Urol Res (1993) 21:439–470

Urological Research

Abstracts

X Congress of the European Society for Urological Oncology and Endocrinology (ESUOE) March 10–13, 1994, Berne, Switzerland

Congress chairman: Prof. U. E. Studer, Berne

Table of contents

Editorial

The ESUOE exists to promote communication between basic and clinical researchers who are interested in urological oncology. The Society has become the focal point in Europe for regular meetings between such researchers. The increasing interest in the Society is reflected in the submission of 150 abstracts for this year's meeting in Berne. This is roughly twice as many as for previous meetings and far too many to present at the Berne meeting. The standard of the abstracts was high, in keeping with Europe's numerous promising research projects. The program committee was thus able to select according to quality and also so as to fulfill the role of the Society by showing the range of Europe's current urological oncology research particularly in benign prostatic hyperplasia, molecular biology and new perspectives in diagnosis and treatment of urological cancers. To try and bring together both basic and clinical researchers and researchers oriented to different organs, in keeping with the Society's aims, the program has been divided into topics such as genetic alterations, tumor suppressor genes, growth factors and immunology. The abstracts show how fast our knowledge of the molecular and biological changes in urological cancers is increasing. There is also increasing evidence that this will soon improve the management of our patients.

Urs E. Studer, Berne

Oral communications: Benign prostatic hyperplasia

O1

3-D MODEL OF BENIGN PROSTATIC EPITHELIAL GROWTH

John Hines, Paul Abel, Christopher Foster.
Royal Postgraduate Medical School, London, UK.

The pathophysiology of benign prostatic hyperplasia (BPH) is poorly understood. There is a lack of representative *in vitro* models of BPH.

Macerated human prostate tissue was enzymatically digested yielding epithelial elements (organoids) without stroma. The organoids were embedded in a 3-dimensional rat-tail collagen matrix and cultured *in vitro* in a serum free, hormonally defined growth medium. The culture system was maintained at 37°C, 100% humidity and 5% CO_2 for up to two weeks, while morphology, antigen expression and DNA synthesis were studied.

During the first 24 hours cellular reorganisation was observed. Subsequently, branched cylindrical structures with a diameter of 4 to 8 cells grew from the organoids. Light microscopy demonstrated morphology similar to the acinar-ductal system of natural benign prostate epithelium and partial internal differentiation was observed by electronmicroscopy. Differential expression of high molecular weight cytokeratins at the periphery of the outgrowths and secretory cell-surface oligosaccharide complexes in the centre of the outgrowths was observed immunohistochemically. DNA replication detected by bromodeoxyuridine incorporation was up to three times greater at the periphery of the outgrowths than in the original organoids.

Acinar and ductal structures morphologically and antigenically homologous to those of intact prostate tissue can be generated *in vitro* using this model. This provides a 3-D model for the study of prostatic epithelial cytodifferentiation and morphogenesis, and may be used to investigate cellular events in benign pathological states of prostate tissue.

O2

EXPRESSION OF TGF-β_1 IN HUMAN PROSTATIC HYPERPLASIA AND PROSTATE CANCER.

Christopher S Foster, Maria Cardillo, Qi Bao, Paul D Abel, Nasir Lalani.
Royal Postgraduate Medical School, London, United Kingdom.

The multi-functional regulatory polypeptide TGF-β_1 inhibits normal epithelial proliferation in human and rodent prostates. Escape from TGF-β_1 inhibition is a potentially important mechanism directly contributing to enhanced growth of prostatic carcinomas. The present study has identified the sites of TGF-β_1 synthesis and expression in conventionally-processed tissue sections from 7 benign hyperplasias and 32 prostatic cancers using a combination of *in-situ* hybridization, immunohistochemistry and RFLP analysis. For the *in-situ* study, an oligonucleotide probe comprising a cocktail of 30mer sequences from exons 6, 7a and 7b of the TGF-β_1 gene was labelled with digoxigenin 11-dUTP by 3' end-labelling and identified immunohistochemically following hybridization. Monoclonal antibodies to the latent form of the TGF-β_1 molecule were employed to define cellular expression of this molecule. RFLP analysis was performed following digestion of genomic DNA extracted from prostatic tissues and from peripheral blood leukocytes by restriction enzymes *EcoR1*, *Msp1* and *Taq1*. Conventional Southern blotting of the digested DNA was performed using a cDNA probe comprising a 1kb fragment of the TGF-β_1 gene. TGF-β_1 message was detected predominantly in well- to moderately-differentiated adenocarcinomas, with no detectable signal in poorly-differentiated tumors. In benign prostatic epithelium, scanty focal signal was detected. Expressed TGF-β_1 was identified immunohistochemically in 2 of the 7 benign hyperplasias and in 26 of the 32 carcinomas. Enhanced expression was identified at the tumor-stromal interface in many regions of the carcinomas. No rearrangements or deletions of the TGF-β_1 gene were identified by RFLP analysis. Although modulation of TGF-β_1 expression appears to be occurring in human prostatic cancers, the mechanism remains elusive.

