPMI 1640 containing 10% fetal bovine serum. When isolated single cells were held in suspension at 37°C for several hours, morphologically distinct secretory cells died as assessed by trypan blue staining. With the use of lectins, it was possible to further monitor the loss of specific populations in suspension. In tissue sections of rat prostatic epithelium, *Griffonia simplicifolia I* isolectin B_4 (GSI-B_4) specifically stained basal cells while *Glycine maximus* (SBA) was specific for secretory cells. Dissociated cells suspended in RPMI 1640 and allowed to plate for 24-48 hours increased the death of SBA-positive secretory cells while permitting the preferential attachment of stromal cells to the well surface. Lectin staining of floating live cells revealed a preponderance of GSI-B_4-positive cells. The surviving cells were replated in WAJC-404 medium supplemented with various factors including insulin (5 μg/ml), transferrin (5 μg/ml), EGF (10 ng/ml) and bovine pituitary extract (30 μg/ml). Epithelial colonies that formed and proliferated from these cells also stained positively for the GSI-B_4 marker and for cytokeratins specific for basal cells as assessed by immunocytochemical staining. These findings suggest that the epithelial cells that survived in suspension and proliferated in culture originated from basal cells of rat prostatic epithelium.

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DIFFERENT PATTERN OF METASTASIS: ORTHOTOPIC VERSUS INTRAVENOUS IMPLANTATION

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Shevrin et al (1988) reported bone metastases after intravenous injection of PC-3 cells while clamping the vena cava (i.v.+clamp). With the technique of orthotopic implantation we never found bone metastases in more than 150 animals. We have compared the pattern of metastasis of i.v.+clamp versus orthotopic tumor cell injection (o.t.).

We injected the human prostate cancer cell line PC-3N in five NMRI nu/nu mice per group either o.t. or i.v.+clamp. The animals were observed for 36 (o.t.) and 60 (i.v.+clamp) days. Prostate, preaortic lymph nodes, spine, liver and lungs were checked histologically.

5/5 animals of the o.t. group developed strong local tumor growth and 100% lymph node metastases. 2/5 lungs presented metastatic disease. After i.v.+clamp we found tumor growth in the lungs in 4/5 mice. 2/5 showed positive lymph nodes and osseous tumor growth.

Intravenous injection of tumor cells while clamping the vena cava leads to the deposition of tumor cell clots in the capillary region of the lung and spine with consecutive local tumor growth. This does not meet a regular in vivo metastatic process which is achieved by orthotopic implantation.

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INCREASED METASTATIC BEHAVIOR: IMPLANTATION INTO THE DORSAL PROSTATE IS SUPERIOR TO VENTRAL APPLICATION

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The mouse prostate consists of three parts, the ventral, lateral and dorsal lobes. Most prostate cancer experiments are done with the ventral prostate. In order the identify the best implantation site in causing metastatic disease we performed orthotopic implantation either in the ventral or the dorsal lobes of the prostate.

We injected $1x10^5$ cells of the human prostate cancer cell line PC3-125-1LN into the prostate of 7-9 weeks old NMRI nude mice (n=36). In order to get a time curve we sacrificed the mice 7-36 days after implantation. Tumorigenicity and presence of lymph node and organ metastases was checked macroscopically as well as by histological examination. Bisbenzimid staining was used to discriminate human from mouse cells.

Tumorigenicity was 100% in both groups. 16/19 (84%) dorsal implanted mice had positive nodes compared to 11/17 (65%). Development of lung metastases was increased in the dorsal group too (21% versus 6%). The first positive nodes were detected after 14 days compared to 21 days after ventral implantation.

We conclude that the dorsal part of the mouse prostate provides a better environment to cause metastatic disease. Orthotopic implantation into the dorsal lobes leads to earlier and increased rates of metastases in NMRI nude mice.

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3A Genetic basis of cancer

P 52

LOSS OF THE CYCLIN-DEPENDENT KINASE INHIBITOR GENES *P15* AND *P16* IN HUMAN BLADDER CANCER CELL LINES.

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Loss of cell cycle control through the structural inactivation of checkpoint genes is a potentially important process in carcinogenesis. The cyclin-dependent kinase inhibitor genes *p15* and *p16* are contiguous on chromosome 9p-21 at the locus of a putative tumour suppressor gene implicated in the early stages of bladder cancer. Fourteen well-characterised established human bladder cancer cell lines were screened by the polymerase chain reaction for homozygous loss of the cyclin-dependent kinase inhibitor genes *p15* and *p16*. In 50% of the bladder cell lines, there was homozygous deletion of both genes; in no case was there differential loss of either *p15* or *p16*. Cytogenetic analysis of the cell lines revealed karyotypes ranging from near diploid to near pentaploid with complex rearrangements of some chromosomes and a high prevalence of chromosome 9p rearrangements. However, all cell lines contained at least one cytogenetically normal 9p-21 region implying that cytogenetically undetectable molecular changes in 9p-21 contributed to the loss of heterozygosity in *p15/p16*-negative cell lines. Homozygous deletion is a mechanism by which both *p15* and *p16* are simultaneously inactivated and may have an important role in bladder carcinogenesis and/or the promotion of cell growth *in vitro*.

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P53 NUCLEAR OVEREXPRESSION AND PROLIFERATION IN BILHARZIAL AND NON BILHARZIAL URINARY BLADDER CARCINOMA

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Tissue expression and mutation patterns of P53 tumour suppressor gene have been shown to reflect the specific carcinogens involved, or the epidemiological background in some cancers. To elucidate the impact of bilharzial infection on the P53 gene expression and mutation in bladder tissues, 32 cases of urothelial cancer associated with bilharziasis obtained from Egypt, 28 cases of western european bladder cancer, 25 cases of bilharzial non malignant lesions, and 10 normal bladder were examined and compared as regard immunohistochemical reactivity to monoclonal antibodies againt P53 (PAb 1801) and monoclonal antibody MiB1 marker of proliferation. Quantitative evaluation of reactivity was done by determining the labelling index (LI) in each case counting 500 nuclei per field/3 fields per slide (400 x magnification).
There was no expression of P53 in normal bladder, but a definite positive staining in 7 of 25 non malignant bilharzial lesions (mean LI = 21%). This staining was associated with epithelial changes (hyperplasia and/or metaplasia). There was a marked LI difference between bilharzial and non bilharzial cancer for P53 (40.54 versus 26.37%) and MIB1 (34% versus 23%) and also when histologic type (squamous versus transitional), histological stage and grade were considered. These data suggest that proliferation and over expression of P53 are frequent events and that these phenomenons may precede neoplastic changes in bilharzial cancers.
These results provide further information concerning the role of P53 abnormalities in the initiation of urothelial transformation and during disease progression.

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EXPRESSION OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN(MRP) GENE IN UROTHELIAL CARCINOMAS: H. Kubo[1,2], T. Sumizawa[1], K. Koga[1,2], K. Nishiyama[2], Y. Takebayashi[1], Y. Chuman[1], S. Akiyama[1], Y. Ohi[2], From the Institute for Cancer Research[1] and Department of Urology[2], Faculty of Medicine, Kagoshima University, Kagoshima, Japan.

Intrinsic or acquired resistance of cancer cells to chemotherapeutic agents is one of the major obstacles to successful cancer treatment. P-glycoprotein(P-gp) encoded by *MDR1* gene is considered as one of mechanisms for intrinsic multidrug resistance(MDR). However, it has become clear that a variety of other mechanisms can also be responsible for MDR. In general, the expression level of P-gp in urothelial cancer is low, therefore we investigated that of Multidrug Resistance-associated Protein(MRP) ,which is a member of the ATP-binding cassette superfamily of membrane transporter proteins. Eleven renal pelvic and/or ureteral tumors, 33 bladder tumors, one metastatic lung cancer from ureter tumor, 7 noncancerous urothelia of transitional cell carcinoma(TCC) patients and one urothelium of renal cell carcinoma(RCC) patient were investigated regarding the level of *MRP*mRNA by slot blot analysis. MRP was detected using immunohistochemistry with a polyclonal antibody. We considered the *MRP*mRNA expression levels in samples positive when those indicate more than 2-fold intensity compared with that of drug-sensitive KB cell line. Five of 11 renal pelvic and/or ureter tumors(45.5%),17 of 33 bladder tumors(51.5%), and 4 of 7 noncancerous urothelia of TCC patients(57.1%) were positive. One metastatic lung carcinoma from ureter tumor and one urothelium of RCC patient indicated low expression levels of *MRP*mRNA. High grade tumors indicated significantly low expression levels of *MRP*mRNA, and there was no significant difference between primary tumors and recurrent tumors. Immunohistochemically, MRP was mainly located on the plasma membrane, but also detected on cytoplasm of cancer cells. MRP has ability to be one of mechanisms involved in the intrinsic drug resistance in urothelial cancer.

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DOMINANCE OF CISPLATIN RESISTANCE AND COMPLEMENTATION OF CISPLATIN SENSITIVITY IN HUMAN CANCER CELL HYBRIDS. John R Masters, Xianghong Wang and Majid Hafezparast. University College London, Institute of Urology and Nephrology, 3rd Floor, 67 Riding House Street, London W1P 7PN, UK

Metastatic testis tumours, in contrast to most other types of cancer in adults, are cured in over 80% of men using cisplatin-based combination chemotherapy. The molecular basis for the differential sensitivity of testis tumours to chemotherapy is unknown. We have shown that testis tumour cells retain their sensitivity to DNA-damaging agents *in vitro*, and the degree of sensitivity is similar to that of cells derived from DNA repair disorders. Because some of the genes responsible for DNA repair disorders have been identified by complementation analysis and expression cloning, we determined if a similar approach could be used to identify the genes responsible for the sensitivity of testis tumours to cisplatin. We fused 3 testis (cisplatin-sensitive) and 3 bladder (cisplatin-resistant) cell lines with D98^{or}C1 (cisplatin-sensitive). D98^{or}C1 was used because it is HAT-sensitive and ouabain-resistant and therefore hybrids with most other cell types can be isolated using a single-step selection. The origin of the hybrids was confirmed by karyotyping and PCR analysis of VNTRs. 6 hybrids of D98^{or}C1 with 3 testis tumour cell lines were uniformly more resistant than the parental cells. This indicates that D98 and the testis tumour cells have different mechanisms controlling cisplatin sensitivity, and that these mechanisms were complemented by the fusion. In 6 hybrids of D98^{or}C1 with 3 bladder cancer cell lines, cisplatin resistance was partially dominant in every case. In keeping with the analogy with the DNA repair disorders, this indicates that genes controlling cisplatin sensitivity might be identified using expression cloning.

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RNA-AP-PCR AS A METHOD TO DETECT LOW ABUNDANCE RNA IN BLADDER CANCER CELL LINES.
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Object: Differential gene expression plays an important role in differentiation and growth control in normal and tumor cells. We considered the investigation of differential gene expression a necessary step to further understand differences in growth pattern under exposure to EGF in two bladder cancer cell lines. These cell lines showed different differentiation, but identical num-bers of an intact and equally phosphorylated receptor.
Methods: RNA was prepared from two cell lines (RT4 and J82) grown with-out and with EGF. In order to identify differential gene expression a PCR-based RNA fingerprint technique was used, the RNA-Arbitrarily Primed PCR (RAP). Here a single arbitrary primer is used for first strand synthesis and subsequent synthesis of cDNA-PCR fragments. An approach with a set of nested primers was applied, which should favorize the amplification of low abundant and differentially expressed populations of RNA species. After gel electrophoresis in a denaturing polyacrylamide gel, PCR fragments were visualized by a non-radioactive silver staining method. Polymorphisms in the band patterns were excised from dried silver stained gels, reamplified, cloned, and finally sequenced on a sequencer (Applied Biosystems).
Results: Differences in band pattern could be visualized in J82 cells in a reproducible pattern and consisted of decreased as well as increased staining. No differences were seen in the cell line RT4 that was not growth stimulated under EGF. 7 enhanced bands of EGF-treated and untreated J82 cells were excised after amplification and 3 have been cloned and sequenced. One sequence revealed a 100% match with that of human fibroblast tropomyosin.
Conclusion: The practicability of the RAP technique could be shown in this preliminary experiment, extending from RNA to possible gene canditates, indicating a new sensitive technique to determine low abundance genes.
-- Work was supported by the: Graduiertenkolleg: "Therapieforschung Onkologie" of the DFG.

P 57

THE ROLE OF E-CADHERIN MUTATIONS IN BLADDER AND PROSTATE CANCER; is E-cadherin a classical suppressor gene ?

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E-cadherin is an invasion suppressor gene, mapped to chromosomal location 16q21. The observation that this locus was frequently involved in Loss of Heterozygosity (LOH) in various cancers including prostate-, bladder-, hepatocellular- and breast carcinoma, made it tempting to speculate that E-cadherin could in fact be inactivated as a 'classical' tumor suppressor gene, i.e. loss of one allele and mutation of the other.

In order to test this hypothesis *mutation analysis* of 28 pathologically well defined human prostate and bladder cancer specimens, as well as, 15 cell lines (4 prostate, 11 bladder), was performed using single strand conformation polymophism PCR (SSCP-PCR) analysis. Since we recently cloned the full length human E-cadherin cDNA (Bussemakers, Biochem. Biophys. Res. Commun. *203*, 1284-1290) this study was now feasible and 9 primer sets covering the entire cDNA sequence were designed. SSCP analysis was done and in case a shift in band motility was observed, the band was excised and nucleotide sequence analysis was performed. A frequent polymorphism at codon 2170 (GCC->GCT) was revealed. In the specimens that were derived from human cancers one mutation that would lead to a change in the E-cadherin polypeptide sequence was found, i.e. 1868 (exon12) GCC-ACC (Ala-Thr). In one prostate cancer cell line (DU 145) the previously reported skipping of exons 9 (Δ 1231-1415) was found. In the LNCaP cell line a mutation was found in a portion of the precursor peptide that is cleaved in the mature E-cadherin. Interestingly, however, the wildtype allele was in all cases also present, hence the biological significance of these mutations is not clear. In the primary cancer specimen contamination with non malignant cells would be an acceptable explanation.

Even though it remains possible that more mutations are found in the intronic splice sequences, E-cadherin mutations do not seem to be a frequent event in prostate and bladder cancer development. Hence, the *predominant mechanism* of E-cadherin inactivation cannot be explained by the 'classical' Knudson hypothesis.

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THE ROLE OF ABERRANT CADHERIN-CATENIN MEDIATED CELL ADHESION IN PROSTATE CANCER

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The gene coding for the cell adhesion molecule e-cadherin is on a chromosomal arm that is frequently mutated in prostate cancer. E-cadherin maintains the normal differentiated phenotype in epithelial cells and this function is in part mediated by interaction with alpha-catenin which links e-cadherin to the cell cytoskeleton. Loss of normal cadherin function is associated with an invasive phenotype in cell culture studies.

We have analysed the expression of e-cadherin and alpha-catenin in prostate cancer to assess the relationship of abnormal expression to stage, grade and survival. Ten benign and 98 malignant prostate specimens obtained at TUR-P during 1984-1991 were analysed.

In benign prostatic epithelium both e-cadherin and α-catenin were expressed uniformly in a clear membranous pattern. The expression of e-cadherin was abnormal in 56% of cancer specimens and the expression of α-catenin was abnormal in 42%. Abnormal expression of both molecules was significantly correlated with Gleason score (e-cad;p=0.0001 and α-cat;p=0.0001), and the ratio of resection chippings infiltrated by tumour (e-cad;p=0.0001 and α-cat;p=0.0005). Abnormal e-cadherin expression was associated with the extent of disease on the initial bone scan (p=0.0001). Univariate analysis showed a lower survival rate for patients with abnormal e-cadherin expression (log rank test;p=0.0003) and for patients with abnormal α-catenin expression (log rank test;p=0.031). In a proportional hazards model abnormal e-cadherin expression was independent of histological grade as an indicator of survival, but not independent of bone scan score.

This study indicates that aberrant cell adhesion is involved in the progression of prostate cancer, and that analysis of e-cadherin expression may be clinically useful.

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DETECTION OF CHROMOSOME 16q DELETION IN HUMAN PROSTATE ADENOCARCINOMA USING FLUORESCENCE *IN SITU* HYBRIDIZATION

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Deletions of chromosome 16q are frequently found in both primary and metastatic human prostatic carcinomas. It seems that a tumor suppressor gene candidate which plays a role in the pathogenesis of prostate cancer might be localised on 16q. E-cadherin is a cell adhesion molecule which is as an inhibitor of tumor invasion, therefore abnormal expression of E-cadherin is significantly correlated to progression and differentiation of prostate carcinoma. The E-cadherin gene is mapped to human chromosome 16q. Studies of both genotype and phenotype disorders are important for the assessment of malignant potential of prostatic carcinoma.

To investigate the roles of chromosome 16q deletion and E-cadherin expression in human prostate adenocarcinoma, 62 primary and metastatic tumor specimens and 12 benign specimens were estimated using dual labeling fluorescence *in situ* hybridization techniques. Probes for centromere 16 and 16q 22q-qter were simultaneously hybridized to the interphase cells. Chromosome 16q deletions were found in sixteen of 39 localized tumors and 16 of 23 advanced malignancy (P=0.03). The loss of 16q was highly correlated to tumor grade (P<0.001) but weakly to tumor stage (p=0.545). The prostate tumor specimens from 57 of 62 patients were immunohistochemically observed for E-cadherin expression. Twenty-four of 36 localized cancers were positively stained, only six of 21 in lymphatic or bone metastasis tumors (p<0.01). We further compared the percentage of 16q deletion to aberrant E-cadherin expression, hence both parameters were stronger associated (p<0.001, r=0.81). These results suggest that deletion of chromosome 16q is one of the steps resulting in abolition of E-cadherin expression which might be associated with loss of tumor suppressor function in human prostatic carcinoma.

P 60

STRUCTURAL ANALYSIS OF THE DNA POLYMERASE B AND CLUSTERIN GENES ON CHROMOSOME 8P IN PROSTATE CANCER.

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It is generally accepted that chromosomal abnormalities in tumors correlate with the activation of oncogenes and inactivation of tumor suppressor genes (TSGs). One of the most frequent abnormalities found in prostate cancer is loss of the complete, or part of the short arm of chromosome 8, indicating the localization of one or more TSGs in this region. We analyzed the structure of the Clusterin gene (CLI) and the DNA Polymerase β gene, in normal cells and in prostate tumors. Clusterin: The genetic position of CLI was finemapped. CLI could be physically linked on several YACs to the markers D8S137 and D8S131, and the novel polymorphic marker D8S242 on chromosome 8p12-p21. Structural analysis of the CLI gene by PCR-SSCP in DNA from 43 locally progressive prostate tumors did not reveal the presence of mutations. However, two SSCP polymorphisms were found, which could be used as markers for LOH (loss of heterozygosity) analysis of prostate tumor DNAs. DNA Polymerase β: The POLB gene is located on chromosome 8p11, which is less frequently deleted in prostate cancer than 8p12-p21. PCR-SSCP analysis of the POLB gene in DNA from the same 43 prostate cancer DNA samples showed the presence of one mutation (a T to A substitution in intron 11). Our findings argue against a major role of CLI and POLB in prostate cancer.

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ANALYSIS OF CHROMOSOMAL INSTABILITY IN PROSTATIC CARCINOMA USING FLUORESCENT MICROSATELLITES TO MAP PREFERENTIALLY ALTERED LOCI.

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Several independent studies have demonstrated preferential sites of genetic lesions in prostate cancers, and localised them to chromosomes 7,8,10,16, 17 and 18. We report here studies of allelic instability at 3 of these loci (Ch8,10 and 16) using highly polymorphic microsatellite markers on a pool of 46 patients with matched tumour and constitutive (Blood lymphocyte derived) DNA. The combination of fluorescent labelling with accurate allele sizing and quantification offered by the Applied Biosystems DNA sequencer, allows a precise measurement of changes in allele content, although amplifications are difficult to determine, in contrast to Fluorescence in situ hybridization (FISH) or Comparative genomic hybridization (CGH). However the extra mapping precision offered by the microsatellites should allow rapid identification of chromosomal regions for more precise genetic analysis, and gene isolation.
The eleven primer pairs used for chromosome 10 have indicated that allelic instability, centred on 10q23 is present in about 60% of well-moderately differentiated tumours, whereas poorly differentiated tumours show a surprisingly lower frequency, of change at 10q23, but an increase in allelic instability at 10qter, which may be linked to telomerase activity changes in carcinomas.
On chromosome 16, where cytogenetic and Southern blotting analyses indicated losses at 16q22.1-qter, 25-30% of patient DNA's contain allelic changes on 16q, rising to more than 50% changes within 16q22.1. Correlation of instability with tumour grade suggests that loss of this locus is an "early" event.
On chromosome 8, we have focused our attention on the region 8p22-pter, since recent data using CGH and FISH have shown that this region is lost in a significantly high proportion of prostatic carcinomas. Recent acquisition of fluorescently labelled genome mapping primer pairs from the Human Genome Project should enable us to finely map this region in our tumour set.
The allelic instability will be compared to tumour grade and rate of tumour progression. The combinatorial approach should enable us to both finely map important regions of chromosomal instability, and to determine the relative significance and the degree of interaction between the various loci in the progression of prostate cancers towards malignancy.

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EXPRESSION OF MEMBRANE-TYPE MATRIX METALLOPROTEINASE IN PROSTATE CANCER SPECIMENS.

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Recently, a new matrix metalloproteinase with a potential transmembrane domain has been cloned (Sato *et al.* Nature **370**, 61-65; 1994). This membrane-type matrix metalloproteinase (MT-MMP) has been shown to induce specific activation of progelatinase A and enhance cellular invasion *in vitro*. Studies in lung tumour samples showed increased expression compared with normal tissue. We have assessed MT-MMP expression in prostate cancer obtained at operation. Cryodissection was used to separate benign and malignant tissue from 14 patients. mRNA was extracted and cDNA synthesised using standard laboratory techniques. Expression was assessed using semi-quantitative reverse-transcriptase PCR with GAPDH as an internal control and MT-MMP primers designed to cross an intron. Products were separated using gel electrophoresis, visualised with ethidium bromide and quantified using computerised densitometry. MT-MMP was expressed by all of the benign tissue. Increased MT-MMP expression compared with benign controls was observed in 64% (9/14) of tumours. Increased expression was associated with bone metastases in 8/9 cases (89%). One case of poorly differentiated metastatic cancer showed no MT-MMP expression.
Prostate cancer was associated with an increased MT-MMP expression. There was a strong correlation between increased expression and the presence of bone metastases. Further investigation into the role of MT-MMP in prostate cancer is indicated.

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CELL TYPE-SPECIFIC MECHANISMS MAY GOVERN THE MORTALITY STAGES 1 AND 2 OF HUMAN PROSTATIC EPITHELIAL AND FIBROBLASTIC CELL IMMORTALISATION: IMPLICATION OF SV40 AND P53.

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Together with invasion and metastasis, immortalisation is thought to be an essential stage in neoplastic progression. To determine the steps involved in the full immortalisation process, we have studied the structure and expression of the tumour suppressor p53 during SV40 large T regulated inactivation or bypass of the mortality stage 1 and 2 (M1/M2) in human prostatic epithelial and fibroblastic cells. The p53 gene was not mutated in any human prostatic cells, either prior to or after immortalisation, and p53 protein was in the wild-type/suppressor form, complexed with SV40 large T, as determined by immuno-precipitation and immuno-blotting. SV40 transfected epithelial and fibroblastic cells overcame M1, leading to an extension of their lifespan up to 7 and 30 passages, respectively. However, none of the fibroblastic strains were able to overcome the crisis or M2 checkpoint, while three human prostatic epithelial cell lines emerged and became immortal: PNT2, PNT1A and PNT1B. This data suggests that a single event mediated by SV40 large T may be sufficient to bypass M1, and that the stabilisation of wild-type p53 restraining its DNA binding and transactivation, may be critical. However, the absence of post-crisis fibroblasts (0/14) proposes that different mechanisms may control the acquisition of an immortal phenotype in the different cell types within human prostate. These results are discussed in view of the extremely low incidence of sarcomas versus the >95% occurrence of adenocarcinomas in many human cancers, including prostate.

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CYTOGENETIC ANALYSIS OF HPV TRANSFORMED EPITHELIAL HUMAN PROSTATE CELL LINES. A NEW MODEL FOR PROSTATE CANCER RESEARCH.

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In tumor the findings of characteristic cytogenetic aberrations may shed some light on the mechanisms of oncogenesis. Several chromosome abnormalities have been associated with prostate adenocarcinoma, however still without a definite common pattern. As a model for the study of prostate carcinogenesis, two new epithelial cell lines have been established using human papillomavirus (HPV) type 18 DNA for immortalization. We report here on sequential cytogenetic analysis of these two cell lines i.e. PZ-HPV-7 and CA-HPV-10 using chromosome banding and fluorescence in situ hybridization (FISH) with painting probes.

PZ-HPV-7 cell line originates from a normal diploid epithelial human prostate cell strain and was studied at passage 15, 38 and 99. PZ-HPV-7 progressed from an initial diploid chromosome number to a modal number of 106 chromosomes, range 103-108. Analysis showed 3 to 5 copies of all chromosomes except X and Y (2 copies each) and chromosomes 5 and 20 (7-8 copies each). There are only few structural changes: 3p- (2x), 3q- (1x) and a third marker possibly a der (16p;12q). The CA-HPV-10 parental cell strain originating from a prostatic adenocarcinoma specimen of Gleason Grade 4/4 showed several abnormalities including an extra Y chromosome and double minutes (DM). At passage 89 the karyotype of CA-HPV-10 was mainly hypertriploid (modal number 72, range 69-75) with a few cells hyperhexaploid and hyper-12 ploid. The karyotype showed consistent markers: del(1)(q12q31) x2, add (9)(p21 or 22) most probably a large Homogeneously Staining Region (HSR) (x2) and a der (11)t(4;11). At passage 14 and 38 the karyotypes varied from hypodiploid to hypotetraploid with a slowly arising mode in the hypertriploid range. The karyotype was unstable with mainly telomeric associations, di- and tricentric chromosomes, isochromosomes and decreasing number of cells exhibiting DM. The latter in late passage were probably replaced by a large HSR on 9p+ marker. Efforts to identify the HSR using flow-sorting of characteristic markers and DOP-PCR amplification as a probe for reverse painting have so far not been successful.

In conclusion, both immortalized cell lines PZ-HPV-7 and CA-HPV-10 showed cytogenetic modifications. Appearently, gene amplification present in the adenocarcinoma strains in the form of DM has been retained as HSR. The latter remain to be identified. These two cell lines constitute an in vitro model that we intend to exploit in our analysis of the mechanisms involved in human prostate carcinogenesis.

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ROLE OF CALCIUM IN RADIATION-INDUCED APOPTOSIS OF PC-3 PROSTATIC CANCER CELL LINE.

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Apoptosis or Active Cell Death (ACD) is a widespread event regulating normal tissular growth. DNA fragmentation is a keystep in ACD, and usually results from activation of a Ca^{2+} /Mg^{2+} -dependant endonuclease which selectively hydrolyses DNA at sites located between nucleosomal units. This activation is thought to be triggered by a sustained increase in cytosolic free Ca^{2+}, an early event in the ACD cascade. Free Ca^{2+} is considered to act primarily as ACD second messenger. The existence of a similar apoptosis-associated rise in calcium during prostatic ACD is based on observation that drugs which increase free cytosolic Ca^{2+} (Ionomycine or thapsigargine) induce an ACD-like cascade. In prostatic tissue, ACD usually results from androgen withdrawal, but can be achieved in androgen-insensitive prostate cancer cell line PC-3 by radiation.

Study Aims

1. To measure changes in intracellular free Ca^{2+} in apoptosis of irradiated PC-3 cells using a direct measurement of a fluorescent Ca^{2+} dye(FURA-PE3).
2. To evaluate the effect of changes in intracellular Ca^{2+} buffering with diffusible BAPTA or EDTA on ACD quantified as the cellular population exhibiting DNA fragmentation following irradiation (1000 rads).

Results

1. No change in intracellular free Ca^{2+} was observed in PC-3 cell line previously incubated with 10 µM FURA-PE3 and irradiated thereafter. Emission of fluorescence (240-280 nm; 180 min acquisition) was measured over a 12 h period, during active DNA fragmentation was triggered.
2. Buffering intracellular Ca^{2+} with BAPTA-AM or EDTA-AM reduced the magnitude of post-irradiation DNA fragmentation. Percent of apoptotic cells assessed by DNA fragmentation detection using *in situ End labeling* technique decrease from 74% to 35%.

Conclusion

Free cytosolic Ca2+ is unlikely to be sole second messenger during radiation induced apoptosis of prostatic cancer cell-line PC-3. Preventing apoptosis with intracellular Ca2+ buffer could result from endonuclease inhibition.

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THE LNCaP MODEL OF HUMAN PROSTATE CANCER: BIOLOGICAL BEHAVIORS AND CYTOGENETIC CHANGES. [1]George N. Thalmann, [1]Robert A. Sikes, [1]Shi-Ming Chang, [2]Sen Pathak, [1]Andrew C. von Eschenbach, and [1]Leland W.K. Chung, Departments of [1]Urology and [2]Cell Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

A series of androgen-independent (AI) human prostate cancer cell lines were derived from androgen-dependent (AD) LNCaP cells. Androgen independence is defined as the ability of prostate cancer cells to grow in the castrated host. One of the sublines, C4-2 was found to be androgen-independent, and acquired a high tumorigenic and metastatic potential. We subsequently demonstrated that this cell line has the propensity to metastasize to the bone.