O3

DIFFERENTIAL LYMPHOKINE RESPONSIVENESS OF NORMAL AND BPH-DERIVED PROSTATIC STROMAL CELL LINES AND CLONES. Georg Steiner, Alexandra Parich, Amna Delalic, Andrea Schöllhammer, Gero Kramer, Michael Marberger. Dept. of Urology, Univ. of Vienna, Austria

Previously we demonstrated that BPH is associated with lymphocytic infiltration. The majority of infiltrating cells belong to the T-cell lineage, known to produce a number of hormone-like growth factors after specific activation by antigen. This suggested to us that the close and continuous contact of stromal cells with activated lymphocytes and their products might modify their responsiveness to growth factors. Therefore various characterized normal (n=2), BPH- (n=5) and Ca-derived (n=1) prostatic stromal cell (PSC) lines and clones (n=11) were stimulated with various cytokines known to be secreted primarily by activated T cells. The percentages given hereafter refer to proliferation seen without stimulation (100%). In summary, normal PSC revealed no proliferative response to IL-2 and IL-7, a slight response to IFN-γ, and a dramatic inhibition induced by IL-4 (13-33%). By contrast, BPH PSC lines consistently showed hyperresponsiveness when stimulated with IL-2, IL-7 and IFN-γ (135-147%) and reduced cytokine-mediated growth inhibition when treated with IL-4 or TNF-α (53-91%), while the responsiveness to bFGF was significantly lower than in normal controls. Analysis regarding the heterogeneity of PSC in BPH using cloned BPH PSC revealed that the 7/11 clones exhibiting rapid proliferation had an even greater responsiveness when stimulated with IFN-γ and IL-2 compared to the polycloncal line of the same patient. By contrast, all 4/11 slower-growing clones revealed a cytokine responsiveness comparable with that of normal PSC lines. Interestingly, whereas IL-4 exhibited a potent inhibitory effect on normal and polyclonal BPH PSC lines, 6/7 fast-growing clones revealed massive proliferation of up to 180%. This was in contrast to all 4 slow-growing lines and the polyclonal source BPH PSC line. These data might indicate that the development of BPH is associated with the occurrence of a connective tissue-forming cell type exhibiting a completely different cytokine responsiveness.

Poster session: Benign prostatic hyperplasia, growth factors, apoptosis, androgen receptors, experimental models

P4

LIPID COMPOSITION IN EPITHELIUM AND STROMA OF HUMAN BENIGN PROSTATIC HYPERPLASIA

Heike Weisser, Sabine Tunn, Kurt Oette *, Michael Krieg
Institute of Clinical Chemistry and Laboratory Medicine, University Clinic Bergmannsheil, Bochum;
* Institute of Clinical Chemistry, University Clinic Cologne, Germany

The androgenic action at the cellular level of the prostate is strongly determined by both the membrane-bound androgen receptor and the membrane-bound 5α-reductase. However, as yet rather limited information is available concerning the composition of cell membranes in human prostate. To get more insight into such membrane composition, the lipid composition in epithelium and stroma of human benign prostatic hyperplasia (BPH) has been investigated. Total lipid extraction was carried out in epithelium and stroma of 15 BPH. Neutral and phospholipids were separated by one- and two-dimensional TLC. After methylation of the phospholipids, fatty acid methyl esters were extracted and analysed by capillary gas chromatography. Results were expressed as means ± SEM. The significance of the differences between the means was calculated by Students' t-test, the significance of age-dependent alterations was calculated by Spearman rank correlation coefficient. $P<0.05$ was considered significant. The main results were: 1. Cholesterol and phospholipids are the major lipids in epithelium and in stroma. 2. The mean phospholipid concentration [mg/g wet weight ± SEM] was significantly higher in epithelium [10.34 ± 0.81] than in stroma [3.76 ± 0.28]. 3. The phospholipid subclasses found in epithelium (E) and stroma (S) were phosphatidylcholine [E: 49.7% ± 4.6; S: 44.6% ± 2.4], phoshatidylethanolamine [E: 26.5% ± 3.1; S: 32.8% ± 3.8], sphingomyelin [E: 7.9% ± 1.4; S: 11.8% ± 1.9], phosphatidylserin [E: 11.2% ± 1.6; S: 6.5% ± 1.4], and phosphatidylinositol [E: 4.9% ± 0.9; S: 4.9% ± 1.6]. 4. Specifying the various fatty acids it became evident that striking differences of the fatty acid composition of phospholipids are given between epithelium and stroma. 5. The fatty acid composition of the phospholipids in both the epithelium and stroma showed a preponderance of C16:0 (E: 20.1% ± 0.3; S: 19.4% ± 0.3) and C18:0 (E: 14.0% ± 0.3; S: 16.8% ± 0.3), while in regard of unsaturated fatty acids C18:1 (E: 24.3% ± 0.8; S: 14.3 ± 0.2), C18:2 (E: 8.7% ± 0.3; S: 7.5% ± 0.2), and C20:4 (E: 9.9% ± 0.6; S: 15.9% ± 0.3) were predominantly found. 6. In epithelium, the phospholipid concentration [mg/g wet weight) as well as the percentual portion of C18:1 decreased significantly with donors' age, but remained constant in stroma.
The differences in membrane lipid composition between epithelium and stroma of human BPH might affect the properties of membrane-bound enzymes and receptors regulating the epithelial and stromal androgenic milieu. In light of earlier findings demonstrating significant differences in the substrate affinity and age-dependent alterations of 5α-reductase activity between epithelium and stroma of BPH, it is attractive to speculate that the activity of 5α-reductase is at least in part dependent on the variation of the membrane composition.