Clinically the lethal phenotypes of human prostate cancer progression are characterized by their androgen-independence and their propensity to form osseous metastases. In this communication we characterized further this animal model of human prostate cancer metastasis. Our results show: 1. that the LNCaP model shares remarkable similarities with human prostate cancer and a comparable metastatic pattern from the primary tumor to the lymph node to the axial skeleton. The predominant phenotype of bone metastasis is osteoblastic. 2. Cytogenetic characterization indicates similarities between human prostate cancer and the LNCaP model. For example, both AD as well as AI LNCaP sublines had 8p allelic loss which has been found to occur frequently in human prostate cancer. All cell lines share common marker chromosomes with the parental LNCaP cell line. Both AD and AI cell lines, however, exhibited many chromosomal gains and losses that are typical of human prostate cancer. 3. Additional LNCaP sublines derived from C4-2 bone metastases maintained osseous metastatic potential. Furthermore, they acquired the potential to grow when injected s.c. 4. In this study, we observed that osseous metastasis in castrated hosts as evidenced by the development of paraplegia in tumor-bearing animals occurs 2-3 times more frequent than in the intact male host. 5. In comparison to AD cell lines, AI cell lines have a 2-3 fold higher invasiveness and anchorage-independent growth potential. 6. All cell lines expressed PSA in vitro and the bone metastasis-derived cell lines expressed various amounts in vivo. All cell lines express mRNA for PSA and human androgen receptor.

In summary, we have established the first human metastatic prostate cancer model in which we characterized further the biologic, cytogenetic, biochemical, and molecular properties of the androgen-independent and osseous metastatic cell sublines.

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EXPRESSION OF GLUTATHIONE-S-TRANSFERASE IN BENIGN AND MALIGNANT PROSTATE. Jeffrey N. Kocurek, Yogesh Awasthi, Randy Mifflin, Judy Aronson, Adekunle Adekosan, and Durwood Neal, Jr. University of Texas Medical Branch, Galveston, Texas, USA.

The glutathione-S-transferases are a group of isoenzymes which may be involved in carcinogenesis. They act by inactivating electrophilic carcinogens and by removing superoxide radicals. We studied the expression of one glutathione-S-transferase (GSTP-1) in prostatic tissue, as it has been reported that the gene is methylated in prostate cancer, and thus not expressed.

The presence of GSTP-1 mRNA was assayed by Northern blot analysis in fresh samples of benign and malignant prostate tissue, as well as in the LNCAP cell culture line, using GSTP-1 cDNA as a probe. Furthermore, paraffin block tissue specimens of benign and malignant prostate tumors were examined using immunohistochemistry (IHC) with a monoclonal antibody to GSTP-1 (Oncor, Inc.).

Malignant prostatic tissue was negative for GSTP-1 by IHC. The benign glandular epithelium was positive in all cases. Interestingly, the tissue that showed prostatic intraepithelial neoplasia (PIN) had variable staining using IHC, with low grade PIN showing some reactivity, and high grade being negative, similar to frank adenocarcinoma. Northern blot showed some positivity for GSTP-1, but the tumors had some benign tissue present as well. Only the LNCAP cell line and one tissue sample were entirely negative.

This data seems to indicate that hypoexpression of GSTP-1 synthesis is a common event in the pathogenesis of prostate cancer and may even have some role as an initiating event. Further studies to examine this are underway in our laboratory.

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ALLELIC IMBALANCE AS PROGNOSTIC INDICATORS IN HUMAN RENAL CELL CARCINOMA.

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The best prognostic factors for renal cell carcinoma (RCC) are stage and grade. Factors like cell type, histological pattern, age at diagnosis, tumor size, have been implicated to affect survival to some extent. To determine the best utilisation of new treatment modalities there is a demand to establish good reliable prognostic factors.

We have examined the prognostic value of molecular genetic factors as allelic imbalance for different chromosomal loci in tumor tissue specimens from 56 patients with localised or metastatic RCC. Twenty-six cases were analysed for loci on all chromosomal arms except for the short arms of the acrocentric chromosomes. In the additional 30 cases only the chromosomes showing the highest frequency of allelic imbalance were analysed (3p, 5q, 10q, 14q, 17p and 18q). Tumor and normal adjacent tissue DNA were processed for Southern blot. Allelic imbalance was examined with the use of restriction fragment length polymorphism (RFLP) markers. The clinical follow up time were at lest five years. The data were also compared with clinico-pathological classifications. Statistical analyses as Kaplan-Mayer, univariat and multivariat analyses by Cox's proportional hazards model were performed.

The highest frequency of allelic imbalances which were regarded as loss of genetic material was found on 3p (74%) followed by 10q, 14q and 18q (>30%). Allelic imbalance which could be regarded as gain of chromosomal material was mainly found on 5q (25%) and 17q (<10%). Statistical analyses showed an increased risk for dying in the tumor disease in cases with 3p deletions and 5q gain.

These data indicates that the loss of 3p and gain of 5q in the tumors are linked to a poor prognosis. Our result suggest attention to these parameters and further studies will clarify their involvement in the pathogenesis of RCC.

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DETECTION OF ALTERATIONS IN THE *APC*- AND *DEL-27*-GENE IN RENAL CELL CARCINOMAS BY POLYMERASE CHAIN REACTION

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Renal cell carcinoma (RCC) is associated with loss of heterozygosity (LOH) on human chromosome 5q. Here the involvement of the tumour suppressor genes *APC*- and *DEL-27*, both located on human chromosome 5q, is assessed.
LOH can be detected by the polymerase chain reaction (PCR) provided that tumour cells are meticulously and verifiably isolated from contaminating non-tumour cells. Non-tumour cells can give positive PCR results and thus obscure the detection of deletions. Using a microdissection technique that allows for accurate and verifiable excision of tumour cell islets for subsequent PCR analysis (Cancer Res 54:1772, 1994), LOH was demonstrated by restriction fragment lenght polymorphism (RFLP, *APC*) or single strand conformation polymorphism (SSCP, *DEL-27*).
Of 24 tumours investigated 11 were heterozygous, i.e. informative, for each locus. Of these six (55%, *APC*) and two, respectively (18%, *DEL-27*) showed LOH. LOH was not detectable if the microdissected tumour cell islets contained more than 20% non tumour cells.
We conclude: 1. Microdissection of RCC and PCR is a feasible technique to detect LOH in small tumour biopsies. 2. The tumour suppressor gene *APC* is involved in the development of RCC. 3. The role of the newly identified putative tumour suppressor gene *DEL-27*, shown to play a role in lung carcinogenesis, cannot yet be assessed in RCC due to the small sample size.

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HUMAN WILMS' TUMOR MODELS IN NUDE MICE: PROPERTIES, IN VITRO GROWTH AND EXPRESSION OF ACTIVIN RECEPTORS. Gert J. van Steenbrugge, Ton A.P. Boijmans, Jan C. den Hollander, Frank H. de Jong, and Rien J.M. Nijman. Depts. Urology and Pathology, Erasmus University; Dept. Pediatric Urology, Sophia Children's Hospital; Rotterdam; The Netherlands.

In spite of considerably improved results in the treatment of children with nephroblastoma (Wilms' tumors), there is still a need for fundamental research into factors involved in the regulation of growth, differentiation and clinical progression of human Wilms' tumor. In an attempt to develop suitable models systems, fragments of surgically removed primary Wilms' tumors and some metastatic lesions were heterotransplanted in NMRI athymic nude mice. This resulted in six positive takes, yielding permanent tumor lines (WT-7, WT-9, WT-11, WT-15, WT-15-LN and WT-16), i.e. tumors which were shown to be serially transplantable. Staining with bisbenzimide, for all models revealed a pattern consistent with human tumor tissue. All six permanent WT lines contained blastemal tissue, and some of the tumors (WT-7-cystic, WT-9, WT-11 and WT-15LN) also included (immature) mesenchymal tissue with variable degrees of epithelial (tubular) differentiation. More specifically, these two components were identified by immunohistochemical staining of tissue sections with vimentin and cytokeratin antibodies, respectively. Recent attempts to bring xenograft-derived tumor tissue into culture resulted in one permanent cell line so far. After 10 passages, this line (designated WT-15LN-C), which originated from a collagenase digest of WT-15LN tumor tissue, was demonstrated to consist of human cells. The WT-15LN-C line could permanently be propagated in serum-free (Ultra-MDCK) medium, its characterization is in progress.

Activins are glycoproteins that are important not only as regulators of follicle stimulating hormone secretion but also as mediators of growth control and cell differentiation in several normal and malignant tissues. The expression of activin receptor-mRNA was studied in the six permanent Wilms' tumor xenografts by an RNAse protection assay. Activin receptor-II (ActRIIA and ActRIIB) mRNAs were differentially expressed in all tumor models. Expression of ActRIIA appeared to correlate positively with the size of the mesenchymal component of the tumor, whereas in the models which were largely composed of blastemal tissue the expression of ActRIIB mRNA was relatively high. It is concluded that the described *in vivo* xenografts tumor lines are representative models for clinical nephroblastoma. The presence of activin receptors in these models suggests that activins are implicated in growth and pathogenesis of Wilms' tumor. For such studies the availability of the WT-15LN-C *in vitro* cell line is of particular interest.

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IDENTIFICATION OF NOVEL CADHERINS IN THE MOUSE TESTIS

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The molecular nature of the cell adhesion systems essential for the maintenance of the seminiferous epithelium remains relatively uncharacterized. Cadherins are a family of integral membrane glycoproteins which mediate cell adhesion by specific homophilic interactions. We have employed a polymerase chain reaction (PCR) study to identify cadherins expressed in the mouse testis. Oligonucleotides encoding amino acid sequences that are conserved among all of the known cadherins were used as primers in PCR, with cDNA preparations of adult, 7-day, and newborn mice testes used as templates. The PCR products of the expected sizes were subcloned into a plasmid vector and sequenced. Based upon the nucleotide sequences of these PCR products, we have identified several cadherins that are expressed in the mouse testis. Epithelial cadherin (E-cad), neural cadherin (N-cad), osteoblast cadherin (OB-cad), and one novel cadherin, designated as SM-9, were present in the adult mouse. In the 7-day animal we found E-cad, N-cad, placental cadherin (P-cad), OB-cad, and SM-9. In newborn mice we have identified E-cad, OB-cad, kidney cadherin (K-cad), SM-9, and an additional novel cadherin, designated as SM-10. We are currently determining the full-length nucleotide sequence of SM-9 and SM-10 cadherins as a first step to gain insight into their biological functions. We believe that these molecules will be found play important roles in mediating cellular interactions within the testis.

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TRANSFECTION OF PROSTATE SPECIFIC AND TISSUE TYPE TRANSGLUTAMINASE cDNAs TO COS AND LNCaP CELLS. Nicole S. Verkaik[1], Hendrikus J. Dubbink[1], Jan Trapman[2], Fritz H. Schröder[1] and Johannes C. Romijn[1]. Department of Urology[1] and Pathology[2], Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Transglutaminases (TGases) are calcium-dependent enzymes that are responsible for irreversible post-translational modifications of proteins. They catalyze the acyl transfer reaction between peptide-bound glutamine residues and primary amine groups, which results in the formation of protein-protein or protein-polyamine cross-links. TGases play a role in a number of different processes that are associated with crosslinking and polymerization events. Examples are blood clotting, terminal differentiation of the epidermis and apoptotic boby formation. In the human prostate, two TGases may be present: tissue type TGase (TG_C) and prostate-specific TGase (TG_P). Although the physiological functions of both TGases are not yet entirely clear, TG_C is believed to have a role in growth regulation and apoptosis, whereas TG_P might control semen coagulation and suppress sperm cell antigenicity.
To elucidate their properties and biological function in human prostate, we performed transfection studies with cDNAs of both TGases. Full length cDNA of TG_C was obtained from Dr. P. Davies (University of Texas, Houston). TG_P was recently cloned in our laboratory. Expression of TG_P appears to be restricted to prostatic cells and is regulated by androgens. For the present study, we obtained the entire open reading frame by ligation of a 0.2 kb EcoRI-EcoRI fragment to a 2.7 kb EcoRI-HpaI fragment and cloned this construct into the eukaryotic expression vector pcDNA3. In parallel, TG_C was also subcloned into the same vector. COS cells were transiently transfected with both constructs by the calcium-phosphate method and TG expression was analyzed by Nothern blot analysis. Control (untransfected) COS cells had a low expression of TG_C and did not have any TG_P. mRNAs with a length of approximately 3.3 kb were found after transfection with TG_P cDNA, whereas TG_C constructs yielded a slightly larger mRNA of about 3.5 kb. With each TGase, six neomycin resistant clones were obtained and used for further characterization. Recently, stable transfectants of the human prostate cancer cell line LNCaP have been made as well. Since the expression of TG_P and TG_C in untransfected LNCaP cells was not detectable, the transfected cell clones will be suitable models to study the effects both types of TGase on the biological behavior of prostate cancer cells.

3 B Mechanisms of endocrine growth control

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ACTIVIN AND ACTIVIN RECEPTORS IN HUMAN PROSTATIC CARCINOMA. R.H.N. van Schaik[1], C.D.J. Wierikx[1], M.A. Timmerman[1], M.H.A. Oomen[2], G.J. van Steenbrugge[2] and F.H. de Jong[1]. *Departments of [1]Endocrinology & Reproduction and [2]Urology, Erasmus University Rotterdam, The Netherlands.*

The possible involvement of the growth and differentiation factor activin (member of the TGFß-superfamily) in prostate tumor progression was studied. The presence of inhibin ß-subunits (necessary for activin synthesis) and activin receptor transcripts was determined by RNase protection assays in primary human prostate cancers, prostatic carcinoma xenografts and in androgen-dependent (FGC) and independent (R and LNO) sublines of the human prostatic in vitro cell line LNCaP.

Primary human prostate tumors expressed activin receptor transcripts, indicating that these tumors may be responsive to activin. Upon analysis of eight human prostatic tumor xenografts, transcripts for activin receptor type IIA (ActRIIA) and type IIB (ActRIIB) were found in all samples. Apart from activin receptor mRNAs, also inhibin ß-subunit transcripts could be demonstrated. Inhibin ßB-subunit mRNA expression levels were positively correlated with androgen-dependence and tumor differentiation status, with an average of 6.6 ± 1.0 (arbitrary units relative to actin, ± s.e.m.; n=3) in the differentiated tumors against 0.9 ± 0.3 (n=4) in the undifferentiated tissues. In all three human prostatic tumor LNCaP cell lines ActRIIA transcript could be detected. Activin was shown to inhibit cell growth in the R-subline. In order to investigate a possible autocrine effect, inhibin ß-subunit transcripts were determined in the three LNCaP-sublines. In FGC and LNO inhibin ßB-subunit mRNA was present, whereas no transcript could be detected in the R-line. Growth of the LNO cell line in DCC-treated medium showed an induction of inhibin ßA-subunit expression and an increase in inhibin ßB-subunit mRNA levels when compared to growth in FCS. These effects were neutralized by growth of LNO in androgen supplemented DCC-treated medium.

In conclusion, these results sustain the hypothesis that activin can play a role in prostate tumor cell growth and differentiation.

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ANDROGEN AND GLUCOCORTICOID REGULATED PROCESSES IN AN ANDROGEN RECEPTOR AND GLUCOCORTICOID RECEPTOR POSITIVE LNCAP SUBLINE.

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Androgens are well known stimulators of specific gene expression in the LNCaP prostate cell line. In addition, it has been well established that androgens stimulate proliferation of LNCaP cells. To study in more detail the specificity of androgen regulated processes, LNCaP cells, which originally lack the glucocorticoid receptor (GR), were stably transfected with a GR expression plasmid. One of the resulting sublines, denoted 1F5, was found to contain approx. 3.10^4 copies of the androgen receptor (AR) and 10^5 copies of the GR. 1F5 cell proliferation could be stimulated by androgens, but not by glucocorticoids. In contrast, expression of prostate specific antigen (PSA) mRNA could be stimulated by both androgens and glucocorticoids in 1F5 cells, whereas PSA induction was androgen specific in the parental cell line. Using the differential display PCR method, a search was initiated for genes which are (differentially) regulated by androgens and glucocorticoids in 1F5 cells. So far, two cDNA clones obtained were studied in more detail. One cDNA, which was up-regulated by both androgen and glucocorticoid, turned out to encode ornithine decarboxylase. A second cDNA was identical to a previously published expressed sequence tag (EST). Expression of the latter mRNA was more prominently stimulated by glucocorticoids than by androgens. These results indicate that the AR, GR positive 1F5 cell line provides a suitable system for studying androgen and glucocorticoid targeted processes.

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A NOVEL FAMILY OF SEMINAL VESICLE TRANSCRIBED GENES.

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In the majority of mammals, the predominant semen proteins are secreted by the seminal vesicles. Although many of these proteins exert a similar function as clot- or gel- forming components, they show a pronounced species variability in primary structure. Despite this, we can now show, that they are encoded by genes belonging to a single gene family. The hitherto identified members of this gene family have their transcription unit split into three exons; the first encoding the signal peptide, the second the secreted protein, while the third exon solely consists of 3' non-translated nucleotides. By sequence analysis and low stringent hybridization it is shown that the nucleotide sequences of the first and third exon are conserved between species, while the second exon display highly differing nucleotide sequence, thereby yielding proteins with highly varying primary structure as well. The genes encoding the human semenogelin I and II, the rat SVSII, SVSIV, SVSV and the guinea pig GP1 and GP2 belongs to this gene family. Selection of different splice sites to generate the major coding exon and repeated duplications of small DNA segments is proposed as the mechanisms for the rapid evolution.

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THE EXPRESSION OF TYPE 1 AND 2 5 ALPHA REDUCTASE mRNA IN BPH AND PROSTATE CANCER AND IN SHORT TERM PRIMARY CULTURES OF PROSTATE EPITHELIAL AND STROMAL CELLS.

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The conversion of testosterone into DHT is essential for both the formation of the complete male phenotype during embryogenesis and for andnrogen-mediated growth of secondary sex organs such as the prostate. In man, two separate genes encoding two steroid 5 alpha reductase isoenzymes with 50% sequence homology, designated type 1 and type 2, have been cloned. However there is considerable controversy on whether both isoenzymes are expressed in the human prostate and if so no one has so far assessed their stroma/epithelial distribution in the gland. We have therefore initiated this study primarily to elucidate some of the gaps in our knowledge and also to identify the factors responsible for the down-regulation of 5 alpha reductase in prostate cancer.

Total RNA was extracted from 9 BPH and 3 prostate cancer specimens and from prostate primary cultures of stroma and epithelial cells at different stages of passage. RT-PCR analysis of total RNA was carried out to demonstrate the presence of mRNA transcripts for either the type I or type 2 isoenzymes. In the case of the 5 alpha reductase type 2, RT-PCR amplification was carried out directly on the total RNA using primers which identified only the type 2 enzyme cDNA but not genomic DNA. Whereas the RT-PCR of 5 alpha reductase 1 necessitated the purification of total RNA to mRNA using a biotinilated Oligo dT/streptavidin Para magnetic bead hybrid. This was necessary to eliminate recognition of 5 alpha R-1pseudogene. The purified mRNA was subsequently reversed transcribed to cDNA and the PCR amplified employing primers from published 5 alpha reductase type 1 cDNA sequences.

The results so far obtained demonstrated that the 5 alpha reductase type 1 and type 2 were present in both human prostate cancer and BPH. However, the primary culture of stroma and epithelial cells presented a different picture, with the type 2 enzyme being expressed only in fibroblast stroma cells. However, the fibroblast cells lost their capacity to express the type 2 isoenzyme after the 5th passage. In contrast, the type 1 isoenzyme was expressed in both stroma and epithelial cells but with prolonged passaging the capacity to express this isoenzyme was also depleted. In short these studies indicate that both isoenzymes are expressed in the human prostate but with a different tissular distribution.

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LIAROZOLE INHIBITS THE GROWTH OF SUBCUTANEOUS AND ORTHOTOPIC ANDROGEN-INDEPENDENT R3327/PIF-1 PROSTATIC CARCINOMA IN THE RAT.

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Liarozole fumarate (LIA) currently under clinical evaluation for relapsed metastatic prostate cancer, blocks the P450-dependent breakdown of retinoic acid (RA). RA is a potent inducer of differentiation and inhibits the proliferation of many cancer cells. LIA enhances the antiproliferative and differentiation effects of RA in human cancer cell lines in vitro. In vivo, LIA increases endogenous plasma and tissue RA-levels causing retinoid-mimetic effects. LIA inhibits the growth of androgen-dependent and -independent subcutaneous (S.C.) prostate tumors in rats and nude mice. The androgen-independent R3327/PIF-1 is a moderately-differentiated, rapidly-growing rat prostatic carcinoma. LIA dietary admixed at a dose of 100mg/kg body weight/day inhibited the growth of S.C. PIF-1 tumors (median inhibition 60% after 65 days; n=20). Similarly, twice daily oral gavage dose-dependently inhibited the growth of PIF-1 tumors orthotopically inoculated in the prostate (n=12/group). Doses of 2x60 and 2x80 mg/kg LIA reduced the weights of PIF-1 inoculated prostate down to that of normal prostate. Lower doses down to 2x15mg/kg significantly reduced PIF-1 inoculated prostate weights compared to vehicle treated inoculated prostates (median inhibition 60%). No macroscopically detectable lung tumors were observed after 65 days daily oral LIA treatment (2x60 mg/kg). Plasma and tissue RA-levels dose-dependently increased. Tumor RA-tissue levels could not be assayed due to the small size of the tumors left after LIA treatment. Untreated rats were sacrificed at different time intervals after orthotopic injection of R3327/PIF-1 tumor cells to evaluate metastasis formation in distant organs. Light microscopy showed all lungs to be positive for tumor foci 7 days after injection already. Evaluation at day 18-28-42-56-70 revealed growing tumors in the lungs, but their number did not increase any further. Metastases in the pancreas and the liver became apparent from day 56 and day 70 respectively. These observations seem to indicate that lung tumors after orthotopic inoculation of a cell suspension into the prostate might be due to blood vessel damage rather than to spontaneous metastasis formation.

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INDUCTION OF TRANSIENT REGRESSION BY LIAROZOLE BUT NOT CYPROTERONE ACETATE IN R3327H PROSTATIC ADENOCARCINOMA RELAPSING AFTER ORCHIECTOMY IN THE RAT.

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The existence of a subset of malignant cell lines derived from the Dunning R3327 rat prostatic carcinoma represents an important tool to study the effects of antitumoral drugs on the different stages of prostate cancer. Indeed these sublines differ by their androgen-dependency, their histological degree of differentiation, their growth rate and metastatic potential. The parental R3327H-line is a well-differentiated, slowly-growing adenocarcinoma with low metastatic potential when subcutaneously inoculated in the rat. These subcutaneous tumors initially respond to orchiectomy but relapse after a few weeks, hereby mimicking the course of human prostate cancer.

The present study compared the effects of liarozole fumarate (LIA), an inhibitor of retinoic acid metabolism versus cyproterone acetate (CPA), a steroidal anti-androgen on subcutaneous R3327H-tumors in active progression after an initial response to surgical castration. LIA treatment (dietary admixture at a dose of 100 mg/kg body weight/day) was initiated as soon as consistent regrowth of the tumors for 3 consecutive weeks was observed. A significant regression of tumor volume lasting for a median duration of 6 weeks (maximum decrease of 70% of median volume) was observed in the LIA group (n=23). No significant difference in growth rate was observed during this period between control (n=22) and CPA-treated rats (dietary admixture at a dose of 50 mg/kg body weight/day) (n=22).

During the following 18 weeks, growth rates of tumors in both the LIA or CPA-groups were significantly higher compared to the growth of the control tumors. Nevertheless, a growth delay of 90 days (time to obtain the same tumor volume as at the onset of treatment) was observed in the LIA group.

Final tumor weights in the CPA-group were significantly greater than in the control group. No significant difference was found between the LIA and the control group.

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Exon Swithcing of Fibroblast Growth Factor Receptor Gene on Established Human Prostate Cancer Cells.

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FGF-R2(IIIb) isoform display a high affinity for FGF-7 which derives from stromal cell of the prostate, whereas FGF-R2(IIIc) isoform display a high affinity for FGF-2 but not FGF-7. An irreversible exon switching from IIIb to IIIc occurs in a malignant progression of rat prostate. To study the mechanism of prostatic cancer cell proliferation, we emamined the expression of mRNA for FGF-R2 (IIIb), FGF-R2(IIIc), FGF-2 and FGF-7 on the androgen dependent prostate cancer cell(LNCaP), the androgen independent one(PC-3) and the primary cultured epithelial cell of human BPH (BPH-E) usingRT-PCR method. As results, mRNA for FGF-2 expressed in BPH-E and PC-3, but not in LNCaP. On the other hand mRNA for FGF-7 expressed in stromal cell derived from BPH. FGF-R2 gene of BPH-E and LNCaP were exon IIIb, while that of PC-3 was exon IIIc.

These results suggests that BPH-E and LNCaP have a mechanism regulated by stromal cell through FGF-7 and FGF-R2(IIIb), however PC-3 has an autocrine proliferation mechanism through FGF-2 and FGF-R2(IIIc).

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OESTROGEN RECEPTOR INDUCTION IN PROSTATIC STROMAL CELLS BY ANDROGEN ABLATION THERAPY.

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The presence of oestrogen receptors (ER) in human prostatic tissue is a longstanding, controversial issue. Exposure to oestrogens results in distinct morphological changes of the prostatic glands, which suggest the presence of ER in prostatic tissue. On the other hand, oestrogens may induce these morphological changes by down-regulation of the production of androgens. Morphological changes due to androgen ablation therapy resemble those induced by oestrogen treatment. We studied ER expression by stromal and glandular cells in prostatectomy specimens of 21 patients with prostate cancer, treated for three months with a LHRH agonist and flutamide, using immunohistochemistry. For comparison, ER expression in a series of 18 untreated prostatectomy specimens was studied as well as a few specimens of patients receiving oestrogen therapy. The specimens of patients treated with androgen blockade showed atrophic changes of the glands as well as basal cell hyperplasia, features characteristic for this therapy. Although stromal cells of untreated prostatectomy specimens were largely ER negative, those of patients exposed to androgen ablation therapy had an intense nuclear ER expression in the stromal cells. Very sporadic epithelial cells lining the prostatic glands displayed some nuclear ER expression, but particularly those glands in the treated specimens with basal cell hyperplasia lacked ER expression. In all treated and untreated cases the carcinoma cells were ER negative. Prostatectomy specimens of patients exposed to oestrogens also demonstrated intense ER labeling of stromal cells. In conclusion, the data imply that the hormonal status of the patient determines the level of prostatic stromal ER. Oestrogens exert their influence on prostatic tissue both indirectly by decreasing androgen levels causing the induction of stromal ER, and directly by their interaction with stromal ER. The profound influence on glandular morphology may be the consequence of a paracrine effect of stimulated stromal cells on the glandular (ER negative) epithelium.

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DEVELOPMENT AND CHARACTERIZATION OF A NEW IN VITRO MODEL TO STUDY THE MECHANISMS OF PROSTATE CANCER PROGRESSION.

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At present, not more than a few permanent human prostate cancer cell lines have been described. The only androgen responsive in vitro cell line is LNCaP, which exhibits an atypical hormone response pattern due to a mutated androgen receptor. The PC-346 xenograft cell line is the only human prostate cell line known thus far in which initial regression upon androgen withdrawal is followed by tumor relapse, suggesting the presence of hormone-dependent and -independent cell populations.

Recently we established in vitro cultures from the PC-346 xenograft model following collagenase digestion of PC-346 tumor fragments from the 12th mouse passage. After plating in Matrigel coated culture flasks with low serum (2%) culture medium, poorly spread epithelial colonies were obtained that could be passaged by trypsinization. Contamination by (murine) stromal cells was no longer detectable after two passages. Currently, this new in vitro cell line, PC-346C, is in its 20th passage. The cells express prostate specific markers such as PSA (resulting in levels >2500 ng/ml in culture medium) and, unlike LNCaP, also prostate-specific TGase.

Hormone responsiveness of PC-346C cells was tested in a modified MTT assay. In the absence of steroids, little if any growth occurred. Dose-dependent stimulation of growth was observed after addition of testosterone, dihydrotestosterone or R1881. Even upon androgenic stimulation, however, growth was relatively slow (doubling time 4-5 days). In contrast to LNCaP, PC-346C cells were not stimulated by estradiol or flutamide. Immunocytochemistry, binding assays and Western blot analysis showed the presence of androgen receptors. Complete sequencing of exons 2 through 8 of the receptor gene demonstrated that mutations were absent in this part of the gene.

Injection of $10x10^6$ PC-346C cells into male nude mice resulted in rapidly growing tumors (doubling time 5.2 ± 1.1 days) within 30 days in 9 of 10 animals. High levels of PSA (>1000 ng/ml) were measured in the serum of these animals. In the same experiment, no growth was detected in females until 95 days post-inoculation. Finally, growth occurred in 2/10 female male. From these tumors, another in vitro line (PC-346CF) was established. PC-346CF cells were not stimulated by androgens in vitro.

Further characterization of this new subline and comparative studies of PC-346C and PC-346CF cells are currently underway. By virtue of their unique properties, PC-346C and its hormone-insensitive variant will be valuable new models to study the mechanisms of androgen-regulated growth and gene expression.

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PROSTATE-SPECIFIC ANTIGEN MODULATES THE GROWTH OF ANDROGEN-SENSITIVE LNCaP, BUT NOT ANDROGEN-INSENSITIVE DU145 AND PC3 IN VITRO MODELS OF HUMAN PROSTATE CANCER

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The influence exerted by prostate-specific antigen (PSA) on the proliferation of androgen-sensitive LNCaP and androgen-insensitive DU145 and PC3 human prostate cancer cell lines was monitored by means of the colorimetric MTT assay. The PSA-induced effect on prostate cancer cell proliferation was assessed by adding increasing concentration of PSA in the culture medium and by adding either monoclonal or polyclonal anti-PSA antibodies in the culture media . The antibodies were used to neutralize the biological activity of PSA secreted by the cells. The influence exerted by dihydrotestosterone (DHT), prolactin (PRL), basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) on PSA expression and cell proliferation in the LNCaP, DU145 and PC3 models was also investigated.
The results show that DHT induced both cell proliferation and PSA secretion in the LNCaP model, while EGF increased cell proliferation and decrease PSA secretion. PRL was without any apparent effect on both LNCaP cell proliferation and PSA secretion. bFGF increased LNCaP cell proliferation but did not significantly modify PSA secretion. DHT, EGF, bFGF and PRL remained without any apparent effect on PSA secretion in both DU145 and PC3 models . Of these four hormones and/or growth factors, only PRL modified significantly cell proliferation in both DU145 and PC3 models , while EGF acted significantly on that of DU145 model only.
PSA significantly modified the cell proliferation in androgen-sensitive LNCaP model, but not in the androgen-insensitive DU145 and PC3 ones.
The addition in the LNCaP cell culture medium of either a monoclonal or a polyclonal anti-PSA neutralizing the biological activity of PSA secreted by the LNCaP cells resulted in an increase of proliferation rate when these antibodies were used at low doses and in a decrease of such proliferation rate when they were used at higher doses. This dual effect exerted by the anti-PSA antibodies on LNCaP cell proliferation suggest a dual role exerted by PSA at both cell gain compartment (mitosis) and cell loss compartment (apoptosis).

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ANDROGENS ARE NOT A DIRECT REQUIREMENT FOR THE PROLIFERATION OF HUMAN PROSTATIC EPITHELIUM *IN VITRO*

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The androgen receptor pathway is known to be a key regulator of growth in normal and pathological prostate. However, the precise mechanisms of this signalling pathway with respect to the different cellular compartments of prostate remain largely unknown. We have used a primary culture system to grow human prostatic epithelial cells of normal, benign, tumour and metastatic origin, and immortalised human prostatic epithelial cell lines, to demonstrate the absence of a direct or indirect effect of androgens on cellular proliferation *in vitro*. In parallel to this observed androgen independence for growth, all cell systems have lost significant expression of androgen receptor, PSA and PAP as shown by immunicytochemistry, immunoblot, immunoradiometric and binding assays. Since the androgen receptor is expressed in the epithelium *in situ*, these studies suggest that the androgens' effects on epithelial cells may be one of prostatic differentiation rather than proliferation, and that the androgen receptor/growth factor pathway act through mesenchymal-epithelial interactions.

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FUNCTIONAL INSERTION OF KGF/FGF7 cDNA IN IMMORTALIZED HUMAN PROSTATIC EPITHELIAL CELL LINE PNT1A PROMOTES INVASION AND TUMOURIGENESIS

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Keratinocyte Growth Factor (KGF/FGF7), a member of the Fibroblast Growth Factor family, is synthesized by fibroblasts in normal tissues. It acts as a potent mitogen on epithelial cells, through interaction with a FGF7 specific receptor, an alternative splice variant of the FGFR-2 gene (bek). FGF7 is thought to be involved in prostate tumor progression, stimulating prostatic epithelial cells as a paracrine/autocrine mediator and relaying the biological effects of androgens on epithelial cells. In consideration of the likely importance of this growth factor on prostate carcinoma multistep progression, we have transfected FGF7 cDNA into SV40 immortalized human prostatic epithelial cell lines. The new PNT1A/FGF7-T1 cell line is stable and expresses high levels of FGF7 mRNA and the corresponding protein. Also, it exhibits functional and physiological changes compared to the original PNT1A: (i) increased doubling time (24.3hrs versus 31.8hrs), (ii) tumourigenicity in nude mice (n=7), (iii) growth in soft agar (122 clones/1000 cells) partially inhibited by neutralizing MAb against FGF7 (44 clones/ 1000 cells), and (iv) invasiveness in gel matrix. Our results suggest a potential role for FGF7 promoting agressiveness in prostate cancer, also provide an original and useful model to study the signalling pathways and functions of FGF7 on prostatic epithelial cells.

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EFFECT OF ZINC ON PROLIFERATION OF HUMAN PROSTATE CANCER CELLS. R. B. Franklin, J. Zou, and L.C. Costello. University of Maryland, Dental School, Baltimore, MD, USA.

The prostate gland of many animals contains an extremely high level of zinc. In addition, an extremely high level of citrate is also characteristic of the prostate. Furthermore, zinc and citrate levels are increased in BPH and decreased in prostate cancer. We have showed that citrate accumulation by prostate results from limited mitochondrial aconitase (m-aconitase) activity and that zinc inhibits m-aconitase activity in prostate mitochondrial preparations. Recently, we proposed a bioenergetic theory of prostate malignancy. The theory proposes that prostate malignancy requires a metabolic transformation of prostate epithelial cells from non-citrate oxidizing cells to citrate oxidizing cells. Recently, we collected evidence that prolactin and testosterone stimulate zinc accumulation in lateral prostate mitochondria that might play a role in the mechanism of citrate accumulation stimulated by these hormones. We began to test the bioenergetic theory by determining the effect of zinc on proliferation of human prostate cancer cells.

LNCaP and PC-3 cells (10^4 cells/well) were plated into 24 well culture dishes. The cells were cultured for 6 days (with one media change at day 3) in RPMI 1640 medium containing 2% charcoal stripped serum and various concentrations of zinc. After the 6 day culture period the wells were washed twice with PBS to remove unattached cells. Attached cells were then collected by treatment with trypsin-EDTA and counted using a Coulter counter.

Zinc inhibited proliferation of both LNCaP and PC-3 cells in a dose dependent manner. Maximum inhibition of proliferation at 20 ug/ml zinc was 50%. Addition of 10^{-10} M prolactin to the medium stimulated proliferation of PC-3 cells. However, the prolactin stimulated proliferation was also inhibited by zinc in a manner parallel to that seen without prolactin. Since we have shown that m-aconitase activity and citrate oxidation are inhibited by zinc, these results are consistent with our proposal that citrate oxidation is essential to the process of prostate malignancy and metastasis. This work was support by grant DK42839 from the NIH.

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IN VITRO CHARACTERIZATION OF THE CO-REGULATORY EFFECTS EXERTED BY THE EPIDERMAL GROWTH FACTOR, DIHYDROTESTOSTERONE AND PROLACTIN IN ORGAN CULTURES OF BENIGN PROSTATIC HYPERPLASIAS

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The morphology and tissue specific functions of prostate can be maintained in short term primary organ culture. This is probably caused by the presence of all tissue components and the interactions of epithelium and stroma.
We investigate whether epidermal growth factor (EGF), dihydrotestosterone (DHT) and prolactin (PRL) exert independent or related influences on proliferation rate of human hypertrophic prostatic tissue (BPH) obtained from 30 patients who had undergone open prostatectomy. The proliferation rate is appreciated by the nuclear labeling with thymidine, and we observe that only the epithelial cells are stimulated.
DHT and EGF induce a significant increase ($p < 0.001$ / Fisher F test) in the proliferation rate of a significant percentage of BPH ($p < 0.001$ / Chi^2 test), while PRL do not. PRL is able to significantly antagonize the EGF-induced stimulatory effect on BPH cells proliferation.
The EGF- and PRL-mediated effects are correlated together ($p = 0.03$; Kendall test), this means that EGF and PRL stimulate the proliferation of the same BPHs. In contrast the DHT-induced effects do not correlate with either the PRL- or the EGF-induced ones.
We can conclude that there exist individual variations in hormone responsiveness of human BPH. We propose the model of organ culture to test in vitro the hormone and drug sensitivity of prostate.
Results of the assessment of finasteride, cyproterone acetate and flutamide will also be presented.

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ANDROGEN RECEPTOR EXPRESSION IN METASTASES FROM PROSTATIC CARCINOMA. Alfred Hobisch, Zoran Culig, Christian Radmayr, Georg Bartsch, Helmut Klocker, and Anton Hittmair; Departments of Urology and Pathology, University of Innsbruck, Austria

Androgen receptor (AR) expression is in several aggressive Dunning prostatic carcinoma sublines and in two of three human prostatic carcinoma cell lines, in contrast to human primary prostatic tumours, reduced or absent. In order to gain a better insight into the mechanisms of androgen independence of advanced prostatic carcinoma we have assessed the status of the AR in 12 lymph node metastases and in 22 distant metastases from prostatic carcinoma by immunohistochemistry. Lymph node metastases were obtained at radical prostatectomy from patients who had not received any kind of endocrine therapy. Primary tumour specimens from these patients were also available. Bone and epidural metastases were obtained from patients showing tumour progression after endocrine therapy. Paraffin-embedded tissue sections were stained for the AR following a streptavidin-biotin-peroxidase protocol with the polyclonal antibody PG-21, which is directed against amino acids 1 through 21 of the rat and the human AR. LNCaP cells served as a positive and PC-3 and DU-145 cells as a negative control. The percentage of AR-positive cells was evaluated on the basis of an arbitrary 4-point scale. All patients' samples except one lymph node metastasis displayed AR positivity. In 8 out of 12 lymph nodes and in 8 out of 22 distant metastases more than 50% of cells were AR-positive. In one case the lymph node metastasis was AR-negative, whereas the primary tumour obtained from the same patient displayed intense staining for the AR.
Our study provides evidence that, unlike androgen-independent prostatic carcinoma cell lines, the majority of prostatic carcinoma metastases does not lack the AR. Together with the results of recent studies on activation of the AR by growth factors, cellular regulators, and by very low concentrations of androgen following long-term androgen deprivation, our findings support the hypothesis that the AR is involved in prostatic tumour progression.

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NEUROENDOCRINE DIFFERENTIATION IN HUMAN PROSTATIC CANCER XENOGRAFTS. MA Noordzij, WM van Weerden, TH van der Kwast, FH Schröder, GJ van Steenbrugge. Erasmus University, Rotterdam, The Netherlands

Neuroendocrine (NE) cells form an intrinsic component of normal and neoplastic prostatic epithelium. Knowledge concerning prostatic NE cells is limited, mainly due to a lack of representative experimental models. The presence of NE cells was studied immunohistochemically in prostatic tumor models under different hormonal conditions.
None of the well known in vitro models (PC-3, DU-145, LNCaP) contained NE cells. A number of human xenograft models recently established at our institution contained variable numbers of NE cells. In the androgen-independent PC-324 and androgen-dependent PC-346 models (both derived from trans-urethral resection material of progressive prostate cancer patients) and in the androgen-independent PC-374 model (scrotal skin metastasis) the NE phenotype appears not to be stable as only early mouse passages contained NE cells. The androgen-dependent PC-295 (lymph node metastasis) and PC-310 (primary tumor) models contained no or only small numbers of NE cells in androgen supplemented mice, but these numbers increased following androgen withdrawal (up to $\pm 40\%$ and $\pm 10\%$ in PC-295 and PC-310 tumors, respectively). Moreover, the NE phenotype in these two models seems to be stable. In all models with NE cells, neurosecretory granules have been demostrated electron microscopically.
Prostatic NE cells do not express the androgen receptor and are therefore considered to be androgen-independent. This may explain the increase of NE differentiation in the PC-295 and PC-310 models following androgen withdrawal. All in all, of 11 in vivo models investigated, 6 displayed a NE phenotype the one way or the other, but in only two models (PC-295 and PC-310) this phenotype is stable in subsequent mouse passages and affected by hormonal manipulation. These two prostatic xenografts are potential models to study the functional implication of NE cells in prostate cancer.

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DIOXIN INHIBITS PROSTATE DEVELOPMENT IN RATS. [1]Barry G. Timms, [1]Lesley D. Barton, [1]Anthony Burbach, [2]Richard E. Peterson, [2]Beth Roman, [3]Fred vom Saal. [1]Department of Anatomy & Structural Biology, University of South Dakota, Vermillion, USA; [2]School of Pharmacy, University of Wisconsin, Madison, USA; [3]Division of Biological Sciences, University of Missouri, Columbia, USA.

Numerous industrial products, and by-products such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are being released into the environment. A number of these chemicals can disrupt the endocrine system and thus pose a threat to fetuses, since the endocrine system plays a regulatory role in organ development. We examined the effect of TCDD exposure during fetal life on prostate morphogenesis. Pregnant Holtzman rats were treated with 1.0 µg TCDD/kg body weight on day 15 of gestation. Male fetuses were cesarean delivered on day 20 of gestation, and their intrauterine position relative to other male and female fetuses recorded. The entire urogenital complex was dissected and processed for morphometry. Serial sections of the urogenital complex were analyzed using computer-assisted reconstruction. We replicated our prior finding that the size of prostatic buds was larger in males that developed in utero between female fetuses (2F males) relative to male fetuses that developed between two other male fetuses (2M males). TCDD produced a significant reduction in the number of prostatic glandular epithelial buds which form from the urogenital sinus, with the result that the total area of buds was also significantly reduced. The most profound consequences were observed in 2F males: 2F control male fetuses had a greater total area of prostatic buds in the dorsal (by 50%) and lateral (by 65%) portion of the urogenital sinus relative to 2F TCDD-exposed male fetuses; these glandular buds differentiate into the dorsal and lateral lobes of the prostate. In particular, glandular buds were lost in the caudal region of the dorsolateral prostate. These findings show that maternal exposure to a single low dose of dioxin alters the growth parameters of the fetal prostate and may predispose the gland for pathological changes during aging.

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Expression of Peptide- and Steroid-Hormone Receptors in Human Prostatic Cell Lines - Correlation with Functional Cell Markers

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Epithelial and stromal interactions seem to be important in the pathogenesis of benign prostate hyperplasia (BPH) and prostate cancer (PCa). The LNCaP cell line represents the epithelial and the P21 cells the stromal compartments of the human prostate. In order to test the hypothesis of epithelial-stromal interactions, we investigated the expression of peptide- and steroid hormone receptors in human prostatic cell lines with RT-PCR and gene-specific primers.

Until now, we have been able to show for the first time the expression of the peptide hormone receptors, calcitonin receptor and the somatostatin receptors I, II and III (SSTR I to III) in LNCaP and P21 cells. The PCR-products were checked with gene-specific cDNA-probes on Southern blot hybridizations. In the case of SSTR I an additional band was found, potentially due to alternative splicing. A small deletion of approximately 50 bp was found with the SSTR II primers. The prolactin receptor was only detectable in the epithelial cell line LNCaP.

The expression of the progesterone receptor (B-Form) was found in the LNCaP cell line, whereas the estrogen receptor was not expressed, neither in the epithelial nor in the stromal cells. Naturally, the androgen receptor was expressed in LNCaP, and in the stromal cells P21, although at a very low level.

In some recent papers of Israeli et al. (Cancer Res. 54, 1994) primers of the prostate-specific membrane antigen (PSM) were used to detect micrometastases of prostate cancer in the blood with nested RT-PCR. We used similar primers of the prostate-specific membrane antigen (PSM) to investigate the prostatic origin of the commonly used epithelial cell lines. Only the LNCaP cell line demonstrated a positive PCR product after Southern blot hybridization, in contrast to the cell lines PC-3 and DU 145.

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CYTOKERATIN STAINING AND IMAGE ANALYSIS OF EPITHELIUM IN RELATION TO STROMA IN TOTAL PROSTATECTOMY SPECIMENS. A STUDY OF THE EFFECT OF NEOADJUVANT HORMONE ABLATION IN LOCALIZED PROSTATE CANCER.

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The surgical specimens from 69 consecutive patients were included in this study. Thirty-one patients were given the GnRH-agonist triptorelin for three months prior to total prostatectomy but 38 received no such therapy. After operation, the glands were step-sectioned into 5 mm slices and whole-mount sections were prepared. Sections parallel to the routinely prepared van Gieson slides were stained with commercial monoclonal antibodies against cytokeratin 8. The borderlines of areas with invasive cancer were marked with a pen and within this area smaller zones with similar tumour density were outlined. Thus the whole tumour area was divided into 1/2-1 cm^2 sections.

All the cancer epithelium was intensely stained, but the surrounding stroma was left unstained. Computer-assisted image analysis was used to calculate the area ratio between immunopositive epithelial cells and the negative stroma. Thus, it was possible to estimate the relative cancer cell volume in each tumour. In the neoadjuvantly treated group 22±12% of the tumour areas showed positive staining compared to 45±11% in the untreated group ($p<0.001$).

In conclusion, neoadjuvant hormonal treatment reduces the amount of cancer cells by about 50%, i.e., considerably more than estimated by techniques, that do not take the tumour cell density into account.

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G PROTEIN-LINKED AND CALCIUM SIGNALLING RECEPTORS IN THE HUMAN TRANSITIONAL-CELL CARCINOMA CELL LINES, T24 AND J82

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In addition to peptide growth factors such as PDGF and EGF acting through tyrosine kinase receptors, agonists activating receptors coupled to guanine nucleotide-binding proteins (G proteins) have been reported to induce cell proliferation in several cell lines *in vitro*, either by themselves or in synergy with peptide growth factors. Among the G protein-coupled receptors, particularly those causing stimulation of phospholipase C (PLC) and increase in intracellular calcium concentration ($[Ca^{2+}]_i$), seem to be involved in stimulation of cell proliferation. To determine to which extent G protein-coupled receptors could influence the proliferation of the human transitional-cell carcinoma (TCC) cell lines, T24 and J82, we investigated in a first approach the expression of G protein-coupled and calcium signalling receptors in these cells, by measuring agonist-induced increases in $[Ca^{2+}]_i$ and PLC activity. Furthermore, we pharmacologically characterized the receptor subtypes, using selective agonists and antagonists. In T24 cells, we identified receptors for lysophosphatidic acid (LPA), bradykinin (B_2 receptor), bombesin (BB_2 receptor), ATP (P_{2U} receptor), histamine (H_1 receptor) and thrombin. Agonist activation of these receptors resulted in differential increases in $[Ca^{2+}]_i$ and inositol phosphate formation. In J82 cells, we also identified receptors for LPA, bradykinin (B_2 receptor), bombesin (BB_2 receptor), ATP (P_{2U} receptor), histamine (H_1 receptor), thrombin and, in addition, for vasopressin (V_1 receptor). Although stimulation of these receptors also resulted in increases in $[Ca^{2+}]_i$ and PLC activity, there were marked differences in coupling efficiency for some of these receptors between the two cell lines. In both cell lines, the LPA- and ATP-induced increases in $[Ca^{2+}]_i$ and PLC activity were, at least in part, pertussis toxin-sensitive, suggesting the involvement of $G_{i/o}$ proteins, while coupling of the other receptors is most likely *via* pertussis toxin-insensitive G_q type G proteins. Studies are in progress to analyze which of these G protein-coupled and calcium signalling receptors can stimulate proliferation of these TCC cells.

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4A Mechanisms of endocrine growth control

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INDUCTION OF LNCaP TUMOR GROWTH IN VIVO BY HUMAN PROSTATE FIBROBLASTS OF DIFFERENT ZONAL ORIGIN. [1]George N. Thalmann, [1]Shi-Ming Chang, [2]L.M. Franks, [1]Andrew C. von Eschenbach and [1]Leland W. K. Chung, [1]Department of Urology, The University of Texas M. D. Anderson Cancer Center,Houston, USA, and [2]Imperial Cancer Institute, London, GB

Epigenetic factors such as stromal-epithelial interaction play an important role in regulating tumor growth, progression, and metastasis. In previous studies, we have demonstrated organ-specificity in the induction of human prostate cancer growth in vivo (Gleave et al., J. Urol., 147: 1151, 1992). Prostate and bone fibroblasts, in the appropriate host hormonal milieu, can induce the progression of prostate cancer cells from an androgen-dependent to an androgen-independent state and confer osseous metastatic potential (Thalmann et al., Cancer Res., 54: 2577, 1994). In the present study, we investigated whether fibroblasts derived from different zones of the human prostate gland can differentially induce the growth and formation of chimeric prostate organs in male athymic hosts. To accomplish this aim, we first established human prostate fibroblast cell lines from histologically normal areas of the peripheral zone (PZF), the transitional zone (TZF), and the central zone (CZF) of radical prostatectomy specimens.

All of the fibroblasts derived from the prostate specimens, when injected alone in intact male athymic nude mice failed to form tumors. When co-inoculated with non-tumorigenic LNCaP human prostate cancer cells, different fibroblasts derived from the TZF induced tumors in 91% (10/11) of the mice. The PZF stimulated tumor growth in 89% (8/9) of the animals, whereas the CZF induced tumors in 50% (1/2) of the hosts. Almost all tumors expressed prostate-specific antigen (PSA) in vivo Tumors were found to stain positively for PSA by immunohistochemical method. Isolated epithelial cells from chimeric tumors expressed mRNA for PSA and human androgen receptor. Histomorphologic analysis of the chimeric tumors indicated that the TZF-induced tumors were anaplastic, invading voluntary muscle and skin; the PZF and CZF-induced solid tumors were trabecular and multinodular tumors, in some instances resembling mouse mammary tumors. PZF had an average doubling time of 5.6 days as compared to 4 days for TZF. Dihydrotestosterone stimulated the growth of TZF and, to a lesser extent, PZF. PZF tend to grow slower than TZF and to senesce in vitro. In an assay for soft agar-colony formation, which is known to correlate with in vivo tumorigenicity, conditioned medium of human prostate-derived fibroblasts induced anchorage-independent growth of human LNCaP and rodent NbE-1 prostate epithelial cells 2-3 fold over that of controls.

In summary, fibroblasts derived from the peripheral, transition, and central zones of the human prostate induced human non-tumorigenic LNCaP cells to form PSA-expressing tumors with histomorphology varying from anaplastic to trabecular. These results illustrate the importance of stromal-epithelial interactions, which affect growth and morphology of prostate cancer.

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STROMAL INHIBITION OF PROSTATIC EPITHELIAL CELL PROLIFERATION NOT MEDIATED BY TRANSFORMING GROWTH FACTOR BETA

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The paracrine influence of prostatic stroma on the proliferation of prostatic epithelial cells was investigated. Stromal cells from the human prostate were previously shown to inhibit anchorage-dependent as well as anchorage-independent growth of the prostatic tumor epithelial cell lines PC-3 and LNCaP. Anti-proliferative activity, mediated by a diffusible factor in the stromal cell conditioned medium, was found to be produced specifically by prostatic stromal cells[1]. In the present study the characteristics of this factor were examined. It is demonstrated that prostate stroma-derived inhibiting factor is an acid- and heat-labile, DTT-sensitive protein. Although some similarities with type ß transforming growth factor (TGF-ß)-like inhibitors are apparent, evidence is presented that the factor is not identical to TGF-ß or to the TGF-ß-like factors activin and inhibin. Absence of TGF-ß activity was shown by the lack of inhibitory response of the TGF-ß-sensitive mink lung cell line CCL-64 to prostate stromal cell conditioned medium and to concentrated, partially purified preparations of the inhibitor. Furthermore, neutralizing antibodies against TGF-ß1 or TGF-ß2 did not cause a decline in the level of PC-3 growth inhibition caused by partially purified inhibitor. Using Northern blot analyses we excluded the involvement of inhibin or activin. It is concluded that the prostate stroma-derived factor may be a novel growth inhibitor different from any of the currently described inhibiting factors.

[1] Prostate, **26**:123-132, 1995.

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DEVELOPMENT OF A NEW *IN VITRO* MODEL TO STUDY THE EFFECTS OF ANDROGENS ON THE PROLIFERATION AND DIFFERENTIATION OF HUMAN PROSTATIC EPITHELIUM.

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The androgen receptor (AR) pathway is a key regulator of growth in normal and pathological prostate. However, the precise mechanisms within this signalling pathway remain largely unknown. To confirm the role of androgen receptor mediated proliferation, differentiation and apoptosis in prostatic epithelium, and to examine the involvement of stromal-epithelial interaction in mediating an androgen induced response, we have introduced and constitutively overexpressed a wild type human androgen receptor gene in immortalised prostatic epithelial (PNT) and fibroblastic (PF1SV1) cells.

The full length human AR cDNA cloned in the vector pGEM3Z was kindly provided by Prof. Liao of the University of Chicago. This has been subcloned into the mammalian expression vector pCEP4 which utilises the cytomegalovirus promoter for constitutive high expression, and the hygromycin resistance gene for stable selection. To confirm that the functional wild-type receptor will be expressed, the AR cDNA in the pCEP4 clone has been completely sequenced. Immortalised human prostatic epithelial and fibroblast cells were then transfected with pCEP4 constructs containing either full length AR, an antisense AR or a CAT reporter gene, using liposomal transfection agents. Following selection and cloning of transfected cells, the presence of the recombinant AR gene was detected in hygromycin resistant colonies using a specific PCR for both the exogenous AR gene and the hygromycin resistance gene. AR mRNA and protein expression levels were then demonstrated using Northern and Western blotting techniques. Expression of the exogenous AR gene was comparable with levels of expression of the promiscuous mutant AR present in LNCaP cells. We have therefore created new lines of prostatic epithelial and fibroblastic origin which overexpress an exogenous AR. These cell lines may augment the present *in vitro* models for the study of androgen and antiandrogen responses.

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INHIBITION OF DHT-INDUCED LNCAP PROLIFERATION AND ENHANCEMENT OF PSA PRODUCTION BY ANTIBODY TO BFGF

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Cells of the human prostate cancer cell line LNCaP cell exhibit a bell shaped growth curve in response to dihydrotestosterone (DHT); at low (0.1 nM) concentration DHT stimulates proliferation and at high concentration (100 nM) it promotes growth arrest coupled with the expression of prostate specific antigen (PSA). It remains unclear whether DHT stimulated PSA production and cell proliferation are two independent or competing events in LNCaP cells. Cells were cultured in the absence or presence of 0.1 and 100 nM DHT for 6 days with media changes every other day. We found that DHT at its proliferative dose of 0.1 nM caused a significant 1.7 fold increase in intracellular bFGF compared to the DHT free control (3.78 ± 0.05 pg/mg vs. 2.25 ± 0.027 pg/mg). In the next experiment we tested if a neutralizing antibody to bFGF was able to abolish the mitogenic effect of 0.1 nM DHT. The neutralizing antibody was able to inhibit the effect of bFGF, providing the efficacy for the antibody we used. Moreover, we found that the same concentration of the antibody was able to inhibit the stimulatory effect of 0.1 nM DHT. In the last experiment we investigated the effect of 0.1 nM DHT and a bFGF neutralizing antibody on the expression of PSA. The presence of neutralizing antibody to bFGF resulted in a significant 1.82 fold increase in PSA expression compared to the DHT treated but bFGF-antibody free control.

Taken together, our results suggest that inhibition of DHT cell proliferation and enhancement of PSA production can be mediated by an antibody to bFGF. These results are consistent with the concept that cell proliferation and PSA are two competing events in DHT action.

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CLONING AND CHARACTERIZATION OF THE RAT KGF (FGF7) GENE PROMOTER

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Keratinocyte Growth Factor (KGF) is expressed by the mesenchymal cells of the developing prostate and is thought to play a major role in prostate morphogenesis through interaction with the KGF promoter which is expressed by the epithelial cells. The sequences coding for the KGF protein are divided over 3 exons in the gene. To study the regulation of KGF gene expression we isolated the first coding exon and upstream sequences from a rat genomic library. Comparison of the cDNA sequence with genomic information showed that part of the 5' UTR of the cDNA is encoded by a separate exon (exon 0). By performing the RACE-protocol on 5'-anchored rat kidney cDNA two transcription start sites were detected. Sequence analysis showed that the major transcription start site is preceded by a CAAT-box and a TATA-box.
Transient transfection of LNCaP cells with a 5.5 kb genomic fragment (containing approx. 4.5 kb upstream sequences, exon 0, intron 1 and part of exon 1) cloned in front of the luciferase reporter gene revealed promoter activity. Cotransfection with androgen receptor cDNA showed a 7 - 10 fold induction of luc activity in the presence of R1881. A 40-fold induction can be observed after cotransfection with glucocorticoid receptor cDNA and dexamethasone treatment. Deletion mapping showed that the major contribution to steroid hormone inducibility of the KGF promoter is located within a 3.5 kb upstream region (between - 4.5 kb and - 1 kb).

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IDENTIFICATION OF THREE REGIONS INVOLVED IN ANDROGEN REGULATED ACTIVITY OF THE PROSTATE SPECIFIC ANTIGEN PROMOTER IN LNCaP PROSTATE CELLS.

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Expression of Prostate-Specific Antigen (PSA) is androgen regulated. We identified three regions in the PSA promoter which are involved in androgen regulation. (i) The promoter of the PSA gene contains at -170 the sequence AGAACAgcaAGTGCT, which is closely related to the HRE (hormone responsive element) consensus sequence (GGT/AACAnnnTGTTCT). This sequence is a high affinity androgen receptor binding site as shown by gel retardation assay and acts as an androgen response element (ARE) as shown by transfection experiments of LNCaP cells with mutated PSA promoter constructs. (ii) Transfection experiments further showed that sequences upstream of the ARE are necessary for maximal androgen inducibility. A 35 bp segment (-400 to -366) was identified, which cooperates with the ARE in androgen induction of the PSA promoter. This segment (ARR: androgen response region) was able to confer androgen induced activity to a minimal PSA promoter and a minimal TK promoter. The ARR was found to contain a low affinity androgen receptor binding site (GGATCAgggAGTCTC). (iii) The identification of a cluster of three DNAse I hypersensitive sites (at approx. -4.5 kb) indicated the presence of a third regulatory region in the PSA promoter. This region increased PSA promoter activity in transiently transfected LNCaP cells. A 1 kb fragment, encompassing the DNAse I hypersensitive region was able to confer androgen responsiveness to a minimal TK promoter and was found to contain at least one high affinity androgen receptor binding site (GGAACAtatTGTATC).