P5

PHENOTYPIC CHARACTERIZATION OF PROSTATIC FIBROMUSCULAR TISSUE CELLS. Georg Steiner, Alexandra Parich, Gerald Hartmann, Andrea Schöllhammer, Gero Kramer, Michael Marberger. Depts. of Urology and Rheumatology, Univ. of Vienna, Austria

It is well documented that prostatic stromal cells (PSC) play a major role in prostatic ontogeny as well as tumor development and dissemination. They are believed to be the key factor in the pathogenesis of the most common types of BPH. In order to comprehensively investigate their phenotypic features, we for the first time used FACS analysis of long-term-cultured PSC lines obtained from normal (n=2), hyperplastic (n=6) and cancerous (n=1) prostates. Marker expression was analyzed using 25 monoclonal antibodies, appropriate isotype controls and flow cytometry on either ethanol-fixed or viable cells. All cell lines exhibited intense intracellular reactivity with anti-desmin and anti-vimentin, known to be specific for fibroblasts and smooth muscle cells in situ. In contrast to previous reports, all cultured PSC lines expressed cytokeratin(Ck)-8 and -18 as demonstrated by immunoblotting and flow cytometry, while antibodies against other Ck were negative on PSC but positive on the corresponding prostatic epithelial cells. Furthermore, despite all reports to the contrary, both all PSC and prostatic epithelial cell lines expressed significant levels of 2a-myosin as demonstrated by flow cytometry and Western blotting. Staining with anti-CD45 was consistently negative, indicating that the analyzed cells were not leucocytes. All 9 PSC lines reacted uniformly with anti-CD29 (β1-integrin), anti-VLA-1, -2, -4 -5 and anti-CD44 at variable expression densities. VLA-6 (β1α6-integrin) was expressed only on the prostate cancer and one BPH-derived PSC line. VCAM-1, known to interact with VLA-4, was only present in a small subpopulation of the cancer-derived PSC line but was uniformly expressed in all other 8 cell lines tested. Furthermore, all PSC lines significantly reacted with anti-ICAM-1 without any stimulation in vitro. Antigen expression as compared with PSC derived from normal prostate differed only in density and was not characterized by a complete loss or new appearance.

P6

PROLIFERATION RATES AND SUPEROXIDE DISMUTASE ACTIVITIES IN BENIGN HYPERPLASTIC HUMAN PROSTATES

S. Claus, M. Wrenger, S. Tunn*, Th. Senge and H. Schulze, Department of Urology and *Institute of Clinical Chemistry and Laboratory Medicine, University of Bochum, FRG

Growth of benign prostatic hyperplasia (BPH) is associated with aging. Superoxide dismutase (SOD) activity is known to decrease with advancing age and correlates with life span of cells. This prompted us to investigate, if changes of SOD activity corresponds with growth of the prostate.
In epithelium and stroma of prostates of 11 men with BPH removed by open prostatectomy (43-94 years old; mean age 67 years) proliferation rates were determined immunohistochemically using the Ki-67 antibody labelling. In the same prostates SOD activity was determined by inhibition of autoxidation of pyrogallol. The enzyme activity was calculated from the difference in the increase of absorbance at 420 nm with and without adding stromal or epithelial homogenates.
Proliferation rates ± standard deviation (SD) in ~~BPH were nearly identical in stromal~~ (~~0.131~~ ± 0.09%) and epithelial cells (0.139 ± 0.07%). However SOD activity ± SD was significantly higher in stromal (360 ± 147 mU/μg DNA) than in epithelial cells (20 ± 8 mU/μg DNA).
High SOD activity corresponds with a low cell death rate. Thus, stromal cells are more protected than epithelial cells and will have a longer life span. Because of the similar proliferation rates in both compartments, this means that the stromal volume in BPH will increase more than the epithelial compartment. This is in accordance with morphometric studies indicating that BPH is a stromal disease.