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INDUCTION OF ANDROGEN RECEPTOR-MEDIATED GENE TRANSCRIPTION IN RESPONSE TO LUTEINIZING HORMONE RELEASING HORMONE. Zoran Culig, Alfred Hobisch, Marcus V.Cronauer, Christian Radmayr, Anton Hittmair, Georg Bartsch, and Helmut Klocker; Departments of Urology and Pathology, University of Innsbruck, Austria

Several polypeptide growth factors and cellular regulators affect the transcriptional transactivation function of steroid receptors. Our group has previously demonstrated that growth factors IGF-I, KGF, and EGF are able to activate the androgen receptor (AR) in the absence of androgen (Culig et al.: Cancer Res 54:5474-5478, 1994). In this study we have addressed the question whether luteinizing hormone releasing hormone (LHRH), which is widely used in therapy for advanced prostatic carcinoma, displays any effect on AR-mediated reporter gene transcription.
DU-145 prostatic carcinoma cells were cotransfected with an androgen-inducible chloramphenicol acetyl transferase (CAT) reporter gene and the AR expression vector pSG5-AR. About 16 hours after transfection the medium was supplemented with either the synthetic androgen methyltrienolone (R1881) or/and LHRH. Since LHRH evokes increase of intracellular cAMP, one portion of cotransfected cells was treated with 8-Br-cAMP. Incubation was continued for about 24 h and, thereafter, reporter gene activity was measured in cell extracts.
LHRH, at a concentration of 1 nM, induced 15% of the maximal reporter gene activity, which was measured after treatment with 1 nM of R1881. Incubation of transfected cells with 2 mM of 8-Br-cAMP evoked 20% of the maximal activity. In control experiments we have observed no increase in CAT activity in the cells transfected with the reporter gene and the empty expression vector pSG5. In experiments in which LHRH was applied together with very low concentrations of R1881 (5 and 10 pM) there was a distinct synergistic effect on stimulation of reporter gene expression. While R1881, at a concentration of 10 pM, was equally effective in inducing CAT activity as LHRH, cotreatment with both substances caused maximal stimulation of reporter gene activity. Similar synergistic effects were observed after coincubation with low doses of androgen and 8-Br-cAMP. The stimulatory effects of LHRH and 8-Br-cAMP on CAT activity were abolished by the nonsteroidal anti androgen Casodex.
Our results provide first evidence of agonistic effects of LHRH on AR activation. Synergistic effects on receptor stimulation observed after treatment with LHRH and very low doses of androgen are of particular importance because of low androgen supply by adrenals in castrated patients with advanced prostatic carcinoma. Considering this pattern of AR activation, we hypothesise that application of a pure AR antagonist together with an LHRH analogue is more beneficial for patients with advanced prostatic carcinoma than application of LHRH alone.

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ANDROGEN-INDUCED APOPTOTIC CELL DEATH IN THE ANDROGEN-DEPENDENT HUMAN PROSTATIC CELL LINE LNCAP.

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This study focusses at the dual action of androgens to induce cell proliferation and cell death in the androgen-sensitive LNCaP-FGC (LNCaP) cell line. We previously showed that androgens exert a biphasic response on growth of LNCaP (passage 70) cells, and that relatively high dosages of androgen inhibited growth of these cells (Prostate 23:213, 1993). The nature of this biphasic response was further investigated with the aim was to assess whether high-dose androgen-induced cell loss has features of apoptotic cell death. Apoptotic cell death was demonstrated by application of the DNA-binding dye, Hoechst 33342 (H33342) in combination with propidium iodide (PI) to intact cell cultures. In addition, a photometric immunoassay ('Cell Death Detection ELISA' (CDDE)) was used, detecting mono- and oligonucleotides in cell lysates, which allows better quantification of apoptotic cell death. In contrast to the apoptotic process known to occur after androgen withdrawal in vivo, in the rat ventral prostate and in human prostatic cancer xenografts, the LNCaP cells when cultured in steroid-depleted (DCC) medium continued to grow at a decreased rate without any evidence of cell death being induced. In cultures grown in the presence of 0.1 nM of the synthetic androgen R1881, a dosage which maximally stimulated growth of LNCaP cells, also some apoptotic cell death was found. The addition of 10 nM R1881, and seemingly not the natural androgen, dihydrotestosterone, led to a substantial induction of cell death and to a decrease in total cell numbers. Experiments with another synthetic androgen are currently done. The observed process of cell death was associated with detachment of cells, which after staining with H33342/PI, as well as electron microscopically were recognized as being apoptotic cells. This androgen-induced apoptosis is a relatively slow process: a maximum of 18 percent apoptotic cells in 10 nM R1881-treated cultures was reached 6-7 days after start of treatment. The staining results could be confirmed and further quantification was obtained using the CDDE assay. The highest 'enrichment factor' (75) of nucleosomes was found after 7 days in 10 nM R1881-exposed cells over DCC cultures as controls, whereas a factor of approximately 20 was measured in cells grown in the presence of 0.1 and 1 nM R1881. In conclusion, androgen-induced cell death in the LNCaP line was demonstrated to be apoptotic cell death. This yet incompletely understood phenomenon seems to be restricted to the synthetic androgen R1881. Supported by Dutch Cancer Society (EUR 90-13).

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Possible bone preserving capacity of high dose intramuscular depot estrogen in the treatment of patients with prostatic carcinoma

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Background : Treatment of prostatic disease with GnRH agonists or orchidectomy has negative effects on bone mass. The beneficial effects of estrogens on bone status in postmenopausal women is well known. High dose intramuscular depot estrogen treatment is an alternative treatment of prostatic disease especially when cost and effects on bone are considered.

Methods: Marker of bone and collagen metabolism were assesses in patients with prostatic carcinoma participating in a randomized two-year study comparing orchidectomy (N=16) with single drug parenteral polyestradiol phoshate 240 mg i.m. every second week for the first two month and then every fourth week (N=17)

Results: Five patients developing progressive disease and/or signs of metastasis were excluded from the analysis. In the remaining patients serum concentrations of osteocalcin, procollagen IIIP (PIIINP) and cross linked carboxyterminal telopeptide of Type 1 collagen (ICTP) increased significantly over time following orchidectomy (N=13). Serum levels of osteocalcin and procollagen (PICP) decreased significantly over time during estrogen treatment (N=15). During treatment serum levels of osteocalcin, PICP, PIIINP and ICTP were significantly lower in the estrogen treated than in the orchidectomized patients.

Conclusions: The induced changes in serum markers for bone and collagen metabolism do clearly indicate an increased bone turnover in orchidectomized subjects. An opposite pattern was found in the estrogen treated patients, indicating a reduced turnover. Estrogens seems to have a bone mass preserving capacity also in elderly males with prostatic cancer.

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GENETIC RESPONSE TO UNILATERAL ISCHEMIA. Min-Wei Chen, Ralph Buttyan, New York, N.Y.; Alan J. Wein and Robert Levin, Philadelphia, PA. (Presentation by Dr. Levin).

Unilateral ischemia results in the progressive hypertrophy of the non-ischemic side of the bladder and the progressive atrophy of the ischemic side. In the current study, we compared the temporal sequence of gene activation of unilateral ischemia for both the ischemic (I) and non-ischemic (NI) sides, as compared to the genetic signals of control bladders (rabbits). We then compared the genetic studies with the effect of unilateral ischemia on protein synthesis (^{35}S-methionine incorporation) and DNA synthesis (^{3}H-thymidine incorporation) of both the I and NI sides. Unilateral ischemia in rabbits was created by surgically placing a ligature on the arteries entering the left side of the bladder. The bladders were excised and studied at 1, 8, and 24 hours following surgery. The results of the genetic study are as follows:

PROBE	CONTROL	ISCHEMIC	NON-ISCHEMIC
hsp-70	1.00	16 (8 hours)	22 (8 hour)
bFGF	1.00	5.14 (8 hours)	3.14 (8 hour)
TGF ß$_1$	1.00	2.0 (1 hours)	.5 (8 hour)
c-fos	1.00	11.8 (1 hour)	10.9 (1 hour)
c-jun	1.00	7.9 (1 hour)	7.9 (1 hour)

Controls assigned an arbitrary value of 1.00, Values in parentheses represent the time after unilateral ischemia signal change is maximal

The ischemic and non-ischemic sides responded approximately the same, reaching a maximum level between 1 and 8 hours following surgery, and returning to near-normal levels by 24 hours. Unilateral ischemia stimulated a similar increase in DNA synthesis (3x control values, maximal increase at 24 hours) and protein synthesis (3x control values, maximal increases at 8 and 24 hours) in tissue isolated from both the ischemic and non-ischemic sides. The tissue incubations were performed on isolated tissue incubated in the presence of oxygen, substrates and ^{35}S-methionine or ^{3}H-thymidine.

This study provides evidence that unilateral ischemia stimulates a uniform stress response in the bladder as a whole as indicated by the level and temporal sequence of gene activation, and that the tissue response to this stress response is increased DNA and protein synthesis. The necrosis and tissue atrophy observed in the ischemic side results from ischemic and hypoxic damage to the affected tissue, and is not a result of the initial genetic response of the tissue. (Supported by NIH RO-1-DK44689)

4 B Metabolic aspects of urological disorders/ New developments in neurourology

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ELECTRICAL SACRAL ROOT STIMULATION FOR BLADDER CONTROL

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In order to restore bladder control in patients who have lost this ability (e.g. paraplegics) the sacral nerve roots can electrically be stimulated to activate the detrusor. However, using a conventional stimulation pattern, voiding is hindered by simultaneous activation of both the detrusor and the urethral closure mechanism since the sacral roots contain nerve fibres innervating the detrusor as well as fibres innervating the urethral sphincter. Our research is directed to the development of a stimulation technique that enables selective detrusor activation without co-contraction of the urethral sphincter.

Since the detrusor is innervated by smaller nerve fibres than the sphincter, selective small fibre activation would result in selective detrusor activation. Selective activation of the small fibres is possible by blocking, distal to the excitation site, the propagation of action potentials in the large fibres (sphincter) using an anodal block. This can be obtained using an tripolar electrode, consisting of a cathode flanked by two anodes, connected to two synchronized current sources. The feasibility of this stimulation method has been demonstrated in computer simulations and in acute animal experiments using dogs. Recently we have shown that the technique can successfully be applied in patients.

The results show that selective activation of the detrusor by sacral root stimulation is possible. When this technique can be used in implanted systems, bladder emptying will be more physiological and at lower intravesical pressures as compared to other stimulation techniques.

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NEUROGENIC BLADDER GENE EXPRESSION IN FEMALE WISTAR RATS AFTER COMPLETE SPINAL CORD TRANSECTION. David L. Bowers, Brenda Mallory, Min Wei Chen, Kathleen O'Toole and Steve A. Kaplan. Columbia Presbyterian Medical Center New York, New York United States of America

We describe the early alterations in the rat bladder gene expression as it may relate to neurogenic bladder dysfunction after T-10 laminectomy and complete spinal cord transection. Completeness of transection was confirmed by histological sectioning. The following c-DNA probes were quantified in the bladders of female Wistar rats at control, 1, 3, 6, 12, 24 and 48 hours, 4 and 7 days post procedure: transforming growth factor-beta (TGF-β) and sulfated glycoprotein-2 (SGP-2). The rat bladders were harvested, weighed and frozen in liquid nitrogen. Ribosomal Ribonucleic Acid (rRNA) was extracted via RNAzol®. The extracted RNA was subjected to a RNA protection assay (Ambion®). Bladder weight increased by 85% over non-operative controls at 7 days post operatively. In the spinalized rat there was a increase in TGF-β seen at the 48 hour, 4 day and 7 day time periods. Expression of SGP-2 decreased at the 1 and 3 hour time periods and slowly returned to baseline pre procedure levels between the 4th and the 7th day. Histological changes consistent with inflammation and squamous metaplasia are noted.

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CONTRACTILITY, MORPHOLOGY, AND CELLULAR PHENOTYPE OF THE RABBIT BLADDER WALL DURING 180 DAYS OF PARTIAL OUTLET OBSTRUCTION. Marleen Roelofs, Francesca Pampinella, Giacomo Passerini-Glazel, and Saverio Sartore; University of Padua, Padua, Italy. Dorothea Rohrmann, Frederick C. Monson, Alan J. Wein, and Robert M. Levin; University of Pennsylvania, Philadelphia, Pennsylvania.

Bladder outlet obstruction induces tissue remodelling in the rabbit bladder wall leading to hypertrophy of the detrusor smooth muscle cells (SMC) and to thickening of the serosa. Hypertrophy of the detrusor SMC is characterized by the expression of two proteins: a particular NM myosin isoform and vimentin. Thickening of the serosa results from proliferation within this tissue and is accompanied by the gradual progressive transition of serosal fibroblasts into SMC of fetal type. These transitions have been followed by means of immunohistology on whole bladder sections and correlated with contractility studies performed on isolated strips of both serosa and detrusor (full thickness) strips.

Partial outlet obstruction was created by surgically placing a silk ligature loosely around the catheterized urethra and removing the catheter. Immunohistochemical and contractile studies were performed at 15-,30-,60-,90-, and 180 days following obstruction.

The results are as follows: the myofibroblasts contained within the serosa (characterized by the expression of SM α-actin), were not contractile to any form of stimulation (15 days); a response to KCl developed when serosal cells expressed SM myosin (30 days; 'fibromyoblast' phenotype); a response to both KCl and bethanechol developed with the homogeneous expression of desmin; i.e. with the appearance of SMC of fetal type. At this obstruction-time, the new SMC were grouped around the elastic membrane to form a ribbon of poorly organized SMC. At 90 and 180 days the contractile response of the serosa was similar to that of the 60 day obstructed bladder. Immunohistological studies show at 90 days the presence of small bundles of adult SMC surrounded by a densily organized 'fibromyoblast'-containing serosal tissue close to the detrusor. At 180 days of obstruction, no serosal SMC were found. However, at the serosal side of the hypertrophic detrusor, relatively small bundles of normotrophic cells that did not express any of the two proteins characteristic of hypertrophic smooth muscle were found. As tested with anti-synaptophysin and anti-neurofilament antibodies, a slight, although not normal, innervation of these bundles was seen.

We conclude that SMC of serosal origin, after having been grouped around the elastic membrane (60 days) and migrated toward the detrusor (90 days) have completed maturation and have been adjoined to the hypertrophic detrusor as normotrophic SMC after 180 days.

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NITRIC OXIDE SYNTHASE ACTIVITY IN THE HUMAN MALE REPRODUCTIVE ORGANS.

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Nitric oxide (NO) has been shown to be a mediator of non-adrenergic, non-cholinergic nerve induced smooth muscle relaxation in the human urogenital tract.

The aim with our study was to investigate NO synthase (NOS) activity and histochemical staining of NOS in the male reproductive tract. We have analyzed the NOS activity using an assay where L-[U^{14}-arginine] is converted to L-[U^{14}-citrulline]. Several different NOS has been suggested and at least three different isoforms, nerve NOS (n-NOS), endothelial NOS (e-NOS) and inducable NOS (i-NOS) have been cloned. n-NOS and e-NOS are Ca^{2+}-dependent whereas i-NOS is Ca^{2+}-independent and is activated as a host defense mechanism. We found a high Ca^{2+}-dependent NOS activity (pmol g^{-1} min^{-1}), in the vas deferens (94±21), prostate (88±15) and seminal vesicle (196±27) but no significant activity could be found in the epidydimis (30±34) or testis (3±4), means ± SEM. No Ca^{2+}-independent activity was found.

NADPH-diaphorase (NADPH-d) histochemistry is a well established method for staining NOS. We found prominent NADPH-d staining of the secretory cells in the vas deferens, seminal vesicle and to a lesser extent in the prostate. The cauda epidydimis also showed NADPH-d staining. NADPH-d staining was also found in some nerves but was sparse as compared to the staining in the secretory cells. Acetylcholinesterase staining colocalized with NADPH-d in some nerve fibres.

In conclusion, we have found NO synthase activity in the vas deferens, prostate and seminal vesicle in the male reproductive organs. The majority of the activity seems to be located in the secretory cells suggesting that NO may be involved in the secretion of seminal fluid although further studies are needed to evaluate the importance of NOS activity in secretory cells.

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NITRIC OXIDE SYNTHASE ACTIVITY IN HUMAN KIDNEY AND RENAL CELL CARCINOMA

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NO synthase activity has previously been described in human gynecological cancers and the activity was inversely correlated with the differentiation of the tumor. The aim of this study was to investigate the activity and localisation of NO synthase in human kidney and renal cell carcinoma. We found significantly ($P < 0.0001$) decreased NO synthase activity in human renal cell carcinoma compared to nonmalignant renal tissue from the same kidney in patients (n=17) undergoing nefrectomy. Calcium-dependent NO synthase activity was found in all nonmalignant tissues (range, 102-884 pmol/min/g tissue). The calcium-dependent NO synthase activity was significantly decreased in renal cell carcinoma (range, 0-75 pmol/min/g tissue), high grade tumors showing the lowest activity. Calcium-independent activity was also found in all nonmalignant tissues (range, 6-194 pmol/min/g tissue) and significantly decreased in the tumors (range, 0-62 pmol/min/g tissue). The decreased activity is not likely due to the presence of an endogenous NO synthase inhibitor, since tumor tissue did not inhibit the activity in normal kidney tissue. Immunohistochemical investigation using antibodies raised against endothelial, nerve and inducible NO synthase was performed. In the normal kidney a dense staining of the endothelium in vessels and glomeruli was evident, whereas in the tumors, endothelial NO synthase could not be demonstrated . We could also show nerve NO synthase-like immunoreactivity in nerves in the normal kidney but not in the tumors. Inducible NO synthase immunoreactivity could not be detected in the normal kidney or tumor tissue. Contrary to human gynecological cancers the NO synthase activity was lower in high grade renal cell carcinoma than in the low grade tumors. We propose that the NO synthase gene transcription and/or translation is suppressed in the renal cell carcinoma neo vasculature.

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NITRIC OXIDE (NO) AND SUPEROXIDE (O_2^-) FORMATION WITHIN THE SPERMATIC VEIN IN PATIENTS WITH VARICOCELE : A POTENTIAL ROLE FOR PEROXYNITRITE ($ONOO^-$) IN SPERM DYSFUNCTION

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The pathophysiology of testicular dysfunction in patients with varicocele remains poorly understood. Concerning the mechanisms involved, a plethora of them has been proposed, varying from induction of testicular hypoxia to retrograde flow of adrenal metabolites and scrotal hyperthermia. We investigated the activities of xanthine oxidase (XO) and nitric oxide synthase (NOS), the NO levels and the serum antioxidant capacity (SAOC) in patients with varicocele.

The study group was consisted of 5 normogonathotrophic men with clinically evident varicocele and moderate to severe oligoasthenospermia. Whole blood samples were drawn from a peripheral vein and the spermatic vein before ligation. NOS and XO activities were measured by a method based on the oxidation of scopoletin that can be detected fluorophotometrically. The NO levels were estimated by luminol enhanced chemiluminescence while SAOC was determined by means of a chemiluminescence reaction using sodium perborate in the presense of horseradish peroxidase. The serum NOS and XO activities were higher in the samples from the spermatic vein than those from peripheral vein (750±50 vs 70±15 nmoles/ml/min and 0.65±0.1 vs 0.09±0.01 mU/ml, respectively). The same was observed with the serum NO levels (625±90 vs 25±4 μM), while SAOC in the serum from the spermatic vein was increased compared to SAOC in the serum from the peripheral vein (Tmax : 400±10 vs 600±20 sec).

The present study suggests that NOS and XO released by endothelial cells within the dilated spermatic vein result in a dramatic formation and subsequent stagnation of NO, O_2^- and H_2O_2 which react to form the noxious oxidant $ONOO^-$. The oxidative potential of $ONOO^-$ could be a causative factor for an impairment in spermatogenesis, sperm function and fertility potential in patients with varicocele.

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CHARACTERIZATION OF EXTRACELLULAR MATRIX (ECM) AND COMPLIANCE CHANGES IN THE AGING FEMALE RAT BLADDER. Diane Felsen, Marc Danziger and E. Darracott Vaughan, Jr. New York Hospital-Cornell Medical Center, NY, NY, USA.

Bladder dysfunction and incontinence are major problems associated with aging. Although much attention has been paid to the neurological and obstructive causes of bladder dysfunction, there has been relatively little study of the contribution of bladder ECM to these conditions. The ECM contributes both to the viscoelasticity of the bladder and to normal propagation of muscle contraction. Studies have shown increases in bladder elastin and collagen in aging females; in aging, increases in bladder connective tissue were found using morphometrics. The aim of the present studies was to study bladder compliance and ECM composition in aging rats (Fischer 344 rats, ages 6 - 22 months). Bladder volume at micturition was not different between 6 and 22 month old animals. However the pressure at micturition was different in the two groups, resulting in differences in compliance (.16 $\pm$ .00 ml/mm Hg in 6 month old compared to .06 $\pm$ .01 ml/mm Hg in 22 months). ECM components localized included Collagens I, III and IV, laminin and fibronectin. No changes were apparent in either the amounts or localization of the ECM components examined. These results suggest that the ECM components examined do not significantly contribute to the decreases in bladder compliance with aging. Other ECM components (e.g., elastin) may be involved, or the direct quantification of ECM components or their synthesis or degradation rates may be required to demonstrate the involvement of the ECM in changes in the aging bladder.

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INVESTIGATION ON ANDROGEN DEPENDENT ERYTHROPOIETIN SYNTHESIS IN THE KIDNEY UNDER HYPOXIC STIMULATION

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Experiments in the isolated perfused kidney model and in hypophysectomized rats demonstrate stimulation of EPO synthesis by testosterone. Furthermore Blanchard et al. previously demonstrated existence of a steroid receptor element in the oxygene responsive 3'flanking region of the EPO gene. We studied the effect of various kinds of androgen ablation on EPO serum levels and mRNA expression in the kidney under hypoxic stimulation.

Material & Methods: Controls and 5 Han Wistar rats /group were treated by orchiectomy, 10 mg cyproteroneacetat (CPA) or 10 mg Flutamide per day. After 14 days of treatment the animals were exposed to hypoxia for 4 hours and killed. EPO was determined by RIA, kidneys were harvested and shock frozen. EPO mRNA expression was determined by RNase protection assay.

Results: Mean EPO serum levels were 191 mU/ml for untreated controls, 275 mU/ml for orchiectomized animals, 275 mU/ml for animals treated by CPA and 300 mU/ml for animals treated by Flutamide. Mean EPO mRNA expression measured in cpm was 929 cpm for controls, 1754 cpm after orchiectomy, 1292 cpm after treatment by CPA and 1678 cpm after treatment by Flutamide.

Conclusion: We could demonstrate that neither low testosterone levels nor androgen receptor blockade by steroidal and non steroidal antagonists have a negative regulatory effect on EPO mRNA synthesis and EPO production, even under hypoxic stimulation. So far stimulation of EPO gene is not modulated by antiandrogene therapy.

Further experiments now must evaluate whether androgen blockade has a direct effect on the colony- and burst forming units in the bone marrow.

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EVIDENCE FOR A POSSIBLE MECHANISM FOR THE ONSET OF INJURY IN THE INTERSTITIAL CYSTITIS BLADDER: INVOLVEMENT OF NITRIC OXIDE SYNTHASE. A. Elgavish, Keith Lloyd and R. Reed. University of Alabama at Birmingham, Birmingham, AL 35294.

The general hypothesis underlying our studies is that exposure of poorly differentiated basal urothelial cells (UB) to bacterial toxins affects their adhesive properties. Our studies showing that UB treatment with bacterial toxins inhibits integrin-mediated cell/extracellular matrix interactions (Elgavish et al., *Amer. J. Physiol.* 266: C1552-C1559, 1994), support this possibility. In the present studies, we have compared untreated epithelial cells isolated from IC bladders (IC-UB) with untreated epithelial cells isolated from control bladders (UB) and UB treated *in vitro* with lipoteichoic acid from *Streptococcus faecalis* (LT-2). UB and IC-UB cultures were organized in colonies. A population of poorly differentiated single cells was lower in IC-UB and LT-2-treated UB as compared to untreated UB. A comparable increase occurred in the percentage of high cytokeratin 8 (CK8)-expressing. i.e. more differentiated large colonies (>10cells/colony) displaying $\alpha_3\beta_1$ integrins at cell-cell contacts, from 0.6+1 (UB) to 15+5.7 (LT-2-treated UB) and 14+4 (IC-UB) percent. The effects of LT-2 were prevented when UB were treated in the presence of L-NAME or dexamethasone, two inhibitors of nitric oxide synthase (NOS), suggesting that NOS activation mediated LT-2 action. Large colonies of IC-UB may be the progeny of a subpopulation of basal IC-UB that had been exposed *in vivo* to bacterial toxins and had acquired enhanced ability to proliferate and differentiate under conditions that are not permissive for control cells. This possibility was supported by foci of high CK8 expressing cells in intermediate layers of the IC urothelium, whereas control urothelia displayed high CK8 only in terminally differentiated superficial cells. In conclusion, toxins at subepithelial foci of infection may enhance the ability of a subpopulation of basal cells to proliferate and differentiate. Redistribution of integrins in these small areas, from contacts with the ECM to cell-cell contacts, may be a contributing factor to detachments in the IC bladder (Supported by NIH grant DK47546 (to AE)).

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CELLULAR ACCUMULATION OF THE 5-AMINOLEVULINIC ACID METABOLITE PROTOPORPHYRIN IX IN NORMAL AND TRANSFORMED UROTHELIAL CELLS

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Tumor cells may be distinguished from their normal counterparts by differences in their metabolism. The external admission of 5-aminolevulinic acid (ALA), a precursor in the biosynthetic pathway to heme, leads to an accumalation of the fluorescent endogenous photosensitizer protoporphyrin IX (PPIX). Following instillation of ALA in the urinary bladder neoplastic tissue can be discerned by fluorescence cystoscopy and/or treated by illumination with light of an appropriate wavelength (photodynamic therapy). To investigate the mechanisms underlying the clinical findings in vitro investigations of PPIX accumulation in cell lines of different urothelial differentiation have been carried out using flow cytometric quantification of intracellular PPIX. The cell line HCV29 derived from normal urothelium exhibited significantly lower fluorescence intensities than their malignant counterparts (cell lines RT4 and J82 derived from a papillary and invasive bladder tumor respectively). The amount of accumulated PPIX was dependent on the growth conditions (subconfluent versus postconfluent monolayers, two- versus three-dimensional culture) and the presence of serum in the incubation medium. Individual fluorescence saturation values (8 8 for HCV29, 56.8 for J82, 200.5 for RT4, arbitrary units) were obtained after 4h of incubation with ALA at different concentrations (30-60 µg/ml for HCV29, 100µg/ml for J82 and more than 400 µg/ml for RT4). In consideration of the mitochondria associated pathway leading from ALA to PPIX the mitochondrial activity of J82 and RT4 cells was compared by measurement of the fluoresence intensity of the mitochondrial membrane potential sensitive dye rhodamine 123. RT4 cells exhibited a significantly higher rhodamine 123 fluorescence intensity as J82 cells indicating a correlation of the metabolic activity with the amount of accumulated PPIX. In addition, absorption spectrometry revealed significant differences in the ALA uptake between the two cells lines which likewise contribute to the observed intercellular differences. In conclusion, ALA metabolism can be successfully exploited to support diagnosis and therapy of urothelial neoplasias and may add further insight into biological factors distinguishing normal from neoplastic cells.

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DETECTION OF EARLY BLADDER CANCER BY ACCUMULATION OF THE 5-AMINOLEVULINIC ACID METABOLITE PROTOPORPHYRIN IX

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Neoplastic cells may be distinguished fom their normal counterparts by metabolic differences. This fact is used in fluorescence cystoscopy based on intravesical instillation of 5-aminolevulinic acid (ALA) which is a precursor in the biosynthetic pathway to heme, and stimulates the accumulation of fluorescent protoporphyrin IX (PPIX). The present study was conducted to evaluate the clinical benefits and biological background of this new diagnostic tool. Therefore 104 patients suspected of bladder cancer received an instillation of ALA (1.5 g in 50 ml $NaHCO_3$) 2 - 3 h prior to cystoscopy which was performed under conventional white light illumination alternating with illumination with violet light suitable to excite the fluorescence of PPIX. 433 evaluable biopsies were taken out of fluorescent and non-fluorescent lesions of the bladder wall. 35 urothelial neoplasms (12 dysplasias, 2 T_{is}, 20 T_a-, 1 T_2-tumor) were missed during routine cystoscopy and solely detected by the fluorescence of the ALA metabolite PPIX. The overall sensitivity of fluorescence cystoscopy was more than 95% which was significantly increased as compared to conventional white-light cystoscopy. Despite the high sensitivity 102 biopsies out of fluorescent lesions were histologically classified as benign. To get further insight into the biology of these false positive specimens the expression of the proliferation associated antigen Ki 67 was evaluated by quantification of immunohistochemical MIB-1 positivity with a true color image analysis system. The proliferation index of false positive biopsies was significantly increased as compared to true negative specimens. On the other hand, papillary tumors with low proliferation indices showed pronounced ALA induced fluorescence. Hence, further investigations are required to draw final conclusions about the biological behaviour of these "benign" lesions with enhanced ALA metabolism. At present, ALA induced fluorescence-guided biopsies can be recommended due to the high sensitivity of the procedure. Whether ALA can also provide a new tool for the diagnosis of pre-neoplastic lesions of the bladder must be proven in the future.

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LIPID PEROXIDATION AND THE EFFECT OF ALLOPURINOL BLOCKADE FOLLOWING RELEASE OF OBSTRUCTIVE UROPATHY .

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Renal blood flow is reduced significantly in acute obstructive uropathy. On release of obstruction blood flow is transiently increased only to fall again to pre-release levels. This type of reperfusion picture in other organs is associated with free radical release. This study investigates free radical production as estimated by Malondialdhyde (a marker of lipid peroxidation) after release of unilateral ureteric obstruction.

METHOD: Seven wistar rats had complete left ureteric obstruction created by silk ligature with a further seven rats undergoing a sham operation. After 24 hours 0.5 ml samples of blood were taken from the left renal vein prior to release, 10 minutes and 20 minutes post release. The same procedure was repeated with the obstructed and control groups receiving 10 mg allopurinol / 100 gm body weight. Samples were analyzed for malondialdhyde levels (MDA) by an H.P.L.C. method.

RESULTS: Av. MDA level (umol/l) and changes (S.E)

Time (mins)	0	10	20
Control	1.13 (0.23)	-0.08 (0.23)	0.08 (0.22)
Obstruction	1.01 (0.13)	1.11 (0.35)	1.09 (0.35)
Mann Whitney		p = 0.006	p = 0.045
Control + allop	1.32 (0.17)	-0.13 (0.10)	-0.13 (0.34)
Obstruction + allop	1.24 (0.33)	0.23 (0.08)	0.26 (0.14)
Mann Whitney		p = 0.03	p = 0.45

Time 0 = MDA level. Time 10 and 20 mins. = change in MDA levels.
Allop = Allopurinol.

CONCLUSION: Free radicals, as expressed by MDA levels, are significantly elevated at 10 and 20 minutes after release of ureteric obstruction with allopurinol partially blocking this response. It is postulated that free radicals have a role to play in obstructive uropathy.

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ALTERED GLOMERULAR ENDOTHELIN RECEPTOR STATUS IN OBSTRUCTIVE UROPATHY.

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INTRO: It is established that renal vasoconstriction occurs in obstructive uropathy. The role of Endothelin-1 (ET-1), a potent vasoactive peptide, in obstructive uropathy is unknown.This study assesses glomerular endothelin-1 receptor status by an autoradiographic method after 24 hours of unilateral ureteric obstruction in wistar rats.

METHOD: Unilateral ureteric obstruction was created by ligation of the left ureter in five adult female wistar rats. A further five rats had sham operations. Both kidneys from the obstructed group and the left kidney from the sham group were removed 24 hours post-ligation and immediately snap frozen. 3 u sections of kidney were pre-incubated with 0.1% B.S.A. in P.B.S. and exposed to I^{125} labelled ET-1 (20pM, sp.act. 2000Ci /mmol) for 20 mins. A proportion of the slides were exposed to an excess of cold ET-1 and any bound radioactivity was defined as non-specific binding. Slides ~~were post-fixed with 2.5% glutaraldehyde and dipped in~~ Ilford G5 nuclear emulsion. After seven days at 4 C the autoradiographs were ~~developed and lightly stained with H&E. Using a Vidas Image analyser (Kontron),~~ the percentage area occupied by silver grains (which equated with bound ET-1) within the glomerular boundary was calculated for 20 outer cortical glomeruli for each slide.

RESULTS:

	1	2	3	4	5
R/CT %	102.4	82.7	119.3	88.6	89.9
OB/CT %	63.6	55.9	58.4	55.4	58.0

(R/CT and OB/CT = Average percentage silver grain area per glomerulus for the right unobstructed (R) and left obstructed kidneys (OB) / average precentage silver grain area per glomerulus of the control kidney).
Stats: Mann-Whitney p=0.009 95% Confidence Limits for reduction in receptor status in obstruction were (24.72% and 55.71%).

CONCLUSION: Endothelin-1 receptors are significantly down regulated in obstructive uropathy.

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OXALATE TRANSPORT ACROSS MONOLAYERS OF LLC-PK$_1$ CELLS

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Hyperoxaluria is a major risk factor in calcium oxalate nephrolithiasis. Oxalate excretion in the urine is governed by free glomerular filtration and renal tubular secretion. In the mammalian proximal tubule (PCT) it has been demonstrated that transcellular oxalate transport depends on distinct anion-exchange mechanisms. At the apical membrane a transport pathway has been described that mediates oxalate/chloride exchange, whereas at the basolateral membrane transporters have been detected that mediate the exchange of oxalate for sulfate and bicarbonate. The tubular fluid that enters the late PCT has a relatively high concentration of chloride, whereas the adjacent peritubular capillaries contain relatively little chloride. This natural transepithelial chloride-gradient in the late PCT has been recognized as driving force for para and transcellular chloride reabsorption in this segment. Since at the apical membrane the exit of oxalate depends on the entry of chloride, such a chloride-concentration gradient could also be a driving force for oxalate secretion. This hypothesis was tested with the proximal tubular cell line, LLC-PK$_1$. Unidirectional [^{14}C]oxalate fluxes were measured across monolayers cultured on porous supports in the presence or absence of a transepithelial chloride concentration gradient. Transport experiments were performed under voltage-clamp conditions in modified Ussing chambers. Under these conditions a secretory flux of oxalate could be detected that was dependent on the magnitude of the chloride gradient and sensitive to the anion-exchange inhibitor 4,4'-diisothiocyanato-stilbene 2,2'-disulfonic acid (DIDS). This model system could provide detailed information about renal tubular oxalate transport mechanisms that are possible involved in hyperoxaluria.

Study supported by the Dutch Kidney Foundation (grant C92.1235).

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TIME-DEPENDENT CHANGES OF MORPHOLOGY AND RESORPTION IN INTESTINAL SEGMENTS INTEGRATED IN THE URINARY TRACT

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Introduction: A report on elevated concentrations of methotrexate in serum of chemotherapy-patients with an ileal conduit (J. Urol. 143: 498, 1990) lead us to develop an experimental model to examine the resorption in intestinal segments, after they were integrated in the urinary tract. Results were compared with the resorption in normal intestine and urothelium.
Methods: 100 female Wistar-rats received a bladder augmentation with ileum. After 2 weeks, 4, 6, 10 and 12 months 20 animals were killed, the augmented bladder and a normal piece of ileum were removed. To characterize the capability of resorption a chamber model was used. The three tissues, bladder wall, augmented and normal ileum, were fixed in the chamber like membranes. The mucosal side was filled with a methotrexate or ofloxacin-solution. For two hours the transport across the membrane was measured. Concentrations on the serosal side were determined by high liquid chromatography. Histological sections were prepared to compare the mucosa of normal and augmented ileum.
Results: Histological examinations of the tissues confirmed the results reported in the literature. The mucous membrane of the augmented ileum underwent morphological changes, characterized by shortening of the villi and deepening of the crypts. There was no time-dependent decrease of resorption by the augmented ileum.Resorption by the intestine was higher than by the bladder, but even resorption by the bladder was higher than expected.
Conclusion: Our experiments confirmed previous reports as mentioned above. The surprising high resorption by the bladder wall will be of importance for the interpretation of intravesical instillation therapies.

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THE EFFECT OF ESTRADIOL BENZOATE AND OXYBUTYNIN ON ISOLATED RAT DETRUSSOR MUSCLES:A rationale for the clinical combination of estrogens and oxybutynin in low capacity bladders

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We studied the effects of estradiol benzoate and oxybutynin on the stimulatory action of acethylcholine on isolated rat detrussor muscles in vitro. Estradiol benzoate was given subcutaneously to female Wistar rats (n=7) in doses of 150 ug/kg for 10 consecutive days. A second group of rats (n=6), being injected saline for 10 days, served as control. On the 11th day the bladders were removed and placed in organ baths for isometric recording. The action of oxybutynin (10 nM) was investigated by adding it to the bath fluid. Estradiol benzoate traetment significantly reduced the amplitude of the contractile responses of the preparations to stimulation with acethylcholine Oxybutynin further shifted the concentration response surve to acethlcholine to the right without any change in its maximum, in preparations taken from estrogen group. These results suggest that oxybutynin potenializes the inhibitory effect of estrogen treatment on the contractions induced by acethlcholine in rat detrussor muscles. In clinical use, combination of estrogen preparations with oxybutynin has a rationale and will be useful in some conditions with low compliance bladder.

5A Molecular prognostic factors

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SIGNIFICANCE OF CONVENTIONAL AND NEW PROGNOSTIC PARAMETERS IN THE CASE OF LOCALLY CONFINED RENAL CELL CARCINOMA.

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The prognosis of patients with locally confined renal cell carcinoma is variable. For improvening the prognostic knowledge and selecting high risk patients additional prognostic parameters are needed.
The significance with respect to survival and tumor recurrence of "classic" and "new" prognostic parameters has been examined by following up the fate of a total of 41 patients after surgical removal of a locally confined renal cell carcinoma (mean follow-up time 5.2 years). The validity of the following parameters was evaluated: tumor stage, malignancy grade, Ki-67 index, PCNA (nulear cell proliferating antigen) index and ^{3}H-thymidine labeling index, the tumor cell ploidy (DNA index), tumor growth after xenotransplantation into nude mice (GAX range), epidermal growth factor (EGF), EGF receptor (EGFR), transforming growth factor α (TGFα) as well as c-erb-B2. The significance of the various prognostic parameters with respect to survival and tumor recurrence was tested using the Kaplan-Meier plots by long-rank-test or by Cox multiple hazard regression analysis.
Tumor stage ($p<0.0025$), malignancy grade ($p<0.005$), Ki-67 index ($p<0.006$) and GAX range ($p<0.00004$) were found to be significant prognostic parameters with respect to both survival and tumor recurrence using the single factor analysis. Applying the multivariate analysis the combination of the "new" factors GAX range and Ki-67 index showed even a higher prognostic relevance than the combination of the "classic" prognosticators. The calculated prognostic index based on the results of the Cox analysis which included Ki-67 index, stage and grade was shown to be highly correlated with survival ($p=0.00002$) and tumor recurrence ($p=0.0004$). Its prognostic validity was studied with the receiver operating characteristics (ROC) procedure and was found to be considerably superior to that of the two conventional prognosticators, tumor stage and malignancy grade.
In conclusion, the additional determination of the Ki-67 index increases the prognostic knowledge concerning patients with locally confined renal cell carcinomas.

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BIOLOGICAL DESCRIPTIONS OF LOCALIZED CARCINOMA OF THE PROSTATE (CAP) ORGAN CONFINED VERSUS NON ORGAN CONFINED DISEASE.

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Evidence has accumulated from radical prostatectomy that grade and finding a non organ confined disease on the step sectionned specimen are the strongest indicators of tumor progression. These data suggest that there is at this stage (i.e clinically localized Cap) different disease with different biological behaviors. In order to better understand the natural history of this disease at this stage, we characterized 35 radical prostatectomy specimen by means of immunohistochemistry using a panel of antibody-markers related to 1) epithelial differentiation cytokeratin8-18 (RGE 53), cytokeratin 5-14 (RCK 103), PAP, PSA, chromogranin A, E-cadherin, androgen receptor; 2) to proliferation Mib1, EGR receptor ; 3) to tumor control genes, P53 and Bcl2.
Results expressed in per cent of tumor cell stained were compared to pathological Gleason score, preoperative PSA and pathological staging.

	pT1-pT2 n = 15	pT3-pT4 n = 14	Capsular invasion n = 9	Positive surgical margins n = 9	Seminal vesical invasion n = 4
RGE 53	90.00	90.00	90.00	90.00	90.00
RCK 103	16.68	7.40	8.09	6.31	10.25
Androgen Rc	50.09	37.20	36.88	40.03	25.70
EGF Rc	13.09	4.74	7.08	5.33	3.28
Chromogranin A	0.81	0.80	0.64	0.86	0.12
MiB1	2.42	3.20	3.54	2.82	3.00
Bcl2	14.89	12.74	8.27	13.03	2.30
P53	0.86	1.36	1.73	1.02	1.40

There was no statistical difference for the expression of any of these markers and global pathological staging. However there is a parrallelism between the evolution of these markers and grade and adverse pathological findings.
It is unlikely that any of these markers is a clinical significant predictor of pathological stage but these findings support the hypothesis that Cap is a progressive, ineluctable, slow growing tumor. This approach may also help to define profile-markers associated with disease progression and molecular target for testing chemoprevention.

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PLASMA LEVELS OF CHROMOGRANIN A AND NEURON-SPECIFIC ENOLASE IN PATIENTS WITH PROSTATIC CARCINOMA.

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Neuroendocrine differentiation in conventionnal prostatic adenocarcinoma is a relatively frequent occurence and may be the predominant phenotype in some metastatic carcinomas. Also, the presence of neuroendocrine cells in the prostatic tumor has been associated to a poor prognosis and resistance to antiandrogen therapy. We report here the plasma level variations of two major neuroendocrine products associated with prostatic malignancy and their usefulness as new markers to follow prostatic carcinoma. Plasma chromogranin A (CGA) and neuron-specific enolase (NSE) were measured by double-site immunoassays in 145 patients with prostatic carcinoma (CaP). Elevated levels of CGA were observed in 3.4% of patients with localized disease (stages A and B, n=28) and in 17% of patients with advanced CaP (stages C, D1 and D2, n=52) before any treatment. High plasma values for NSE were also found in, respectively, 10% and 15% of patients in these two groups. After radical prostatectomy, all patients (n=18) recovered normal levels of CGA and NSE. Furthermore, in patients treated by androgen withdrawal therapy (n=20), 11% retained elevated concentrations of CGA and NSE. In hormone independent CaP (stage D3, n=27), plasma CGA and NSE were elevated for 55% and 30% of patients, respectively. In this group, Kaplan-Meier survival curves showed statistically significant difference between CGA (+) and CGA (-) groups (p=0.002) but not between NSE (+) and NSE (-) groups. Plasma chromogranin A appears to be complementary to PSA in monitoring CaP treatment. Although it does not seem helpful in predicting tumor progression, plasma chromogranin A levels may be useful to select patients for therapy using agents more specifically effective against neuroendocrine differentiation.

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CLINICAL SIGNIFICANCE OF CHROMOSOMES 8p AND 10q DELETIONS IN PROSTATE CANCER Hideyasu Matsuyama[1], Katsusuke Naito[1], Yi Pan[2], Bernhard Tribukait[2], Satoru Yoshihiro[2], Chunde Li[2], Ulf S.R. Bergerheim[2], Peter Ekman[2] ([1]Yamaguchi University, Ube, Japan, [2]Karolinska Hospital, Stockholm, Sweden)

In order to identify the clinical significance of deletions of specific chromosomal regions in prostate cancer, chromosomal deletions on 8p (LPL/8p22 and D8S7/8p23-pter) and 10q (D10S27/10q24-qter), putative tumor suppressor genes in prostate cancer, were studied in 53 patients with prostate cancer (30 prostates, 18 lymph nodes, 5 bone metastases) by fluorescence in situ hybridization (FISH) technique. The deletion data were compared with patient prognosis (tumor progression) in 26 patients whose tumors were confined to pelvic cavity before operations. All cases were followed up for 3 years or more after operations.

Out of the 53 specimens, 39 (74%) showed deletions of one or more probes of 8p. The distal deletion (D8S7) was detected in 25 cases (47%). Deletion of LPL was detected in 34 (64%), of which 14 (26%) retained the D8S7 (interstitial deletion). Deletion of D10S27 was seen in 26 (55%) of the 47 cases.

Significantly shorter progression-free survivals were observed in patients with LPL deletion than in those who retained LPL ($p<0.05$, generalized Wilcoxon's test). A Cox proportional hazard model revealed 8p22 deletion to be a significant prognostic factor predicting tumor progression, while neither D8S7, nor D10S27 showed any correlation to tumor progression.

These data support the hypothesis that the chromosomal region 8p22, in which a tumor suppressor gene may be located, plays a crucial role in the development of tumor progression, hence determination of 8p22 deletion might become as a diagnostic tool to predict patients at risk for tumor progression in prostate cancer.

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AUTOMATED IMAGE ANALYSIS OF PROSTATIC CARCINOMA.

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Introduction: In a prospective study the value of cytochemical and morphometrical parameters in the diagnosis of prostatic carcinoma was determined. Thirty human prostatic malignancies and 5 human prostatic tumor lines were investigated before and after endocrine therapy, using an automated image analyzer.

Material and method: Biopsy material of human prostatic tumors and of 5 humane xenografts (PC-82, PC-EW, LNCaP, PC-133, PC-135), permanently transplantable in athymic nude mice, were stained for DNA (Feulgen) and protein (Napftalin) and passed an optical controlled image analyzer (Becton-Dickinson Discovery). In each specimen DNA-ploidy, nuclear protein content, nuclear roundness and nuclear area was determined in 150 tumorcells.

Results: The DNA-ploidy showed a good correlation with histological grading: grade 1 tumors appeared diploid in 86%, Grade 2 tumors in 31% . Grade 3 tumors were aneuploid in 83%. The large number of aneuploid tumors in grade 2/3 tumors made DNA-ploidy as a single parameter of limited prognostic value. Under endocrine therapy and in cases of tumor progression an increase in ploidy was observed. A correlation was found between nuclear protein content and tumor progression, independent of PSA levels. Nuclear roundness showed considerable variation independently of tumor grade and tumor progression. A nuclear area > 200 um^2 was found in tumor cells only; no correlation with tumor progression was found.

Conclusions: A combined morphometrical and cytochemical analysis of prostatic tumors provides important information on tumor differentiation and cell kinetics.

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KGF-R, A GROWTH FACTOR RECEPTOR, IS LOST IN AGGRESSIVE TRANSITIONAL CELL CARCINOMA (TCC)OF THE BLADDER

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Growth factors and growth factor receptors can play a role not only in the growth of cells but also can have many other functions. They can act for example as scatter factors, inhibitory factors or differentiation factors. It is therefore not astonishing that growth factors and their receptors can be both positive and negative regulators in cancer.

KGF receptor is one of the splice variant of the FGFR2 receptor. It has been found in several epithelia. We have investigated the presence of KGF-R in the normal urothelium and in transitional cell carcinoma (TCC). Total RNA has been obtained from normal urothelium, lamina propria, muscle of normal bladder (N = 5) and various TCC (N = 57). A semi quantitative RT-PCR analysis, using TFIID as an internal standard, shows that in normal urothelium KGF-R was only present in the epithelium. In most of the carcinoma KGF-R mRNA was either unchanged or decreased: 15 tumors had a KGF-R level below 20% of the value found in normal urothelium. The same results were found using GAPDH as another internal standard or by Northen blot analysis. We also showed that Bek, the other plicing variant of the FGFR2 gene was not expressed in any tumor, thus the decrease of KGF-R mRNA is not due to a change in the splicing of the FGFR2 gene.

Patients with tumors having low level of KGF-R (below 20% of the normal value) had a poorer prognosis than the other patients. The difference was still significant when only patients with invasive tumors were considered.

We are now trying to determine if KGF-R plays an active role in the control of the tumor progression.

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MDM2 AND P53 IN EARLY BLADDER CANCER

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Earlier investigations have demonstrated, that inactivation of the p53 tumor suppressor gene (TSG) occurs in approximately 50% of advanced bladder tumors. Furthermore, p53 accumulation can be observed in a significant number of dysplasias and carcinomata in situ (TIS). The interaction between the gene products of the MDM2 gene and p53 TSG is well established. The aim of this study was to investigate the status of these two genes in early bladder cancer. The 146 specimens from 66 patients incorporated in this study include 8 dysplasias, 20 TIS, 63 Ta and 36 T1 lesions and 19 cases with advanced bladder tumor. The specimens were deparaffined and immunohistochemistry was performed using monoclonal antibodies DO1 and MDM2 (AB-1), clone IF2. Slides with more than 5% stained tumor cells were regarded as positive. No staining was observed for p53 and MDM2 in normal urothelium. While alterations of both genes were rare in dysplasias and in low grade papillary tumors P53 alterations were observed in approximately 40% of the other tumor stages examined. MDM2 overexpression increased from 15% in TIS to 53% in T1 tumors but was presented in only 26% of advanced tumors. Regarding both genes alterations were seen in 12% of the low grade tumors but increased to 80% in high grade lesions. To investigate the prognostic relevance of alterations of these genes 54 patients with superficial bladder tumors were followed for at least more than 2 years (mean 55 months). Uni- and multivariate analysis demonstrated that p53 accumulation was significantly correlated with tumor recurrence and tumor progression ($p<0.001$), while MDM2 overexpression alone had no prognostic significance. This study suggests a potential interaction between P53 and MDM2 in the progression of bladder tumors which will be subject of further in vitro experiments.

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TRANSFORMING GROWTH FACTOR-ß1 AND BASIC FIBROBLAST GROWTH FACTOR IN SERA OF BLADDER CANCER PATIENTS AND IN SUPERNATANTS OF PRIMARY BLADDER CELLS.

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Transforming growth factor-ß1 (TGF-ß1) and basic fibroblast growth factor (bFGF) have been described as potent stimulators of angiogenesis that have the ability to advance proceeding and metastasis of a tumor.
Using ELISAs we measured significantly elevated TGF-ß1 levels in sera of bladder cancer patients with invasive disease (T_2-T_4: 67.2 ng/ml; p=0,0001). Serum TGF-ß1 levels in patients with superficial tumors (T_a/C_{is}=26.2 ng/ml; T_1=35.9 ng/ml) however did not differ from the control group (36.3 ng/ml). Furthermore we noticed a correlation of TGF-ß1 amount in serum with tumorgrading. Poorly differentiated G_3-tumors had increased levels of TGF-ß1 (56.6 ng/ml) in comparison to G_1-tumors (33.3 ng/ml).
bFGF serum levels of bladder cancer patients were elevated (range: 8.34 to 15.75 pg/ml) in comparison to the healthy controls (1,37 pg/ml; p=0,003) but without any significant correlation with staging or grading, respectively.
Although both TGF-ß1 and bFGF were elevated in patients' sera compared to the controls we could not find a direct correlation of the two cytokines. In 4 patients treated with open cystectomy we noticed a striking similarity of the time courses of TGF-ß1 as well as of bFGF serum levels after surgery. Within 9-14 days after surgical removal of the tumor (pT_3G_3) TGF-ß1 serum levels increased continuously from 22,8-67,0 ng/ml to 66,8-154,9 ng/ml and finally fell off again (63,0-90,95 ng/ml). bFGF levels in contrast neither increased nor decreased but remained elevated to the control level despite tumor removal.
Using cell cultures we tried to elucidate the origin of TGF-ß1 and bFGF by measuring the 2 cytokines in supernatants of different human primary bladder cells. Both TGF-ß1 and bFGF were slightly higher in supernatants of normal epithelial cells (TGF-ß1: 100-1600 pg/ml; bFGF: 1-320 pg/ml) in comparison to the tumor cells (TGF-ß1: 4-520 pg/ml; bFGF: 1,5-18,5 pg/ml). Moreover, relatively high levels of TGF-ß1 (810-4000 pg/ml) were found in supernatants of stromal cells.
In summary, our results indicate an important function of TGF-ß1 and bFGF in bladder cancer formation and differences in cytokine production among different cell types.

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PREDICTIVE VALUE OF P53 MUTATIONS ANALYZED IN BLADDER WASHINGS FOR PROGRESSION OF SUPERFICIAL BLADDER CANCER.

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The tumor suppressor gene p53 acts as a cell cycle control protein at the level of G1 to S phase transition. If DNA damage occurs, p53 protein levels rise and block cells in the G1 phase to permit DNA repair processes or to initiate apoptosis and eliminate the abnormal cell clones that could lead to cancer. Mutations in the p53 tumor suppressor gene, which abolish the wild type p53 function, have been shown to occur in bladder cancer and are associated with both grade and stage. The relatively higher frequency of p53 mutations found in T2-T4 tumors compared to T1 tumors suggests its involvement in the progression of T1 tumors to invasive disease. However, the precise timing of p53 inactivation in the cascade of tumor progression from superficial (Ta) tumors to lamina propria invasive (T1) and muscle invasive (T2-T4) tumors is still unclear.

In order to get more insight into the timing of p53 mutations in this cascade, we analyzed p53 mutations (exon 5-9) by means of Single Strand Conformation Polymorphism analysis (SSCP) in bladder washing specimens of superficial bladder cancer patients. In our hospital, the follow up of patients with superficial TCC is done routinely by quantitative karyometric analysis of bladder washings (Quanticyt). In the group of patients that were followed by Quanticyt prospectively, progression was found in 14 patients. A control group of patients (n=14) was chosen with similar Quanticyt score (sex/age/follow-up matched), that showed no progression. Furthermore the transurethral resection material of accessory tumors of a number of patients were analyzed for p53 mutations, to investigate the reflecting capacity of the bladder washings for the genotype of the histological samples.

The preliminary results show that 9 out of the 14 patients that clinically progressed, have a p53 mutation, which was already shown in the bladder wash sample prior to progression. In the control group (no progression) 2 out of 14 patients showed a p53 mutation. A good concordance between the bladder washings and the accessory tumors is observed (sensitivity: 77.8%; specificity: 85.7%). These results implicate that the presence of p53 mutations in bladder washings reflects the status of p53 in the histological samples. Moreover p53 mutations are indeed involved in the process of tumor progression and have a positive predictive value for the risk of progression.

5 B Approaches to biological and gene therapy

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Characterization of the prostate specific membrane antigen (PSM) promoter. Warren D.W. Heston, Sai L. Su, C.Thomas Powell, and William R. Fair. Urologic Oncology Research Laboratory, Memorial Sloan-Kettering Cancer Center, New York, N.Y., USA 10021.

We have identified and isolated the promoter region of the Prostate-Specific Membrane Antigen gene. Sequencing of a 3 kb genomic DNA clone that contained 2.5 kb upstream of the transciption start site revealed that two stretches of about 300 b.p. (-260 to -600: and -1325 to -1625) have substantial homology (79-87%) to known genes. The promoter lacks a GC rich region, nor does it have a consensus TATA box. However, it contains a TA-rich from position -35 to -65. We identified several consensus recognition sites for general transcription factors such as AP1, AP2, NFKB, GRE and E2-RE. Chimeric constructs containing fragments of the upstream region of the PSM gene fused to a promoterless chloramphenicol acetyl transferase gene were transfected into, and transiently expressed in LNCaP, PC-3, and SW620 (a colonic cell line). With an additional SV40 enhancer, sequence from -565 to +76 exhibited promoter activity in LNCaP but not in PC-3 nor in SW620. (Supported in part by NIH grant DK/CA 47650 and the Koch Foundation.

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HIGHLY EFFICIENT GENE TRANSDUCTION OF BLADDER EPITHELIUM MEDIATED BY A RECOMBINANT ADENOVIRAL VECTOR

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A variety of molecular lesions have been demonstrated to be associated with carcinoma of the bladder. In this regard, tumor suppressor genes and dominant oncogenes have been shown to be associated with this tumor type. Based upon this concept, it is logical to consider gene therapy strategies as an approach to carcinoma of the bladder. As a first step towards this goal, methods must be developed to achieve efficient *in situ* gene delivery to bladder epithelium. To achieve this end, the utility of recombinant adenoviral vectors for urothelial transduction was examined. Initial analysis of vector efficacy was based upon *in vitro* transduction of human (HT 1197, HT 1376 and T24) and murine (MBT-2) transitional cell carcinoma cell lines employing a recombinant adenoviral vector. Transduction of these cells was accomplished employing an adenoviral vector encoding the firefly luciferase reporter gene (AdCMVLuc). In this study, bladder cell lines exhibited high levels of expression of the reporter gene after vector transduction. An analysis of the kinetics of heterologous luciferase gene expression demonstrated expression for up to 7 days post-transduction. To examine the efficacy of this vector at a higher level of stringency, primary cells derived from the bladder of humans and mice were evaluated for their susceptibility to adenoviral-mediated gene transfer. *In vitro* infection of these cellular targets demonstrated the additional efficacy of the vector in this context. Thus, efficient transduction of bladder cell lines and primary cells from mice and humans can be efficiently accomplished with recombinant adenoviral vectors. It is recognized that gene therapy strategies for bladder cancer in a clinical setting will require *in vivo* gene delivery of the bladder epithelium. In this regard, the recombinant adenoviral vectors have been shown to be capable of achieving efficient *in vivo* gene transfer in a variety of organs including liver, lung, and CNS. We thus evaluated the utility of the adenoviral vector for *in situ* transduction of the bladder after direct *in vivo* delivery. For this analysis, the AdCMVLuc vector was administered through the transurethral route to adult female BALB/c mice. Subsequent analysis evaluated the temporal pattern of the reporter gene expression after harvesting from the transduced bladders. Results indicated that high levels of reporter gene expression could be detected in the bladders of mice treated in this manner. Reporter gene expression could be detected for up to one-week post-vector delivery. As an additional assay of the magnitude of *in vivo* transduction, a Sprague-Dawley adult female rat was treated by the transurethral route with an adenoviral vector encoding the ß-galactosidase reporter gene. Histochemical analysis of the LacZ gene product in the treated animal demonstrated effective transduction of bladder epithelium. Extensive areas of stained cells indicated a relatively high rate of *in situ* transduction by this method. These studies demonstrate the utility of the adenoviral vector for the purpose of gene transfer to bladder epithelium cells. The demonstration of the capacity to accomplish efficient *in situ* gene delivery to the bladder provides a strategy to implement gene therapy approaches for carcinoma of the bladder.

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HIGH EFFICIENCY NEOPLASTIC REVERSION OF BLADDER CARCINOMA CELLS MEDIATED BY AN ANTI-ras RIBOZYME ENCODED IN A RECOMBINANT ADENOVIRAL VECTOR

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A variety of highly characterized molecular defects have been identified in the context of carcinoma of the bladder. In this regard, mutational lesions of the ras oncogene have been shown to be a key determinant of neoplastic transformation and progression in this neoplasm. Based upon this knowledge, a strategy has been developed to achieve ablation of the activated H-ras oncogene based upon the employment of a hammerhead ribozyme designed to cleave the mutant sequence in codon $12^{gly\text{-}val}$. This approach has been shown to be capable of achieving specific cleavage of the H-ras transcript in EJ bladder carcinoma cells with the reversion of the neoplastic phenotype *in vitro*. These studies, however, have been accomplished exclusively *via* the derivation of stable clonal populations of bladder carcinoma cells expressing the anti-ras ribozyme. For this method to be of practical utility in the content of anti-cancer gene therapies for bladder cancers, methods must be developed that yield high efficiency delivery of the anti-ras ribozyme to achieve neoplastic reversion of a population of neoplastic cells. To accomplish this, we have constructed an E1A-deleted, replication incompetent recombinant adenoviral vector expressing the anti-ras ribozyme. This ribozyme encoding vector (AdGT6) was thus employed to transduce H-ras EJ bladder cells. Subsequent analysis of vector transduced cells included parameters related to the molecular efficacy of the encoded anti-H-ras ribozyme and the phenotypic alterations of the EJ cells. Analysis of transduced cells confirmed that the AdGT6 vector was capable of inducing expression of the anti-H-ras hammer head ribozyme in the EJ target cells. Evaluation for the presence of the H-ras transcript demonstrated that the AdGT6 vector induced a marked down-regulation of this target. This inhibition was specific in that the AdGT6 virus did not induce alterations in the levels of control transcripts in EJ cells. A recombinant adenovirus encoding an irrelevant gene (E.coli LacZ) had no effect on the levels of H-ras transcripts. Thus, the AdGT6 vector could achieve a specific, targeted ablation of the H-ras transcript after adenoviral mediated gene delivery. The vector was then employed to infect EJ cells to analyze the phenotypic consequences of ribozyme expression within the neoplastic cell population. Evaluation of cell growth kinetics demonstrated that the AdGT6 vector could induce a marked inhibition of growth in the EJ cells. This effect was not seen upon transduction with irrelevant adenovirus. To determine if this proliferative arrest correlated with tumorigenicity abrogation, transduced cell populations were transplanted heterotopically to athymic nude mice. In this analysis, animals receiving untranduced control EJ cells or EJ cells transduced with irrelevant adenovirus demonstrated progressively growing tumors. In marked contrast, animals infected with the AdGT6 treated cells did not develop any visible tumors. These studies demonstrate that adenoviral vectors are capable of achieving high efficiency delivery of a functional ribozyme to bladder tumor cells. Further, this approach was able to achieve highly efficient reversion of the malignant phenotype of a population of tumor cells with abrogation of tumorigenicity. Thus, the development of high efficiency delivery vehicles may allow development of gene therapy strategies for bladder cancer on this basis.

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THE INFLUENCE OF INTERLEUKIN-1 BETA ON THE ADHESION OF RENAL CELL CARCINOMA TO ENDOTHELIUM IN VITRO AND IN VIVO

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Formation of hematogenous metastases requires in part direct adhesive interactions between tumor cells and vascular endothelium. Several cytokine-inducible endothelial cell adhesion molecules (e. g. VCAM-1, ELAM-1) seem to be important in this interaction. This study investigated the adhesion of three RCC-lines to lung microvasculature after stimulation with rIL-1ß and the adhesion molecules involved in these interactions. In addition, we examined the effect of rIL-1ß on the expression of VCAM-1 in nude mice and the organ distribution and arrest of radiolabeled tumor cells. In vitro, rIL-1ß stimulation of lung endothelial cells resulted in a 1.4 to 1.8 fold increased adhesion of RCC cells to lung endothelium. In vivo, rIL-1ß treated nude mice demonstrated 1.8 fold higher levels of radiolabeled tumor cells in the lungs as controls. Flow-immunocytometry studies revealed a significant enhanced expression of ELAM-1 and VCAM-1 on the lung endothelial cells after rIL-1ß stimulation and the presence of the ligands sialyl-Lewis X and VLA-4 on the RCC-lines. Furthermore, immunohistochemistry examinations of the rIL-1ß treated nude mice revealed a significant increased expression of VCAM-1 in the lung vessels. In order to determine the effect of these cell adhesion molecules on the interaction between RCC cells and lung endothelium in vitro, monoclonal antibodies (MoAb) directed against the ELAM-1/sialy-Lewis X and VCAM-1/VLA-4 system were used. MoAb directed against the ELAM-1/sialyl-Lewis X and VCAM-1/VLA-4 system resulted in a 30% ($p<0.05$) and 44% ($p<0.05$) decrease of RCC adhesion to lung endothelial cells, respectively. These results demonstrate that rIL-1ß increases adhesion of RCC-cells to lung microvasculature in vitro and in vivo, and that this increased adhesion is mediated in part by the ELAM-1/sialyl-Lewis X and VCAM-1/VLA-4 system in vitro.

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GROWTH SUPPRESSION OF RENAL CELL CARCINOMA CELL LINES BY A DOMINANT NEGATIVE H-*ras* MUTANT.

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N116Y, which derived from the v-H-*ras* oncogene by substituting the asparagine-116 with tyrosine, has been shown to exhibit a dominant negative activity and suppress the transformed phenotypes of NIH3T3 cells induced by overexpression of the H-*ras* protooncogene and several PTK oncogenes (Ogiso *et al.*, Exp Cell Res 208, 415-421, 1993). Since *ras* genes are highly conserved among mammals, N116Y can be a potent suppressor of human tumor cells. We therefore examined whether N116Y can inhibit the growth of renal cell carcinoma (RCC) cell lines.

In order to examine whether high expression of a dominant negative H-*ras* mutant, N116Y, affects tumor cell proliferation, we constructed an efficient N116Y expression vector, pZIP-N116Y, and transfected two RCC cell lines (ACHN, NT-2) by the lipofection procedure. Transfection of pZIP-N116Y completely inhibited the colony formation of ACHN and NT-2 and no cell survived after G418 selection. Although pZIP-N116Y may be a potent suppressor of RCC cells, it is possible that the suppressor activity of pZIP-N116Y depends on *neo* gene inactivation. To exclude this possibility, we examined the colony forming ability of 6 RCC cell lines (ACHN, NT-2, OSRC2, SMKTR-2, SMKTR-3, SMKTR-4) containing both pZIP-N116Y and pSV2*neo* by cotransfection of these plasmids at the rates of 10:1 and 0:1. Total amounts of DNA were adjusted to 11 µg with salmon sperm DNA. The numbers of G418-resistant colonies were 4% (ACHN), 6% (NT-2), 10% (OSRC2), 13% (SMKTR-2), 20% (SMKTR-3), and 9% (SMKTR-4) in comparison with the control transfection (0:1).

These results suggest that a dominant negative H-*ras* mutant, N116Y, can suppress the proliferation of RCC cell lines.

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VINBLASTINE AND DEXVERAPMAIL COMBINATION TREATMENT IN METASTASIZED RENAL CELL CANCER. MA Noordzij[1], A van der Gaast[1], KH Koehrmann[2], H Kupper[3], FH Schröder[1], GH Mickisch[1]. [1]Academic Hospital, Rotterdam, The Netherlands; [2]Mannheim Hospital, University of Heidelberg, Germany; [3]Knoll pharmaceutics, Ludwigshafen, Germany

Renal cell cancer (RCC) is known to be insensitive to a large variety of chemotherapeutic agents which is thought to be due to the overexpression of P-glycoprotein (Pgp), the product of the MDR_1-gene. Inhibitors of Pgp could enhance the results of chemotherapeutic treatment of several tumors. The first generation Pgp inhibitors displayed considerable side-effects, however. The ability of the second generation Pgp inhibitor dexverapamil (DVPM), a calcium entry blocker, to enhance vinblastine (VBL) cytotoxicity was studied in patients with vinblastine resistant RCC. In addition, pharmaco-kinetic studies of both VBL and DVPM were performed.

30 patients with histologically proven, progressive metastatic RCC were included in the study. They were treated with VBL cycles (5-day continuous infusions, 16 days recovery) as long as the tumor responded. If VBL-resistance was observed, oral DVPM was added, thus, all patients were their own control. DVPM doses were escalated, if possible. Tumor responses and side-effects were classified according to the WHO criteria. 22 patients were evaluable (2 or more cycles VBL + DVPM). 1 partial response, 3 minor responses, 8 stable diseases and 10 progressive diseases were noted. All serious side-effects were VBL related. Cardiovascular parameters changed significantly after adding DVPM to the treatment scheme. Hypotension was the dose-limiting factor for DVPM. VBL (1.4mg/m^2/day) + the maximum tolerated dose of DVPM was generally felt to be save and well tolerated. The mean peak concentration of DVPM was $\pm 6\mu$M; serum VBL levels decreased significantly if DVPM was added (from 3.8 to 1.8ng/ml), which may indicate higher intra-cellular VBL concentrations.

Application of a second generation chemosensitizer (DVPM) resulted in serum levels previously thought difficult to reach and may have led to higher intra-cellular VBL concentrations. Although the clinical effectivity of this particular treatment schedule was only limited, this approach needs further attention. Newer trials based on this idea are now in progress.

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RADIOIMMUNOLOCALIZATION OF HUMAN RENAL CELL CARCINOMA WITH I-131 LABELED CHIMERIC G250 MONOCLONAL ANTIBODY.

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In previous studies excellent localization of the murine monoclonal antibody (mAb) G250 in Renal Cell Carcinoma (RCC) tumors was shown (Oosterwijk et al. J Clin Oncol 11, 738, 1993). In all patients studied a HAMA reaction was observed, irrespective of the antibody protein dose administered (0.2-50 mg), hampering multiple administrations and thus preventing multiple dosing in radioimmunotherapy with this antibody. To circumvent the occurence of HAMA, a chimeric version of this mAb (c-G250) has been developed.

We have shown that chimeric G250 binds with the same affinity (K_{av}=4*10^9/M) to the same epitope as murine G250. Recently a phase I protein dose escalation study has been initiated to investigate the *in vivo* characteristics of c-G250.

Patients received a single intravenous infusion of 6 mCi I-131 labeled c-G250 (^{131}I-c-G250). Up to now, 9 patients have been studied. Planar images were acquired on day 1,2,3,5 and 7. Normal tissue samples and tumor biopsies were obtained at surgery. The immunoreactive fraction following iodination was $\geq$90%. In one patient the immunoreactivity of plasma cG250 was monitored up to 7 days p.i. and remained higher than 80%. In six patients clear tumor imaging was seen 24 hours post injection and in one patient the tumor and a known bone metastasis were visible as early as one hour after injection. Image quality improved over time. Two patients had an immunohistochemically confirmed antigen-negative tumor which were not visualized. Primary as well as metastatic renal tumors were imaged. Plasma clearance of ^{131}I-c-G250 was comparable to ^{131}I-m-G250 clearance with a $t_{1/2\beta}$ of 42 $\pm$ 8 hours. Gammacounting of the tumorous kidney removed at surgery showed remarkable high overall tumor uptake (range: 2.4 - 9.0 %ID). ^{131}I-c-G250 uptake in tumors was heterogeneous with focally very high uptake (0.34, 0.08, 0.04, 0.03 % ID/g in pts #1, #2, #4 and #7). A tumorous lymphnode also showed high uptake (0.0136 %ID/g, pat. #8) whereas uptake in all tumor-negative lymphnodes remained low, comparable to other non-tumor tissues (range 0.0006 - 0.0061 %ID/g, pat. #8). Tumor-to-serum ratios as high as 485 were observed 7 days p.i. As with the m-G250, saturable liver uptake was observed. Adverse events did not occur.

In conclusion, the *in vivo* behavior of ^{131}I-c-G250 in RCC patients seems comparable to the murine progenitor. Since the tumor uptake of c-G250 is far superior to almost all monoclonal antibodies, c-G250 seems to be a promising vehicle for radioimmunotherapy.

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ADOPTIVE IMMUNOTHERAPY OF HUMAN PROSTATE CANCER USING THE SCID MOUSE MODEL.

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Although adoptive immunotherapy (AIT) has been investigated as a modality for the treatment of a variety of human tumors, the study of this treatment for prostate cancer has been minimal. A major hindrance to the evaluation of AIT as a means to treat any cancer is the lack of controlled *in vivo* studies. To date, *in vivo* studies have been confined to the use of animal models of a limited number of human tumors or to relatively few clinical trials in limited patient populations. Animal models have had their limitations that include the inability to consistently expand and activate lymphocytes from all tumor models and the absence of many of the lymphocyte markers on animal cells that are available on human cells for use in the identification of cell populations. We have developed an *in vivo* model of human prostate cancer by growth of the tissue in the immunodeficient *scid/scid* mouse. Eighty eight percent of the *scid/scid* mice will grow human prostate cancer tissue following implantation. In addition, *scid/scid* mice injected with interleukin-2 (IL2) activated cells will demonstrate the survival of these transplanted cells. We have utilized this model system to study the *in vivo* effectiveness of AIT in the treatment of prostate cancer. We obtained prostate tumor tissue and the draining obturator lymph nodes (LN) from patients undergoing radical prostatectomy. A portion of the tumor was used to prepare a cell suspension by enzymatic digestion, and the remaining tumor implanted subcutaneously into C.B 17 *scid/scid* mice. The LN and tumor cells were cultured together in medium containing 1000 units/ml of human recombinant IL2. After four weeks of culture, the cells were counted, a portion used to flow cytometric analysis, and the remaining injected i.p. into the tumor-bearing mice. The mice received a single injection of 1-3 x 10^6 IL2-activated lymphocytes and five twice-daily injections of additional IL2. Controls received cells alone, IL2 alone, or no treatment. The treatment of IL2-activated cells plus IL2 produced a decrease in the size of tumors (28-45% decrease). The tumors in the control mice continued to grow beyond the size at the start of treatment (range from 18.7% to 186% increase). The spleen cells of the mice that received the human lymphocytes had a mean of 16.7% human CD45+ cells, 53% of which were CD3+. These data indicate the ability to use adoptive immunotherapy for the treatment of prostate cancer. Further investigations using this model system are aimed at increasing the efficacy of the treatment.

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EFFECT OF CANARY-POX (ALVAC) VIRUS-MEDIATED CYTOKINE GENE EXPRESSION ON PROSTATE TUMOR GROWTH

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The effect of local cytokine production on the growth of the mouse prostate tumor, RM-1, was investigated. Cytokine genes (IL-2, IFNγ, TNFα) and the co-stimulatory molecule, B7, were incorporated into the canarypox viral vector, ALVAC (Virogenetics, Inc). ALVAC is a canarypox viral vector that does not undergo productive replication in mammalian cells, although the protein production of extrinsic genes by infected cells is high. Parental ALVAC (CPpp) and ALVAC vectors containing cytokine or B7 genes were incubated with RM-1 cells for five (5) hours. Because preliminary studies using ALVAC-IL2 (vCP275) and -B7 (vCP268) showed these vectors induced high and equivalent expression of inserted genes with varying infection ratios, the 5:1 ratio was used for the following studies. Infected tumor cells were inoculated subcutaneously in the back of C57BL/6 mice, and tumor growth and survival were monitored. The growth of tumor cells infected with ALVAC-IL2, -IFNg (vCP271), TNFa (vCP245) and -B7 was significantly delayed ($p < .05$) compared to parental virus control. Optimal anti-tumor activity was observed with the ALVAC-TNFa vector; however, all animals ultimately died of tumor. Studies were performed to determine the effect of ALVAC-TNFa combined with either ALVAC-IL2, -IFNg, or -B7. Combination therapy with ALVAC-TNFa and -IL2 induced complete tumor regression in 60% of treated mice. The regression resulted in complete cure since no tumor recurrence was observed during an observation period of 100 days. Histological analysis of the tumor regression site revealed mononuclear infiltration and necrotic change. No cytotoxic T lymphocyte (CTL) activity toward RM-1 cell was observed in mice rejecting RM-1 tumors, although allogeneic CTL were observed to kill RM-1 cells. Furthermore, no protection against subsequent tumor challenge was present in RM-1 cured mice. Finally, RM-1 cells were demonstrated to be resistant to the lytic effects of TNFa in vitro. These data suggest that the ALVAC vectors efficiently transferred inserted genes into RM-1 cells and expressed these genes, and the combination of ALVAC-TNFa and -IL2 induced nonspecific immune mechanisms that resulted in tumor elimination. Further studies are in progress to assess the anti-tumor effects of ALVAC-GMCSF (vCP319) and -IL12 (vCP1303) alone and in combination with other cytokines.

6A Molecular prognostic factors

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Characterization of Cadherin/Catenin complexes in Renal Cell Carcinoma Cell Lines

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Cadherins are cell-cell adhesion molecules important for the maintenance of tissue integrity and cell differentiation. E-cadherin, the epithelial specific cadherin, is considered as an invasion suppressor gene, and is down regulated during the progression of many carcinomas. Renal cell carcinoma (RCC) is thought to originate mainly from renal proximal tubules, epithelium which does not express E-cadherin. Accordingly, most RCCs do not express E-cadherin. On the other hand, α and β catenins which are cadherin associated proteins are expressed both in proximal tubules and in many RCCs. To characterize the cadherin/catenin complexes present in RCC, we analyzed 7 RCC cell lines by immunoprecipitation with an anti α catenin antibody. In all the lines, β catenin coprecipitated with α catenin. In 2 cell lines a faint 120 kDa band corresponding to E-cadherin was seen. Interestingly in all cell lines, a higher molecular weight band was also present (in 2 cell lines this band migrated slightly faster than in the 5 others). On immunoblot, these bands reacted with an anti pan-cadherin antibody, suggesting that it represents a member of the cadherin family. RT-PCR, using degenerated oligonucleotides corresponding to peptides well conserved among the cadherins, generates a 160 base pair product expected for a cadherin. This will allow the identification of these cadherins and their cloning if they are unknown members of the family of cell-cell adhesion protein.

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HIGH LEVEL EXPRESSION OF THE CD44 VARIANT SHARING EXON V10 IN RENAL CANCERS. Masaharu Kan, Hiro-omi Kanayama, Kin-ya Akiyama, Seiichi Naruo, Susumu Kagawa. University of Tokushima, Tokushima, Japan.

Recently, cell surface glycoprotein CD44 has been reported to participate in a wide variety of cell-cell or cell-matrix interactions including tumor metastasis and showed differential expression of the splice variants in various cancers and normal tissues. To examine whether renal cell carcinoma display altered CD44 expression we performed reverse transcription-polymerase chain reaction (RT-PCR) analysis of CD44 in 38 specimens from renal cancer, normal kidney and metastases of 19 patients and 6 renal cancer cell lines. To detect the CD44 variants, we utilized the RT-PCR Southern blot method reported by Matsumura et al. One out of 19 (5.3%) renal cancer specimens expressed a larger molecular weight band than 1kb with probe S1, and our finding is different from previous reports in colon and breast cancer. The band patterns emphasized in RT-PCR were different in 13/16 (81.2%) cases between normal kidney and tumors, and about a 700bp band was especially emphasized in 12/16 (75.0%) tumor specimens and 4/6 (66.7%) cell lines. By cloning and sequencing of the 700bp band, we found that this variant is identical to the CD44 variant sharing only exon v10. Examination by Northern blot analysis has revealed that all tumors express a higher level of CD44 mRNA than paired normal kidneys. These findings suggested that the CD44 variants sharing exon v10 play some roles in renal cancer and are considered to be a useful marker in renal cancers.

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P53 GENE ALTERATION AS A PROGNOSTIC FACTOR FOR RENAL CANCER

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For renal cell cancer mutational inactivation of the p53 gene was described with extremely varying frequency, ranging between 6 - 60 % of investigated tumors using moleculargenetic techniques including SSCP - analysis and DNA - sequencing. To exclude the influence of genetic background signals such as tumor infiltrating lymphocytes on the outcome of moleculargenetic analysis we have established a microdissection technique which allows the retrospectively performed analysis of formalin fixed and paraffin embedded tumor tissue. Tumor areas for analysis were isolated from tissue sections by a microscopically guided "scraping technique" according to histopathological criterias as well as the immunohistochemical staining reaction for the CD3 antigen and the p53 oncoprotein (pAb 1801). Following the selection of about 100 tumor cells, PCR - directed molecular genetic analysis was performed. A new highly informative allelotyping approach for the detection of allelic loss (LOH), determining BstUI- and VNTR - polymorphisms, a 100 bp marker directly localized in intron 1 of the p53 gene, as well as the screening for mutations by single strand conformation polymorphism analysis (rSSCP) in exons 5 - 8 were used. In total, 33 of 44 (75 %) of renal cancer specimen were informative for PCR - directed RFLP - analysis. Allelic loss at the p53 gene locus was observed in 10 of 33 cases (33 %). In 2 tumors the presence of point mutations at the p53 gene locus as indicated by mutational shift during rSSCP - analysis, was confirmed by DNA - sequencing. In 5 of 10 cases allelic loss at the p53 gene locus was observed in tumor stages > pT2. A significant correlation between p53 gene alteration and the T - stage or the histological grade as well as the histological differentiation could not be observed. Alteration of the p53 gene as detected by a moleculargenetic as well as immunohistochemical approach has been tried to be correlated with overall survival time. During univariate analysis (log rank test) only the histological grade, the lymph node status and the presence of distant metastases could be identified as prognostic parameters for overall survival. For patients revealing allelic loss at the p53 gene locus just a tendency towards a decreased long term survival was evident. Summarizing these results, it seems unlikely that p53 gene alterations will serve as an important new prognostic factor for the clinical prognosis of the patients with renal cell cancer.

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GENE-SPECIFIC DNA REPAIR AND SENSITIVITY TO CISPLATIN IN HUMAN TESTIS TUMOUR CELLS. [1]John R Masters, [1]Beate Köberle, [2]Lloyd R Kelland, [3]Keith A Grimaldi and [3]John A Hartley. [1,3]University College London, [3]Department of Oncology and [1]Institute of Urology and Nephrology, 3rd Floor, 67 Riding House Street, London W1P 7PN, UK and [2]CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK.

Metastatic cancer in adults is usually fatal. In contrast, 80-90% of patients with metastatic testicular germ cell tumours are cured using cisplatin-based combination chemotherapy. Sensitivity to DNA-damaging agents is retained by human testis tumour cell lines *in vitro* (Masters *et al.*, Int J Cancer **53**: 40, 1993). Cisplatin is also the most effective single agent for bladder cancer, but few if any patients with metastatic disease are cured. Cisplatin binding to whole genomic DNA was compared in 3 testis and 3 bladder cancer cell lines using atomic absorption spectrophotometry (AAS). Paradoxically, initial cisplatin binding to DNA was lower in the testis tumour cells. However, all the bladder and one testis tumour line (SuSa) rapidly removed cisplatin from their genome, whereas the other testis tumour cell lines (833K and GCT27) showed little evidence of DNA repair over a 48h recovery period. Using quantitative PCR (Q-PCR), we measured the repair of an inactive (*CD3*) and an actively transcribed gene (*N-RAS*). The amount of initial binding and the rates of cisplatin adduct removal were similar for both genes in each of the cell lines. In complete agreement with the data obtained by AAS of the whole genome, the bladder lines and SuSa were repair-proficient, whereas the other 2 testis tumour cell lines showed little capacity for repair. These findings indicate that, at least in part, the sensitivity of testis tumour cells to DNA-damaging agents is due to a reduced capacity to repair DNA damage.

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CYTOKERATIN 20 EXPRESSION IN UROTHELIAL DYSPLASIAS AND CARCINOMAS

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Cytokeratin 20 (CK20) expression is restricted to the terminally differentiated superficial cells in normal urothelium. We have investigated its distribution in the early stages of urothelial neoplasia and have found that CK20 expression was deregulated in neoplasia, with loss of restriction of expression pattern.

In dysplasia (n=31), CK20 expression was seen through the full thickness of the urothelium, including basal cells. This pattern was never observed in normal (n=11) or inflamed urothelium with proliferative lesions (n=6).

CK20 was expressed in a proportion of non-invasive TCCs (19/29). Patterns of CK20 expression were predictive of non-recurrence in 57% of patients who did not develop further tumours on 5 year follow-up.

We have therefore shown the potential of this marker:

1. as an objective criterion in the diagnosis of dysplasia. This could serve to reduce the recognised inter- and intra- observer variation and identify patients at risk of tumour development;

2. in the distinction between recurrent and non-recurrent non-invasive transitional cell carcinomas (TCCs), in order to distinguish benign versus malignant behaviour and to determine treatment strategies.

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MULTIPARAMETER-DNA-ANALYSIS OF *IN VITRO* AND *IN VIVO* UROTHELIAL TUMORS WITH UROTHELIAL-ASSOCIATED GLYCOPROTEINS.

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Object: A panel of urothelial-associated glcoprotein was tested on in vitro and in vivo bladder cancer material in dual parameter staining with DNA to judge on cell cycle relation of the antigens as well as to define distribution patterns in bladder cancer focussing on papillary G2 carcinomas.
Method: Cloned cell lines with different urothelial differentiation were grown to different growth states (exponential and plateau monolayers, threedimensional multicellular spheroids [MCS]), and consequently dissociated to single cell suspensions and double stained with either Uro1, Uro5, Uro9, Uro10 (all Signet) and propidiumiodide (PI). Similarly 57 freshly dissociated urothelial neoplasms were stained with these antibodies and and PI . Suspensions from *in vitro* and *in vivo* material were measured on a FACScan (Becton Dickinson [BD]) using Cell-Fit and Lysis software (BD).
Results: *In vitro* a cell cycle relation of Uro1 and Uro10 was found in contrast to none in Uro5 and Uro9, emphasizing the role in differentiation of the latter two. In contrast to the *in vivo* situation Uro10 was expressed in all cell lines independant of differentiation, indicating a selection for more aggressive cells *in vivo*, since this antibody did not stain any highly differentiated *in vivo* tumors. It did show, however, an interesting variability in G2-superficial tumors that has to be related to prospective clinical analysis.Uro5 turned out top be a very selective lineage marker for urothelium, showing no cross reactivity to other cells, and being maintained *in vivo*.
Conclusion: Phenotying of cancer can be supported by multiparameter flow cytometry, and may help define biologically significant subgroups in bladder tumors.

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DUAL-PARAMETER IMMUNO FLOW CYTOMETRY IN MONITORING OF BLADDER CANCER PATIENTS.

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Apparent limitations of the current follow-up examinations in monitoring of patients with high risk bladder tumors necessitate the development of new, preferably non-invasive techniques for the diagnosis of transitional cell carcinoma (TCC). Dual-parameter immuno flow cytometry (DPI-FCM) is a method based on the utilization of two monoclonal antibodies (mAbs) used for preselection of urothelial cells (mAb Due AUT 2) and analysis of the expression of an differentiation antigen within the preselected group of cells (mAb Due ABC 3). DPI-FCM, previously shown to have high sensitivity (95%) and specificity (93%) detecting malignant cells in bladder barbotage specimens from bladder cancer patients with endoscopically visible tumors, was examined in patients previously treated for bladder cancer. DPI-FCM employing mAbs Due AUT 2 and ABC 3 was performed in bladder washings from 30 patients with endoscopically normal appearing mucosa six weeks after TUR-BT. According to an increased amount of Due ABC 3-positive cells, 16 patients were considered abnormal. Rebiopsy confirmed tumor presence in 10 of these patients and in two with negative DPI-FCM findings, yielding sensitivity of the method in this group of patients of 83%. In the remaining six positive patients histology revealed dysplastic urothelial alterations in three of them and urothelial hyperplasia in the remaining three.
All of these six patients had previously multifocal and/or high grade tumors and further examinations disclosed tumor recurrence at 4 and 12 weeks after DPI-FCM examinations in two of them. Further observations of the patients and ongoing clinical investigations should determine the potential prognostic value of abnormal DPI-FCM results in monitoring of TCC patients. Our study has shown clinical significance of DPI-FCM in the detection of tumor recurrence in the follow-up of patients with bladder cancer.

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DECREASED E-CADHERIN EXPRESSION IS AN INDEPENDENT PROGNOSTIC INDICATOR IN BLADDER CANCER

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Down regulation of E-cadherin has been related to ability of cancer cells to metastazed in vitro and to prognosis in human neoplasms, specially bladder cancer. We compared expression of E-cadherin at the protein and in RNA level by mean of immunohistochemistry and RT-PCR on 36 specimens. There was a concordance between immunohistochemical pattern (positive, heterogenous, negative) and the level of mRNA expression mesure by semi quantitative RT-PCR.
The prognostic value of E-cadherin expression was further studied on 111 TCC by immunohistochemistry with the following pattern of staining according to stage and grade.

	Ta	T1	T2	T3	T4	G1	G2	G3
Positive	13	27	5	3	2	16	23	11
Négative / heterogenous	2	18	15	19	7	13	9	39

E-cadherin abnormal expression is associated with adverse prognosis regarding recurrence ($p = 0.004$) and progression ($p < 0.001$) using Kaplan Meier estimates for event-free survival. Using stepwise logistic regression this parameter was found to provide independent prognostic information compared to stage and grade ($p = 0.005$, OR = 2).

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Does Catenin Expression Provide Additional Prognostic Information over E-Cadherin alone in Human Bladder Tumors?

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Homotypic intercellular adhesion in epithelia is mediated primary by the E-cadherin (E-cad)/catenins complex. In a previous study we have shown that E-cad expression has prognostic value in bladder cancer. The function of E-cad is thought to be regulated by its associated cytoplasmic proteins including α-,β- and γ-catenin. In this study we have compared the expression of E-cad and three catenins in 38 human bladder tumors by immunohistochemistry using anti E-cad, α-, β- and γ-catenin antibodies. E-cad and catenins expression patterns were graded as normal (N;uniformly positive) or aberrant (A;heterogenous or uniformly negative). The abnormal expression of these molecules was significantly correlated to stage and grade of the tumors. Kaplan-Meier survival analysis showed that aberrant expression of catenins was strongly correlated to poor prognoses of the patients as well as the expression of E-cad ($p<0.01$). Comparative analysis of the E-cad and catenin expression patterns are illustrated in the table below. Even though there was a high degree of concordance, discrepancies in expression between E-cad and catenins were also observed in 10 (26.3%) out of 38 bladder tumors. Since those patients with abnormal expression of either E-cad or catenins had poor prognoses, it is suggested that the evaluation of the catenins expression pattern can give additional information over E-cad evaluation alone as a prognostic factor.

E-cad \ catenin	N	A
N	19	3
A	7	9

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P53 NUCLEAR OVEREXPRESSION IN UROTHELIAL BLADDER TUMORS

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Introduction: Mutations in the p53 gene leading to accumulation of the p53 gene product are often found in invasive urothelial bladder tumors. In this study we determined p53 overexpression in epithelial tumors with different grade and stage. Purpose of this study was to stratify groups of patients in view of prognostic value.
Methods: From 77 samples of epithelial bladder tumors we chose those paraffin blocks representing the most aggressive tumorpart in regard of infiltration and differentiation. P53 nuclear overexpression was analysed immunohistochemically on 2μm sections using the monoclonal antibody DO-1 (Oncogene Science). Tumors were devided into three groups according to their p53 nuclear expression; 1) no expression, 2) <20% of the nuclei expressing p53 and 3) >20% of the nuclei expressing p53.
Results: P53 expression was never found in normal urothelium. None of the G1 tumors (0%), 22 of 40 G2 tumors (55%) and 17 of 27 G3 tumors (62%) showed p53 overexpression. 25% of the G2 tumors showed overexpression with >20% of the nuclei positive as did 50% of the G3 tumors. At differentiation according to stage, the same relation was found (pTa: 13/34 = 38%; pT1: 15/23 = 65%).
Conclusion: Nuclear p53 accumulation in epithelial bladder tumors correlates with differentation grade and pathological tumor stage. This might add to the prognostic differentiation of patients having tumors with identical grade and stage.

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PRELIMINARY SIGNIFICANCE OF p53 OVEREXPRESSION IN T1 BLADDER CANCER. A PRELIMINARY STUDY.

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In bladder cancer it is of utmost importance to determine patients prognosis in early oncogenetic stages, as different treatment approaches may be disposed of. In our preliminary study we have analyzed p53 overxpression by immunohistochemistry with the DO-7 monoclonal antibody in 38 consecutive T1 bladder cancer patients who came under our observation in the years between 1985 and 1987 and who have been subsequently followed in our Institution up to five years. Of the 38 cases, 12 did not show any tumor relapse or progression, 18 had a T1 relapse and 8 had tumor progression. Two (16%) out of 12 no relapsing, 11 (60%) out of 18 T1 relapses and 7 (87%) out of 8 progression have shown p53 positivity of nuclear and/or cytoplasmic pattern. Among patients with T1 relapse, p53 positiva ones show an augmented relapse risk of abaut 35%, our data actually indicate that, in a five years period, 58% of the p53 negative cases will still be free from replase, while 38% of the p53 positive ones will not (p=0.07 log-rank). In the progression group, where only one patient was p53% negative, a progression time curve has been obtained, showing that in a five years period the progression risk for p53 positive patients is of about 35%. In conclusion, our data, suggest that: p53 overexpression in T1 bladder cancer cases could be related to both relapse and progression. Also if, statistically significant data must be obtained on larger series of patients to confirm our results, the treatment of "early" bladder cancer might be differentially oriented in relation to p53 positivity.

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NONINVASIVE DIAGNOSIS OF BLADDER CANCER USING THE COMET ASSAY.

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Current methods for the noninvasive diagnosis of bladder cancer are not sufficiently sensitive. To address this problem we have used the Comet Assay (single cell gel electrophoresis assay). This is a rapid, simple and sensitive technique for demonstrating and quantifying nuclear damage in individual mammalian cells.

Voided urine and bladder washing specimens were obtained from 71 patients. Samples from 15 control patients and 9 bladder tumour patients were found to contain sufficient cells for analysis. These cells were embedded in layers of agarose on specially prepared microscope slides and the cell membranes were lysed. The cells were then exposed to electrophoresis at high pH. After staining the nuclear DNA with ethidium bromide, the resultant fluorescent "comets" underwent image analysis.

DNA damage as quantified by the Comet assay was found to be significantly higher in bladder tumour compared to control specimens. Comet optical density was also observed to be increased in bladder tumour specimens relative to controls, indicating DNA ploidy.

The findings of this study indicate the potential value of the Comet assay as a noninvasive means for the diagnosis of bladder cancer. Comet optical density is a potential measure of ploidy, correlating with tumour grade and thus prognosis.

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CHEMICAL INDUCED BLADDER CARCINOMA IN MICE- AN EXPERIMENTAL APPROACH FOR LOCALLY ADVANCED DISEASE

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We used BBN (β-hydroxybutylnitrosamine) as carcinogen (0.05 % BBN/drinking water x 10 weeks) in female mice (strain B6D2F1) and found that 11/27 (41 %) mice developed locally advanced transitional cell carcinoma (TCC, ≥ pT3). In order to improve drug therapy we compared two different treatment regimes in BBN model system

	n	TCC ≥ pT3b	p-value
control	27	11 (41 %)	
IFN α 2b[1)]	27	10 (37 %)	> 0.5
MMC [2)] APV [3)]	28	2 (7 %)	< 0.01

1) IFN α 2b: Interferon alpha 2b 10 x 3 x 10^5 IE i.p.
2) MMC: Mitomycin C 5 x 0.06 mg i. p.
3) APV: acellular pertussis vaccine 5 x 0.08 ml i. p.

The intraperitoneal (i.p.) immunotherapy with IFN α 2b has no influence on tumor progression. On the other hand we determined reduced local progression in the combined treatment regime.
The BBN model is a useful experimental approach in mice for locally advanced bladder cancer disease.

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ANALYSIS OF A NEW CDNA-SEQUENCE WITH PARTIAL HOMOLOGY TO PROSTATE SPECIFIC ANTIGEN

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The prostate specific antigen (PSA) is known as the most useful tumor marker in prostate carcinoma (CaP). RT-PCR with primers against PSA for the detection of hematogenous micrometastases was shown as a new useful tool for the prognosis of patients with CaP. In an attempt to establish such an assay we used an upstream primer from exon 2 and a downstream primer from exon 4 of the PSA gene, in regions with only low homology between PSA and other proteins of the kallikrein family. In initial experiments the crude RNA from LNCaP cells was isolated and reverse transcribed with random primers. From this cDNA, PCR with the choosen primer was performed and the resulting fragments were analysed by gel electrophoresis. The PCR lead to two fragments with different molecular weights. Size determination showed that one fragment had the expected molecular weight refering to an amplification from PSA-RNA. The other fragment was cloned and partially sequenced. The analysis of the DNA-sequence revealed identity of the fragment to PSA in the primer region. In other parts of the fragment there was no homology found between it and PSA or other known DNA sequences. RT-PCR with RNase digested RNA showed that this fragment resulted from cDNA-synthesis of RNA and not from DNA contamination. Other RT-PCRs revealed that the accompanying RNA is expressed in LNCaP cells and in benign prostate tissue. In conclusion we could find a cDNA from prostate tissue which is in part homologous to PSA but not to other known DNA sequences. In further studies it will be investigated wether this marker is useful in the diagnosis of CaP.

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PSA in aspiration biopsies is a good predictive test for treatment success in patients with prostatic carcinoma.

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Background: In order to choose the best treatment for patients presented with prostatic carcinoma it should be of value to use a predictive test that could select patients that will benefit from a special treatment.

Methods: We have retrospectivly evaluated a number of different ways of using PSA as a marker in 110 patients treated for prostatic carcinoma beginning 1986 to 1990. Patients were TGM classified and samples taken before treatment were analysed regarding PSA (total and free) and total PSA /µg DNA in aspiration biopsies.

Results: No correlation was found between serum-PSA and PSA in aspiration biopsies.
A inverse correlation was found between concentration of PSA in aspiration biopsies and grading. Of the markers used total PSA /µg DNA in aspiration biopsies was the best test to predict time to regression and time to death.

Conclusion: Measurment of total PSA /µg DNA in aspiration biopsies can give a new way of using PSA as a predictive test for prostatic carcinoma.

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RELATION BETWEEN TISSUE AND SERUM LEVELS OF PSA/PAP AND PROSTATE VOLUME IN PATIENTS WITH PROSTATIC DISEASES

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Introduction:The relation between PSA/PAP levels in serum and tissue as well as the volume of the prostatate may be clinically important for the diagnosis and prognosis of patients with prostatic diseases. In this study we investigated PSA/PAP expression in prostatic tissues by means of immunohistochemical analysis and related the findings to serum PSA/PAP levels and prostate volume.
Methods: Immunohistochemical analyses using monoclonal and polyclonal anti-PSA and anti-PAP antibodies was performed on paraffin embedded prostate tissue of 45 benign prostatic hyperplasia (BPH) and 42 prostatic carcinoma (pCa) patients. Serum PSA/PAP levels were determined using an enzyme immunoassay and prostate volume was calculated from the three largest diameters x 0.52 of the surgical specimens.
Results: The proportion of stained cells with anti-PSA as well as anti-PAP in BPH was 90% In well differentiated pCa the percentage was 65% and in poorly differentiated pCa 20%. Also the intensity of staining was strongest in BPH and reduced with dedifferentiation of pCa. Mean serum PSA in BPH patients was 6.18 +/- 0.73 ng/ml, in patients with well differentiated pCa, 6.66 +/- 1.17 ng/ml and in patients with poorly differentiated pCa, 11.80 +/- 1.12 ng/ml. No difference in serum PAP levels could be measured in the different patient groups. Mean prostate volume in BPH was 75 cc and in pCa 45 cc.
Conclusions: Immunohistochemical staining of PSA/PAP in pCa tissues is significantly lower than in BPH. Dedifferentiation of pCa thereby is associated with a decrease of intraprostatic PSA/PAP. In serum however the opposite is found: PSA levels increase with dedifferentiation whereas serum PAP levels are found to be constant. Mean prostate volume in BPH is significantly larger than in pCa indicating that no relation exists between prostate volume and PSA/PAP levels. High concentrations of serum PSA or high serum PSA/prostate volume ratio's, or a weak immunohistochemical PSA/PAP staining suggest a poorly differentiated pCa and possibly a bad prognosis.

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HETEROGENEITY OF E-CADHERIN EXPRESSION IN PROSTATE CARCINOMA: An immunohistochemical study in radical prostatectomy specimens

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Objective: At present, there is no reliable way to predict the clinical course of patients with prostate cancer. To address this problem, markers are sought that have a predictive value for prognosis. Of particular interest is E-Cadherin (EC), a cell adhesion molecule which decreased expression is associated with invasive tumor phenotypes. However, the degree of EC heterogeneity within the prostate tumor is not well documented. Analysis of EC heterogeneity in prostate tumors is important for future implementation of the staining on biopsies. Therefore we evaluated intratumor heterogeneity of prostate cancer based on EC expression in a clinically relevant setting.

Materials and Methods: A series of 25 radical prostatectomy specimens was included in this study. Immunohistochemistry with EC antibodies was performed on formalin-fixed, paraffin-embedded material. The EC staining pattern in a tumor was scored as complete normal or complete lost. A threshold of approximately 10% aberrant staining tumor cells was defined as a heterogeneous expression.

Results: Most cases had a similar, diffuse EC pattern (either normal or lost) throughout the entire tumor. In a substantial number of cases, however, intratumor EC expression was heterogeneous. The heterogeneous pattern most often identified was two separate tumor cell populations within a cancer lesion. The minority of heterogeneous cases had a diffuse pattern. The amount of aberrant EC expressing tumor cells in a heterogeneous tumor varied from 10-90%.

Conclusion: Although the presented results are preliminary, intratumor EC expression as detected immunohistochemically was frequently heterogeneous. Based on this observation, we deduced that EC expression of prostate biopsies may not always be representative for the EC expression of the entire tumor. We will continue to evaluate this by including both the prostatectomy specimens and biopsies of the same patients.

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TENASCIN EXPRESSION IN PROSTATIC INTRAEPITHELIAL NEOPLASIA (PIN): IS TENASCIN EXPRESSION ASSOCIATED WITH TISSUE REMODELLING IN PROSTATIC NEOPLASTIC TRANSFORMATION ?

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BACKGROUND: Prostatic intraepithelial neoplasia (PIN) is currently defined pre-malignant lesion related to prostatic adenocarcinoma (PCa)(Brawer 1990). According to the multi-step theory of neoplasia (Medline and Farber 1981), PIN is suggested to be a histological process proceeding the transformation from benign tissue to neoplasm. We assume that in this remodelling process, there is a disturbance in the interaction between epithelium and extracellular matrix (ECM). Our previous study indicated that tenascin (Chiquet Ehrismann et al. 1986), as an ECM glycoprotein, was induced in prostatic lesions with active epithelial-stromal interaction. This prompted us to further evaluate the possible role of tenascin as a marker in tissue remodelling process presuming associate with PIN.

METHOD: Semi-quantitative immunohistochemistry was applied on paraffin tissues from radical prostatectomy. We analyzed the distribution pattern and intensity of monoclonal antibody against tenascin in 38 PIN lesions, which were compared with about equivalent amount of non malignant fields (normal-looking pre-existing gland, benign prostatic hyperplasia, BPH) and PCa lesions.

RESULTS & CONCLUSION: 1) Tenascin expression increased from non-malignant through PIN up to PCa (GG2,3). In particular, the values in PINlow were close to those in pre-existing and BPH glands where as the expression of tenascin in PINhigh close to and partly overlapped with those in PCa (GG2,3). 2) The induced tenascin immunoreaction was most pronounced directly surrounding the basement membranes of PIN lesions indicating that the stimulation for tenascin synthesis and secretion came mainly from the reshaping PIN lesions. However, the paracrine effect from the adjacent tumors was not negligible. 3) PINlow was not different from non-malignant group, in term of tenascin expression. This could be interpreted that since PINlow has an almost intact basement membrane, interaction of epithelium and ECM was as low as non-malignancy, remodelling activity was therefore not pronounced. These results suggest that tenascin can be useful in classifying PIN lesions on basis of its apparent usefulness in indicating tissue remodelling process. From tenascin gene expression pattern point of view, as a precursor of tumor, PIN links non-malignant and PCa.

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PRETREATMENT P53 IMMUNOREACTIVITY DOES NOT CONFER RADIORESISTANCE IN PROSTATE CANCER

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Purpose To test, in a clinical context, the hypothesis that p53 mutations, expressed by immunoreactivity, is related to radioresistance as suggested by several experimental studies (1).

Patients and Methods: Sixty patients with prostate cancer who underwent transurethral resection of the prostate or biopsy prior to definitive external beam therapy were retrospectively identified. The endpoint in the study was cancer specific survival. The nuclear accumulation of the aberrant p53 protein was determined by immunohistochemistry with the pantropic, monoclonal Ab-6 anti-p53 antibody (clone DO-1, Oncgene Science) on tissues obtained through TUR or biopsy prior to radiation.

Results: No significant difference was found in cancer specific survival between the p53 positive tumors (109 months) and the p53 negative tumors (99 months), log rank 0.25, N.S.

Conclusions: No survival disadvantage was seen for patients with p53 immunoreactive tumors, implicating that p53 immunoreactivty does not cause radioresistance in prostate cancer. Thus p53 inactivation may be a less important determinant of tumor response to radiotherapy in some humane cancers than in the hitherto studied experimental situations. Other mechanisms may be more important in determining outcome after radiation. However, the series is small and data should be interpreted with caution.

Ref: 1 Lowe et al. p53 status and the efficacy of cancer therapy in vivo. Science 266; 807-810; 1994.

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IMMUNOHISTOCHEMICAL AND MUTATION ANALYSIS OF P53 EXPRESSION IN HUMAN PROSTATIC TUMORS

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Introduction: Contradictory results have been reported concerning the incidence and relevance of p53 mutations in prostatic carcinoma. In this study we performed an immunohistochemical screening of p53 mutations in human prostatic carcinoma specimens and related the results to genetic analysis of the p53 gene in the same tumor areas using Single Strand Conformation Polymorphism (SSCP) as well as sequencing techniques.

Methods: Immunohistochemical analysis using the monoclonal antibody DO-1 (Oncogene Science) was performed on paraffin embedded tissues of 50 primary tumors and 15 lymphnode metastases. Mutation analysis of tumor areas with nuclear p53 overexpression was performed by means of SSCP and DNA sequencing using standard techniques.

Results: 9 of 50 primary tumors showed nuclear p53 accumulation. Except of one case in which elaborate tumor areas were found to be positive, overexpression was focal and heterogeneous. 4 out of 15 lymphnode metastases were found to be positive for p53 and overexpression in these samples was found in almost all nuclei. SSCP and sequence analyses confirmed the immunohistochemical data.

Conclusions: p53 overexpression was found in about 25% of the lymphnode metastases indicating an association with progression of prostatic tumor formation. Only 4% of the primary tumors showed elaborate tumor areas with nuclear staining. The biological meaning of the focal overexpression in the other primary tumors is subject of further analyses.

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Expression of Bcl-2 and androgen receptors in carcinoma of the prostate.

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The bcl-2 proto-oncogene is said to contribute to malignant cell expansion primarily by prolonging cell survival rather than by increasing the rate of cellular proliferation, in other words by delaying or blocking apoptosis. It has been studied in a number of malignancies including breast, thyroid, neuroblastoma and lung where loss of bcl-2 expression would appear to correlate with poor prognosis. In contrast, some studies in prostate cancer have suggested that high levels of expression are associated with disease progression following androgen ablation. We set out to examine bcl-2 and androgen receptor (AR) expression in prostate cancer with a view to relating expression to clinical outcome.

We studied 10 cases of benign prostate, 7 androgen - dependant cancers and 8 cases of progressive cancer following androgen ablation. Using immunohistochemistry, paraffin tissue sections from transurethral prostatectomy specimens were stained for bcl-2 and AR using mouse monoclonal antibodies. Antibody specificty was confirmed by Western blotting. The benign cases all stained similarly for bcl-2 with basal glandular cells and lymphocytes positive. For AR there was good nuclear staining in the glands with weak nuclear/cytoplasmic staining in the epithelium of the prostatic ducts and urethra. Smooth muscle from prostate stained better than smooth muscle from bladder neck.

In the cancers, only areas of malignant disease were studied. Of the 7 androgen-dependant cancers, 2 showed moderate staining for bcl-2, 3 were weakand 2 negative. In the 8 androgen-independant cases, 1 showed moderate staining, 1 was weak and 6 negative. In regard to AR, only 1 of the androgen-dependant group was negative, in contrast to half of the androgen-independant group. In general, the solid, poorly differentiated cancers were negative but AR expression did not correlate well with bcl-2 expression.

Our results suggest that in prostate cancer, as in other cancers, loss of bcl-2 expression correlates with disease which is less responsive to treatment. We are currently examining larger numbers of both treated and untreated patients and are correlating our findings with the number of apoptotic cells in each case.
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PREDICTING PROSTATE CANCER STAGE UTILIZING ANGIOGENESIS, GLEASON SCORE AND PSA

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A preoperative method of predicting tumor stage has been shown. The method incorporates digital imaging and analysis to characterize an area of carcinogenic prostate tissue taken from a needle biopsy. A retrospective, blinded, multisite study involving 500 cases was conducted. Radical Prostatectomy samples at 3-5mm sections, were independently reviewed by blinded referee pathologists to verify pathologic stage. Five (5) staging classes were defined: 1) Tumor Organ Confined, 2) Capsular Involvement, 3) Extra Capsular Extension, 4) Lymphnode metastases and, 5) Bone metastases. Seminal Vesicle involvement was also recorded for each stage. One H & E and two unstained slides from each positive needle core were supplied for image analysis. Each 5 um thick sample was formalin fixed and paraffin embedded and then stained for Factor VIII related antigen. At least 3 fields of cancer were required for analysis. Microvessels were counted at 400x magnification by two independent technicians. Microvessel density (MVD) was calculated as the number of microvessels per square millimeter of tissue. 100,000 fields were measured. MVD was shown to be an independent predictor of tumor stage. A statistical model combining MVD, Gleason Score and pre-operative PSA was used to produce a quantitative result that was strongly predictive of tumor stage. The result significantly enhanced the accuracy of tumor stage prediction over Gleason Score and PSA alone.

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A RETROSPECTIVE STUDY OF HIGH MOBILITY GROUP PROTEIN I(Y) AS PROGRESSION MARKER IN PROSTATE CANCER DETERMINED BY IN-SITU HYBRIDIZATION

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In a previous study using RNA in-situ hybridization, we found a significant correlation between high mobility group protein I/Y, (HMG-I(Y)) mRNA expression and tumor stage and grade in prostate cancer patients, suggesting that HMG-I(Y) might be a potential prognostic marker in prostate cancer. However, our clinical follow up was limited because cryopreserved material was used. Assessing the potential prognostic value of this molecule is of importance because the clinical course of prostate cancer patients remains unpredictable. Here we describe our results on paraffin embedded archival material from a group of 102 patients undergoing radical prostatectomy. These were evaluated for the presence of HMG-I(Y) using RNA in situ hybridization and a follow up of 12-92 months (average 53 months), was available. In 2 of 14 prostate cancers of Gleason grade 1-2 a high HMG-I(Y) expression was observed, whereas in 19 of 23 Gleason grade 3, and 34 of 35 Gleason grade 4-5 tumors, high HMG-I(Y) mRNA levels were detected (chi-square= 38.78, $P < 0.0001$). Moreover, of tumors that expressed high HMG-I(Y) levels, 25% were organ confined (T1-2) in contrast to 74.5% of the invading tumors (T3, chi-square = 15.8, $p < 0.001$). Furthermore, 87% of recurrent tumors showed high HMG-I(Y) expression. However, a multivariate regression analysis including Gleason grade, tumor stage, HMG-I(Y) expression, and PSA levels showed Gleason grade as the most accurate predictor of progression. These results, confirm our previous findings in a cohort of radical prostatectomy patients. High HMG-I(Y) levels measured by RISH were indicative for a worse prognosis, albeit that additional value over the more subjective grading methods was not evident.

6 B Miscellaneous

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PRENATAL ENDOSCOPIC INTRAUTERINE THERAPY; A MONKEY MODEL

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Prenatal endoscopy was performed in a model for experimental surgery in primates. In the rhesus monkey midtrimester endoscopic intrauterine access with 3 canula could be successfully achieved in 8 rhesus monkeys (macaca mulatta). Timing was controlled by physical examination and ultrasonographic biometry by which intrauterine growth curves could be obtained. Using a seldinger technique and a 1.2 mm introduction sheet with fibertopic endoscope, intrauterine inspection could be performed and a second and third operative 4.5 mm port could be introduced under optical control. Fetal conditions were monitored by ultrasonography, doppler investigations of the umbilical cord and arterial uterina bloodflow measurements. After partial amniotic fluid exchange, adequate fetoscopy was always possible. Two monkeys aborted in the 2nd and 6th postoperative day respectively. Measurements of electrical uterine activity 24 hours postoperatively in the first 5 animals showed no uterine contractions. Postoperative prophylactic tocolysis has been abandoned since then. Serial ultrasonic investigations for fetal biometry showed no disturbance of the intrauterine growth patterns. We currently conclude that the rhesus monkey model for experimental intrauterine endoscopic surgery seems to be a suitable model in which developmental abnormalities of the genitourinary tract can be studied.

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PREDICTION OF RECURRENCE IN SUPERFICIAL BLADDER CANCER BY QUANTICYT ANALYSIS OF BLADDER WASHINGS

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Superficial bladder cancer shows a high tendency to recur after transurethral resection. Quantitative cytological analysis of bladder washings can aid in the early diagnosis of bladder tumor recurrences. In earlier studies two karyometric features were found to predict the presence of cancer in the bladder: the 2c Deviation Index (2cDI) and a nuclear shape feature. Sensitivity of the karyometric analysis for the detection of tumor by bladder washings was 90.6% and specificity was 77.5%.

The somewhat lower specificity of the karyometric analysis could implicate a predictive value for the later recurrence of bladder cancer. To test this hypothesis we analysed the follow up data present in the QUANTICYT database. The QUANTICYT system has been operational since 1991 and 4200 bladderwash samples have been analysed in 1600 patients. Bladder washings were fixed in Carbowax fixative, mailed to the laboratory where they were Feulgen stained and karyometrically analyzed. The median follow up was 17 months (range 2-45). In approximately 4.7% of samples QUANTICYT analysis could not be performed due to an abundant number of leucocytes of low number of urothelial cells.

Overall recurrence rate was 7.5%, and 1.6% of patients showed tumor progression to invasive disease. The recurrence rates were correlated with the QUANTICYT risk score: 1 year recurrence rates were 2%, 8%, 13% for low, intermediate, and high QUANTICYT risk respectively. One year progression rates were 0.02%, 2%, 10% for the respective QUANTICYT risk groups.

From these data in an extensive population we conclude that QUANTICYT analysis can provide additional diagnostic value in superficial bladder cancer.

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PROLIFERATION ANALYSIS (MIB-1) AND QUANTICYT KARYOMETRY OF BLADDER WASHINGS

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High proliferation assessed by PCNA-staining of the tumor cells is associated with poor prognosis of bladder carcinoma. Recently it has become possible to stain archive paraffin sections for an other proliferation marker (Ki-67), MiB-1, using microwave heating of mounted sections, therefore it appeared to be attractive to try this method on bladder washings which are widely used for our prognostic studies. QUANTICYT, a karyometric analysis system for bladderwash cytology applies, next to a descriptor of nuclear shape, the 2c deviation index for tumor detection. The MiB-1 proliferation marker is compared to the karyometric analysis using the QUANTICYT system and routine cytology.

Bladder washings can be used for simultaneous karyometry and proliferation analysis using MiB-1 as a proliferation marker. In particular papillary cell groups showed clear staining patterns. We analyzed 42 cases in which a discordance between routine cytologic and karyometric classification (QUANTICYT) was evident. Moreover, 25 concordant cases were analyzed: 5 normal samples, 6 low-grade tumors and 7 high-grade tumors. A median follow up of 20 months (18-22) was available. 15/42 (36%) Patients showed tumor recurrence at a median time of 5 (1-18) months. One patient received cystectomy for an invasive tumor.

All normal samples and all low grade tumors had labelling indices below 10%, and all high grade tumors over 10%. For the discordant low-grade tumors, the QUANTICYT classification correlated better with the MiB-1 labelling than the cytologic diagnosis. There was a clear correlation between 2cDI and MiB-1 labelling index. Followup analysis showed no correlation between the 2cDI nor MiB-1 labelling index and tumor recurrence. The chance on tumor as calculated from the 2cDI and nuclear shape was significantly higher in patients with recurrent tumors (P=0.006). In a multivariate analysis both 2cDI and MiB1 in combination with nuclear shape were useful in predicting prognosis.

It was concluded that although MiB1 labelling index performed better in the detection of tumor and prediction of recurrence than routine cytology, in combination with nuclear shape the best prediction of both was obtained. The MiB1 staining can be useful for the detection of tumor proliferation in dense papillary cell groups were QUANTICYT analysis can not be performed

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KARYOMETRIC ANALYSIS OF INTRA-TUMOR HETEROGENEITY IN PROSTATE ADENOCARCINOMA

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It has been postulated that the different types of localized cancer are related to their anatomical location within the prostate. The prostate is composed of three zones: the peripheral, central, and transition zone. Tumors originating in the peripheral zone would be more readily diagnosed by digital rectal examination, whereas, incidental cancer found in transurethral resection material develop in the transition zone of the prostate. Quantitative interpretation of cell nuclear features by image analysis techniques (karyometry) showed correlation of nuclear size and shape with prognosis and improved grading reproducibility. In particular variation of karyometric features within the tumor were of predictive value

The intra-tumor heterogeneity in different locations in the prostate was determined by karyometric image analysis and compared with local tumor progression in a retrospective analysis of 65 patients with localized adenocarcinoma of the prostate. In these 65 radical prostatectomy specimens 290 tumor locations were documented. Of each location tumor volume was estimated and Gleason grade determined. Quantitative image analysis of nuclear size, shape and chromatin pattern was performed. As measure for intra-tumor heterogeneity, differences in Gleason grade and karyometric feature values were evaluated. Moreover, of each tumor area the location within the prostate was documented. Gleason grade and karyometric features varied widely in the prostate. Tumor locations in the apex (n=92) had significantly larger and more irregular-shaped nuclei ($P < 0.005$) compared with basally located tumors (n=49). Significant differences in nuclear shape were also found in different locations in the equatorial plane of the gland. Tumor heterogeneity in chromatin pattern features was found to correlate with local extension (seminal vesical invasion, extracapsular tumor growth, positive resection margins) and lymph node metastases. This correlation was even stronger in case more pathological features were present.

Analysis of different tumor locations, however, revealed considerable intra-tumor variation. In particular tumors located to the apex showed increased nuclear shape irregularities. Whether this has prognostic implications remains unclear. Heterogeneity in karyometric features was overall the best predictor of local progression, independent of tumor volume.

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RHENIUM-186-HEDP IN A RAT MODEL, TO INVESTIGATE THE INFLUENCE OF RADIO NUCLIDES ON PROSTATIC TUMOR SKELETAL METASTASES.

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Introduction: By microscopically investigating tumor location concomittant osteoblastic activity and Rhenium-186-HEDP (Re-186) distribution, it is possible to rationalise dosimetry of radionuclides and investigate the effect of Re-186 on micro metastases.

Method: Eighteen Copenhagen rats were inoculated with monodispersed R3327-MatLyLu prostate tumor cells via the tail vein under concomittant occlusion of the vena cava inferior. In three groups of six animals, Re-186 administered after six, eight and ten days respectively. Careful daily examination of the animals revealed early signs of hindleg paralysis and bulging of the bladder as a sign of spinal root compression by bone metastases. On the fourteenth day the animals were sacrificed and a bone scan was made. Subsequently autopsy was performed and followed by transversal sections of different vertebrae for light-microscopy and auto radiographic phosphate imaging. These images were digitally processed.

Results: In group I, four out of six rats stayed without symptoms. In group II and III this was two out of six. In the histological sections we can see the tumor spread and concomittant osteoblastic activity; the highest concentration of radio activity is located in the same region.

Conclusion: Although these are only interim results, the early administration of Re-186 seems to be more effective in reducing clinical signs. This model enables us to investigate microscopically the relation between the distribution of radioactive sources and targets (tumor) and to develop dosimetrical models. We also have the opportunity to use radio sensitisers in combination with Re-186 (and other radionuclides), to evaluate the clinical effect and to arrive at new treatment strategies.

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THE CYTOTOXICITY OF NATURAL KILLER CELLS EX VIVO AGAINST ISOLATED AUTOLOGOUS HUMAN UROTHELIAL BLADDER-TUMOUR-CELLS

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Aim Of The Study:Some reports have demonstrated the resistance of many bladder-tumour-cell-lines against NATURAL KILLER cell mediated cytotoxicity.The NK cell activity can be enhanced by IL-2 and *Bacillus Calmette Guèrin* (BCG).In the present study,we investigated the sensitivity of primary bladder-tumour-cells against NK cells,lymphokine activated Killer cells and BCG activated Killer cells.

Material And Methods:Primary bladder-tumour-cells were isolated from 10 patients with urothelial bladder cancer.The established bladder-tumour-cell-line BT-A was also used as target cells.Autologous NK cells,allogenic NK cells,LAK cells and BAK cells of healthy donors were used as effector cells.Effector and target cells were cocultured at a ratio of 40:1.Afterwards the specific lysis was calculated.

Results:The NK cell mediated cytotoxicity was low against both primary bladder-tumour-cells and BT-A.There was no significant difference between autologous and allogenous effector cells (13% vs 12% against primary cells,16% vs 20% against BT-A).We could demonstate comparable NK cell activity of patients as well as of healthy donors using the NK sensitive cell-line K562 (45% vs 47%).LAK (24%) and BAK (20%) cells showed an increased cytotoxicity against primary bladder-tumour-cells as compared to both autologous and allogenous NK cells ($p<0.05$).There was no significant difference between the cytotoxicity of LAK and BAK cells against primary bladder-tumour-cells.LAK cell mediated cytotoxicity against the established cell-line BT-A was higher than those of BAK cells (32% vs 25%,$p<0.05$).

Conclusions:The present results demonstrate the resistance against NK cell activity of both the established cell-line BT-A and also of primary bladder-tumour-cells.IL-2 and BCG are able to enhance these cytotoxicity.Therefore, we conclude that BCG activated Killer cells may play an important role at the tumour rejection during intravesical BCG therapy.

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SEQUENCING OF ERYTHROPOIETIN (EPO) mRNA OF RENAL CELL CARCINOMAS (RCC) OF PATIENTS WITH AND WITHOUT POLYCYTHEMIA BUT ELEVATED EPO SERUM LEVELS

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46% of patients with RCC have elevated EPO serum levels. Paraneoplastic polycythemia is observed in only 3% of these patients. In order to study whether mutations of the tumor derived EPO gene is responsible for this discrepancy we sequenced EPO mRNA from RCCs of patients with elevated EPO serum levels and with and without polycythemia.

Using the Trizol method, RNA was isolated from four RCCs, two of which were from polycythemic patients. The EPO mRNA expression was detected by the RNase protection assay, the EPO concentration in the serum was determined by RIA. A 591 base pair fragment, including the complete EPO coding region was amplified using reverse transcription polymerase chain reaction (RT-PCR). The PCR fragment was isolated after low-melting agarose gel electrophoresis and recovered by GELase digestion. Sequencing of the reamplified PCR-product was carried out using T7-polymerase.

All four tumors expressed EPO mRNA detected by the RNase protection assay. The EPO serum level of the polycythemic patients were 31 and 35 mU/ml while it were 51 and 40 mU/ml, respectively, for the non-polycythaemic ones. The RT-PCR fragment comprising the EPO coding sequence was of the expected size excluding gross alterations (e. g. splice variants). Sequencing analysis did not reveal mutations for the segments examined.

The discrepancy between EPO serum concentration and clinical appearance of paraneoplasia is not caused by alterations of the EPO mRNA sequence. As a consequence it seems reasonable to assume that posttranslational modifications, e. g. alterations of glycosilation, are responsible for the lack of biological activity of tumor derived EPO. Transfection of established cell lines of these RCCs using an EPO expression plasmid and analysis of the EPO protein are necessary for further enlightenment.

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Retinoids do not show antiproliferative effects on renal cell carcinoma cellines

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Treatment of metastasized renal cell carcinoma remains difficult. Using cytokine the rates of partial and complete responses have been increased up to 36 %. Nevertheless new investigations have shown, that the overall two year survival rate of 20 % might not be lengthened. Following the good results with retinioids in patients with acute promyelocytic leukemia, some prospective clinical trials already started on patients with metastasized renal cell carcinoma. Here retinoids were combined with cytokines and vinblastine or 5-fluorouracil.

We investigated the antiproliferative effects of four retinoids (isotretinoin, tretinoin, acitretin and etitrenate) on 12 cellines of renal cell carcinoma. The retinoids were soluted in DMSO using concentrations from 10^{-6} M to 2×10^{-4} M. After investigating the cytotoxic effects of the retinoids alone we combined them with vinblastine, 5-fluorouracil and interferon α. Using the MTT cytotoxity test as well as microcalorimetric investigations we examined the antiproliferative activities of these pharmaceutics.

The concentrations of all drugs started at the expected blood levels using the recommended doses rising up to at least ten fold higher concentrations.

The slightly antiproliferative effects of the retinoids (2×10^{-4} M) could not be proven to be significant different to the effect of DMSO alone. Furthermore we did not see additional cytotoxicity neither for the retinoids nor for interferon α when combined with vinblastine and 5-fluorouracil compared to the antiproliferative effect of vinblastine or 5-fluorouracil alone. However there was a significant higher cytotxicity of vinblastine compared to 5-fluorouracil in regard with the expected blood levels of these chemotherapeutics (SAS, Wilcoxon Test; $p<0,01$).

In conclusion we could not demonstrate any antiproliferative effects of retinoids on the cellines in vitro. Vinblastine showed significant higher cytotoxity than 5-fluorouracil on cellines of renal cell carcinoma in vitro.

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SYNERGISTIC EFFECTS OF ESTRAMUSTINE IN COMBINATION WITH VINBLASTINE OR DOXORUBICIN ON PROSTATIC TUMOR CELLS

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Estramustine (EM) is an antimitotic agent widely used as monotherapy for prostatic cancer. The effects of combinations of EM with vinblastine (VLB) or doxorubicin (DOX) on cellular uptake, cellular retention and cell survival were investigated in Dunning hormone-insensitive rat AT-1 tumour cells and DU-145 human prostatic tumour cells. Whereas uptake was measured using relatively high concentrations of drug over a short period, (2 h) both drug retention and cell survival experiments were conducted using low concentrations of drugs over a long period (24-96 h). Accumulation of VLB and DOX by AT-1 cells was less than one half of that in DU-145 cells. Inclusion of EM (5-25 μM) considerably increased the uptake of both VLB and DOX in AT-1 cells but not in DU-145 cells. Verapamil, prenylamine and tamoxifen also potentiated VLB uptake in AT-1 cells. These results indicate that compared with DU-145 cells, the uptake of VLB and DOX is restricted in AT-1 cells but this can be greatly enhanced by the addition of EM. Clear synergistic antiproliferative effect on AT-1 cells was obtained when relatively low concentrations (5-10 nM) of VLB were combined with EM. This synergistic effect was clearly reflected in the concentration of ^{3}H-VLB retained by the cells. However, a close examination of the data suggested that other factors (mechanisms) in addition to the enhancement of VLB uptake in the presence of EM might make a contribution to the observed synergy. The results suggest that EM may be combined not only with VLB but also with DOX to improve the therapeutic index and reverse resistance.

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SELECTION AND CHARACTERIZATION OF A CISPLATINUM RESISTANT BLADDER CANCER CELL LINE RT112/CP

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An increase in the expression of metallothioneine (MT) as a possible resistance mechanism of secondary cisplatinum resistance of malignant tumors is under investigation. MT expression is induced by heavy metals like cadmium and zinc. To investigate the role of MT in cisplatinum (CDDP) resistance, we established and characterized a CDDP resistant bladder carcinoma cell line.

The bladder carcinoma cell line RT112 was selected in increasing dosages of CDDP (RT112/CP3). The chemoresistance of the parental line and the subline against substances of the MVAC protocol was evaluated in a MTT assay. The intracellular amount of MT in both lines was measured by capillary electrophoresis.The expression of P-170-glycoprotein in resistant and sensitive cells was compared by immunohistochemistry (MAb C219). The influence of the glutathione metabolism on the CDDP resistance was assessed by coincubation of CDDP with buthionine sulfoximine, which inhibits the rate limiting enzyme in glutathione synthesis, g-glutamylcysteine-synthetase.

The selected subline was about 10-fold higher resistant against CDDP than the parental line RT112. (IC_{50} 10 mg/ml versus,0,7 mg/ml). It also displayed a cross resistance against methotrexate. An increased expression of P-glycoprotein could not be found in either of the cell lines and coincubation with BSO did not modulate CDDP resistance in the subline. The level of MT was markedly increased in the CDDP resistant subline compared to the parental line.

Our results indicate, that CDDP resistance in the bladder carcinoma line RT112/CP3 is not mediated by P-glycoprotein or glutathione. MT is highly expressed in our CDDP resistant subline and might be a major resistance mechanism by binding and inactivating CDDP.

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ENHANCED CYTOTOXICITY OF IDARUBICIN COMPARED TO DOXORUBICIN AND EPIRUBICIN IN ANDROGEN INDEPENDENT PROSTATE CARCINOMA CELLS.

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Epirubicin has been used in the therapy of hormone refractory prostate carcinoma with good, although limited, subjective responses. We have compared the *in vitro* cytotoxic effectiveness of the novel anthracycline idarubicin (ida) with epirubicin (epi) and doxorubicin (doxo) in different sublines of the Dunning rat prostate carcinoma using the MTT assay. We determined the drug concentrations, that were necessary to kill 50% of the cells (IC_{50}) in the hormone sensitive subline G and the hormone independent sublines AT.1, AT 3.1, MatLu and MatLyLu. In G cells ida was about 10-fold and 2-fold more effective than doxo and epi respectively, whereas it was about 60-fold and 67-fold more effective in AT1 cells, 36-fold and 56-fold more effective in MatLyLu cells, 40-fold and 32-fold more effective in MatLu cells and 150-fold and 47-fold more effective in AT 3.1 cells than doxo and epi, respectively. In drug selected KB-cells, which express high levels of the multidrug resistance mediating P-glycoprotein and are highly resistant against other anthracyclines (IC_{50}doxo: 2600 ng/ml, IC_{50}epi: 1000 ng/ml), ida proved to be very effective (IC_{50}: 20 ng/ml). Circumvention of P-glycoprotein-mediated multidrug resistance might also explain the effectiveness of ida in hormone insensitive prostate carcinoma cells. Taken into account the low cardiac toxicity and the possibility of oral administration, idarubicin may be superior to epirubicin in prostate cancer therapy.

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CYTOTOXICITY, CELLULAR UPTAKE AND BINDING TO DNA OF KW-2149, A NEW MITOMYCIN-C ANALOGUE.

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KW-2149 is a new mitomycin-C analogue, currently in phase I clinical trial for the treatment of superficial bladder cancer. Compared to mitomycin-C (MMC), it has similar or better activity against murine tumours and human tumour xenografts, and shows little or no cross-resistance with MMC-resistant tumours *in vivo* and *in vitro*. We found dramatic differences in cytotoxicity when human bladder cancer cells (RT112) were exposed to KW-2149 in different batches of serum. In human serum and one batch of foetal calf serum (FCS) the cytotoxicity of KW-2149 was approximately 8x that of MMC (comparing the concentrations or IC_{50}s of each drug required to reduce colony forming ability by 50%). In another batch of FCS and in mouse serum KW-2149 was 3-5 times less cytotoxic than MMC. These sera did not influence the cytotoxicity of MMC. The mechanism by which serum influences cytotoxicity was investigated by measuring the uptake of [^{3}H]-KW-2149 into RT112 cells over a 2h exposure period. Cytotoxicity was associated with uptake: in the absence of serum uptake was low at 3.0 dpm min^{-1} (IC_{50} 3992 ng ml^{-1}), in one batch of FCS moderate at 6.8 dpm min^{-1} (IC_{50} 952 ng ml^{-1}) and in another batch of FCS high at 12.5 dpm min^{-1} (IC_{50} 27.4 ng ml^{-1}). The linear *Taq* stop polymerase assay was used to analyse sequence-specific binding of KW-2149 to plasmid DNA. At least 100x more adducts were observed in the presence of reducing agents. KW-2149 was targetted to runs of 2 or more guanines, and the reducing agents did not modify the adducts formed.

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COMPARISON OF THE EXPERIMENTAL ANIMAL LABORATORY BLADDER AND MUSCLE STRIP RESULTS WITH HUMAN DETRUSOR CONTRACTILITY.

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Clinically is speculated that in patients with bladder outlet obstruction (BOO) due to prostatic enlargement the bladder initially compensates for the increase in outlet resistance and gradually decompensates, resulting in incomplete emptying. Laboratory experiments demonstrate that the normal contraction of a detrusor muscle consists of an initial phase with a rapid pressure increase, supported by anaerobic breakdown of ATP, followed by a plateau phase responsible for emptying depending on oxidative processes. Anoxia is a cause of almost immediate (1-2 min) failure of the muscle to maintain 'pressure' (or contraction). In vivo, detrusor contraction power can be deduced from the result of urodynamic investigation. A calculation, using intravesical pressure (cmH_2O), bladder volume (ml) and flow (ml/s), results in the 'detrusor power factor' (Watts Factor -WF). This WF (W/m^2) can be displayed and allows observation of the detrusor power, per surface area, during micturition. WF_{max} is the observed maximum. BOO can be quantified in grade 0 (none)- 6 (severe) through analysis of the pressure/flow ratio. Voided fraction of bladder capacity is quantified by Void%; Void% at WF_{max} is the fraction emptied before the moment of WF_{max} and END% is the voided fraction after WF_{max}. The results of 242 patients are presented in the table:

BOO:	0	1	2	3	4	5	6
WF_{max}	10.5	9.1	9.5	12	13.9	16	16.9
Void%	82	84	83	71	70	58	26
Void%WF_{max}	55	49	43	38	27	18	10
END%	27	35	38	32	43	40	16

WF_{max} increases with increasing BOO but the Void% diminishes. We observed a decrease in Void% at WF_{max} and no decrease in END%. Synthesis of these observations with the experiments, provides the innovative conclusion that when BOO exists the first (anaerobic) contraction phase is instantaneously forced into 'high power', with reduced effect on emptying and that the second phase is unchanged, despite incomplete emptying. Possibly contraction in this aerobic phase is limited by shortage of oxygen or metabolites.

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OVINE INTRACORTICAL VARIATION OF RENAL BLOOD FLOW AS ASSESSED BY THE MICROSPHERE METHOD.

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INTRO: The microsphere method for experimental measurement of renal blood flow is well documented. Calculation of blood flow based on samples as opposed to the total amount of tissue available is based on the premise that renal blood flow is evenly distributed. However in the pig, perfusion is inhomogeneous. Renal blood flow distribution is previously undocumented in sheep. This study addresses this question.

METHOD: Paired kidneys from eight anaesthetized sheep were studied. 45 minutes after insertion of carotid and femoral arterial lines, radioladelled 15u microspheres were injected into the left ventricle and flushed with 30 mls of 0.9% saline. A reference blood sample was withdrawn at 20 mls/min. for two minutes. On the experiment's completion both kidneys were removed and fixed in buffered formalin for 4 days. Each kidney was dissected into 14 segments. Each segment's cortex was weighed and counted along with the reference blood sample for radioactivity on a Packard Cobra QC 5003 gamma counter. Blood flow was calculated for the overall average renal blood flow as well as for each individual segment. The total amount of injected microspheres were sufficient to ensure that more than 1500 microspheres were present in each segment.

RESULTS: Average total renal cortical tissue weight =107.92gm (s.d. 13.15) and average segmental weight = 3.85gm (s.d. 1.46). There was a significant difference in the inter-segmental blood flow variation within the study group.(Friedman's test $p<0.001$).

The Range in the percentage deviation of the segments from the overall average blood flow within each of the right kidneys is shown below. This data will be presented in full.

Kidney	Range (%)	Kidney	Range (%)
1	-13.5 to 16.5	5	-66.6 to 32.4
2	-50.8 to 31.7	6	-10.6 to 15.2
3	-18.3 to 32.8	7	-7.1 to 12.7
4	-13.3 to 15.8	8	-11.9 to 18.9

CONCLUSION: Wide ranges in blood flow for both the inter-segmental analysis of the study group and intra-renal blood flows within each kidney suggests that samples of only a few grams in weight are not sufficiently representative of the whole ovine kidney.

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CELL CYCLE ASSOCIATED CHANGES IN A HORMONE RESISTANT PROSTATE CANCER CELL LINE TREATED WITH VINBLASTINE AND COLONY STIMULATING FACTORS

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Cell cycle associated changes in vinblastine (vlb) treated hormone resistant prostate cancer cell line (PC3), and the effects of concomitant administration of colony stimulating factors (G-CSF or GM-CSF) were investigated by flow cytometry and histomorphological examination. Flow cytometric analysis of exponentially growing PC3 cells revealed a pronounced decrease in proliferative index by vinblastine treatment from 54.9% to 17.8% ($p < 0.001$). Morphological examination of these cells revealed an apoptotic index of 44.4% with vlb treatment. Addition of G-CSF and GM-CSF to these cultures resulted in a significant increase in proliferative index from 17.8% to 88.3% and 87.5%, respectively which accompanied by a significant reduction in apoptotic index of these cell populations (24.5% and 16%, respectively, $p < 0.001$). This observation also coincided with an increased BrdU labeling index (18.7% for vlb treated cultures and 51.3% and 45.6% for vlb+G-CSF and vlb+GM-CSF, respectively, $p < 0.001$). The deficit in the proportion of cells in proliferative phase coupled with high apoptotic and low BrdU labeling indices resulting from vlb treatment suggests that cells in this phase was preferentially effected by this treatment. Present data also indicates a protective effect of colony stimulating factors on cells treated with vlb which raises the possibility of decreased effectiveness of this agent due to this interaction in clinical setting.

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RADIOFREQUENCY INTERSTITIAL TUMOR ABLATION : A NEW MODALITY OF TREATMENT OF CANCER USING INTERSTITIAL BIPOLAR RADIOFREQUENCY IN A HUMAN KIDNEY MODEL.

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Parenchymal organs and tumors can be successfully ablated by interstitially delivered radiofrequency energy that allows to create very localized necrotic lesions. RF has been recently used in Urology to selectively ablate prostatic tissue (TUNA). Size of lesions depends on properties and vascularity of each tissue.

Therefore, the extent of the lesions must be carefully studied. Radiofrequency interstitial tumor ablation (RITA) has been used in four human kidneys in an ex vivo experiment. Our aim was to assess the necrotic lesions produced by the bipolar radiofrequency on a model close to the physiological conditions prior to in vivo human experiments. Energy was delivered by a generator connected to two needles introduced parallel to each other in the renal parenchyma. A thermocouple was inserted between the two active electrodes. Size of the lesion has been studied in relation with the power delivered and the duration of the treatment. The renal artery has been perfused with a physiological renal blood flow maintained at a constant temperature of perfusion of 37° by a computer-assisted hot-line apparatus. Two lesions in each pole of each kidney including the cortex and the medulla have been produced. In one kidney, a tumor has been ablated by this interstitial approach. Active needle deployment was 3 cm for an interneedle distance of 3 cm. Macroscopic examination showed large discoloured lesions after RITA treatment. When treating the tumor, all the area was affected by the necrotic lesion but the adjacent tissue was unaffected.

Microscopic examination showed a stromal oedema with intensive pycnosis. Cytoplasmic eosinophilia was observed in both glomerular cells and tubules. Maximum temperature at the active needle ranged from 84 to 133° with 10 to 14 watts applied during 10 to 14 min. Lesions created were on average 2.2 x 3 x 2.5 cm.

In conclusion, this study demonstrates the ability of RITA to produce localized extensive necrosis in kidney parenchyma. Future studies will be conducted in in vivo animal models in order to asses the safety of this approach prior to human studies in renal cancer.

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ARGON LASER-INDUCED AUTOFLUORESCENCE OF NORMAL AND TUMORAL HUMAN UROTHELIAL CELLS : IN VITRO SPECTROFLUORIMETRIC STUDY.

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Early detection of occult urothelial precancerous lesions such as severe dysplasia and carcinoma in situ is still a challenge for urologists. Recently, laser-induced autoflorescence spectroscopy has been reported to discriminate with a high sensitivity normal from tumoral tissues. In order to determine whether laser-induced autofluorescence may be used as a diagnostic method in urology, we have compared the emission characteristics-fluorescence maximum and intensity- of several normal and tumoral human urothelial cells by using laser microspectrofluorimetry. In all cases, the fluorescence spectrum of a single living cell excited at 488nm (Ar^+ laser line) appeared as a broad band of green fluorescence with a maximum in the 550-560nm spectral range corresponding, most likely, to oxidized flavoproteins emission. Only slight differences were noted between emission maxima but, the half-band width of normal cells was found to be about 40% larger than that of tumoral cells. However, a striking difference was observed in autofluorescence intensity of normal and tumoral urothelial cells : the fluorescence intensity of all tumoral cell types was drastically decreased – 10 times , ($p<0.0001$) – compared with normal cells. These results are discussed in view of a possible decrease of oxidized flavoproteins concentration in tumoral urothelial cells, suggesting the use of argon laser-induced autofluorescence as a diagnostic method for occult urothelial precancerous lesions.

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PROTECTION OF ISCHEMIC RAT KIDNEYS BY INHIBITION OF ANGIOTENSIN CONVERTING ENZYME

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Increased renal vascular resistance with severe reduction of reperfusion after renal ischemia importantly promotes the establishment of postischemic acute renal failure. The role of direct actions of vasoconstrictors like catecholamines, endothelin or angiotensin II remains unclear. This study was performed to evaluate the functional role of the renin-angiotensin system in acute ischemic renal failure.

Male Sprague-Dawley rats (200g) underwent catheterization of carotid artery, jugular vein and urinary bladder under halothane anesthesia. They were injected either with placebo (NaCl 0.9%, n=12, group 1) or the angiotensin-converting-enzyme inhibitor Enalaprilat (MSD, Munich) (2mg/kg, n=10, group 2). 30 minutes after application the left renal artery was clamped for 60 min. The intact right kidney was removed after release of the clamp. In the conscious animal blood pressure and renal function (inulin-, hippurate-clearance, doppler-flow) were measured 90, 120, 150 minutes and 48 hours after ischemia.

All pretreated animals survived, 4 placebo-animals died during 48 hours post ischemia. After 48 hours blood pressure was not significantly different between the groups. Group 1 showed very low glomerular filtration rate and renal blood flow (8% and 3% of preischemic kidney values, respectively) whereas group 2 showed significantly ($p<0.05$) higher values (33% and 32%, respectively). Renal vascular resistance in group 1 was maximally increased (81-fold of preischemic values), whereas group 2 showed significantly lower levels (5-fold of preischemic value, $p<0.05$). However preischemic levels were not reached in the treated animals after 48 hours.

Preischemic inhibition of angiotensin converting enzyme (ACE) results in significantly better postischemic renal function with a pronounced effect on renal vascular resistance compared to control. Therefore a pathophysiologic role of angiotensin II in postischemic renal vasoconstriction is suggested although unspecific effects of ACE-inhibitors (bradykinin activation) must be discussed. The results could lead to new pretreatment-protocols for surgical procedures on kidneys under temporary ischemia (e.g. organ-preserving surgery for renal cell carcinoma).

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PURIFICATION, CHARACTERIZATION AND PSA-MEDIATED PROTEOLYSIS OF THE MAJOR GEL FORMING PROTEINS IN HUMAN SEMINAL PLASMA, SEMENOGELIN I AND II.

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At ejaculation, the bulk of the human ejaculate is turned into a viscous gel in which the spermatozoa are entrapped. The gel liquefies within a few minutes and the spermatozoa are released. The liquefaction is mediated by PSA (prostate specific antigen) and is the result of limited proteolysis of the gel-forming proteins by PSA. PSA is a serine protease displaying extensive structural similarity with the trypsin-like glandular kallikreins but has a chymotrypsin like enzymatic activity, hydrolyzing peptide bonds at the C-terminus of certain tyrosine and leucin residues.

The major gel-forming proteins are semenogelin I (SgI), semenogelin II (SgII), and fibronectin, all three proteins synthesized in the seminal vesicles. The SgI molecule, composed of 439 amino acid residues and with a molecular mass of 52 kDa, displays extensive structural similarities with SgII. The SgII molecule is composed of 559 amino acid residues and occurs in two molecular forms with masses of 71 and 76 kDa.

Human seminal plasma was collected directly after ejaculation, the gel immediately dissolved in the presence of urea, under reducing conditions and at alkaline pH. Spermatozoa were separated by centrifugation and SgI and II purified by affinity chromatography on heparin-Sepharose in the presence of urea at alkaline pH. Most seminal plasma proteins did not bind to the matrix and the semenogelins were eluted with a sodium chloride gradient. Further purification was achieved by FPLC chromatography on Superose 6 in the presence of urea. The molecular mass of SgI was 49,958 Da and that of the dominating form of SgII 63,539 Da when determined with mass spectrometry. The amino termini were blocked but the N-terminus of respective semenogelin was identified after cleavage with pyroglutamate aminopeptidase. Both the N-terminal sequences and the amino acid compositions of SgI and SgII were in agreement with the respective sequence of SgI and SgII cDNA. The two molecular forms of SgII were shown to be due to differences in glycosylation. The extinction coefficients (280nm, 1%, 1cm) were 5.5 and 5.4 for SgI and SgII, respectively, and their isoelectric points approximately 9.5.

The PSA-mediated proteolysis of the semenogelins was studied by incubating purified SgI and II, respectively, with PSA at pH 7.4, for four hours, in the presence of 0.5 M urea. Proteolytic fragments were isolated by HPLC using a C_4 column. Each fragment was subject to amino-terminal sequence analysis and molecular mass determination with mass spectrometry. Thirteen cleavage sites in SgI and 16 in SgII were identified. Many cleavage sites occurred at corresponding positions in both SgI and SgII. Cleavage sites were found C-terminal of certain tyrosin and leucine residues but were not exclusively restricted to these amino acids. Our data indicate that the enzymatic activity of PSA is similar to that of chymotrypsin but also quite distinct from both chymotrypsin and trypsin since no cleavages C-terminal of either arginine, lysine and tryptophan were identified.

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PRODUCTION OF RECOMBINANT PSA AND HK2 AND ANALYSIS OF THEIR IMMUNOLOGIC CROSS-REACTIVITY

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Human glandular kallikrein-2 (hK2) is a kallikrein-like serine protease with 80% structural similarity to prostate-specific antigen (PSA). Both the proteins are androgen regulated and expressed almost exclusively by the prostate epithelium. Measurements of PSA in serum are widely used to monitor patients with prostate cancer. Discrepancies in serum PSA concentrations obtained with differently designed assays complicate interpretation of the results. This may partially be due to attenuation of the assay response when PSA has formed complexes with protease inhibitors, but may also relate to whether or not mono- or polyclonal anti-PSA IgGs cross-react with hK2. We have expressed hK2 and PSA in eucaryotic cells by the Semliki Forest Virus expression system. The proteins were glycosylated and secreted into the culture medium and had an apparent mass similar to that of PSA from seminal fluid on Western blots after SDS-PAGE. The recombinant proteins were used to study the specificity of 18 monoclonal anti-PSA IgGs. Five of them cross-reacted with identical affinities to recombinant hK2 whereas 13 monoclonal anti-PSA IgGs recognized PSA alone. All of the antibodies that recognized both PSA and hK2 bind to a region of the protein that is exposed when PSA is complexed to ACT.

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An Internet Program Allowing Global Access to Computational Resources in Urological Decision Making

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Computational modeling is becoming one of the most powerful tools allowing Urologists to classify patients by disease outcomes, thus predicting prognosis and suggesting treatment strategies. We have been investigating one such modeling tool, neural computation. A neural network is a computer program that mimics the function of neurons in a biological network such as the brain. These programs may be 'taught' a set of training data, and if the program performs well on test data, it may be used in real world urological problems. We have built such neural networks to prognosticate mortality and new metastases in patients with renal tumors, to predict testis biopsy results from azoospermic patients and to predict outcomes from patients undergoing varicocelectomy. Training real world problems generally involves the use of high-speed state-of-the-art computers which may not be available to the practicing Urologist. We have thus built an interface to our neural computational tools which allows these programs to be accessed worldwide by any computer connected to the Internet and using a World-Wide-Web multimedia browser such as Mosaic or Netscape. Our interface consists of a suite of programs written in the perl text-based programming language. The flexibility to handle laboratory results from different centers with different upper and lower limits of assay normal is a key feature of the suite, as well as exploitation of the 'user friendly' forms standard allowed in common World-Wide-Web browsers. Usage of our system is described so that other investigators may use these programs as a template for Internet access to their urological data models.