P7

THE EFFECT OF FINASTERIDE (PROSCAR®) ON 5α-REDUCTASE IN EPITHELIUM AND STROMA OF HUMAN BENIGN PROSTATIC HYPERPLASIA

Heike Weisser, Sabine Tunn, Michael Krieg
Institute of Clinical Chemistry and Laboratory Medicine, University Clinic Bergmannsheil, Bochum, Germany

The development of human benign prostatic hyperplasia (BPH) is at least partly under androgenic control. Therefore, the inhibition of 5α-reductase, which catalyzes the conversion of testosterone to the most potent androgen 5α-dihydrotestosterone, is a promising concept in the treatment of BPH. Finasteride is a specific 5α-reductase inhibitor that has been shown to reduce the size of BPH. However, at present it is not known whether the epithelial or the stromal 5α-reductase is the preferred target of finasteride. Therefore, the aim of our in vitro study was to describe the effect of finasteride on 5α-reductase in these cellular compartments.
BPH tissue (n=5) was separated in epithelium and stroma. 5α-reductase activity was determined using five different testosterone concentrations (14 - 582 nM) alone or in the presence of varying concentrations of finasteride (1 - 125 nM). The androgen metabolites were separated by HPLC. IC_{50} and K_i were derived from inhibition curves and Lineweaver-Burk plots, respectively. The main results were: (1) 5α-reductase activity in epithelium and stroma was dose-dependently inhibited by finasteride. The mean IC_{50} values determined in the presence of various testosterone concentrations were generally 2- to 4-fold lower in epithelium than in stroma. (2) In epithelium as well as in stroma a competitive inhibition of 5α-reductase was found if finasteride was added in a concentration >5 nM. The mean inhibition constant K_i [nM ± SEM] for that competitive inhibition in epithelium and stroma was 7 ± 3 and 31 ± 3, respectively. (3) In the presence of finasteride concentrations ≤5 nM, in epithelium 5α-reductase seems to be inhibited in an uncompetitive manner, whereas in stroma also at such low finasteride concentrations a competitive inhibition occurs.
The present study demonstrates that the inhibitory effect of finasteride on 5α-reductase is much stronger in epithelium than in stroma. At present, it remains unclear whether the different inhibition mode in epithelium and stroma at finasteride concentrations ≤5 nM is due to the existence of different 5α-reductases or due to different posttranslational modifications of a unique 5α-reductase in epithelium and stroma of BPH. Moreover, the question arises whether under in vivo condition such inhibition actually occur, giving a daily dose of 5 mg of finasteride.

P8

EFFECTS OF AROMATASE INHIBITION ON PROLIFERATION RATES IN HUMAN BENIGN PROSTATIC HYPERPLASIA

S. Claus, Th. Senge and H. Schulze
Department of Urology, University of Bochum, FRG

Estrogens are believed to play a critical role in pathogenesis especially on stroma of benign prostatic hyperplasia (BPH). Inhibition of estrogen synthesis by selective blockade of the aromatase enzyme could be an effective systemic mean of reducing estrogen production. The influence of estrogen deprivation on proliferation rates was studied on BPH in men.
14 patients with large, symptomatic BPH were treated with the aromatase inhibitor Atamestane (1-methyl-androsta-1,4-diene-3,17-dione) (600 mg/day p.o.). After 3 months of treatment 4 men had an open prostatectomy due to insufficient symptomatic improvement. In epithelium and stroma of these prostates proliferation rates were determined immunohistochemically using the Ki-67 antibody labelling (group 1). The results obtained were compared to data obtained in 11 age-matched, previously untreated open prostatectomy specimens (~~group~~ 2).
Proliferation rates (PR) ± standard deviation of group 1 was distinct higher in epithelium (0.200 ± 0.24%) than in stroma (0.095 ± 0.07%), while in group 2 there was no difference between epithelium (0.121 ± 0.06%) and stroma (0.120 ± 0.09%). Thus, compared to the untreated prostates aromatase inhibition led to an increased PR in epithelium and a diminished PR in stroma.
The results indicate, that estrogen suppression could influence the BPH by decreasing the stromal proliferation. However, these effects may be overcome by epithelial stimulation due to an increase of serum androgens, known to occur after aromatase inhibitor administration.

P9

TRANSURETHRAL NEEDLE ABLATION (TUNA) : HISTOPATHOLOGICAL, RADIOLOGICAL AND CLINICAL STUDIES OF A NEW OFFICE PROCEDURE FOR TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

C.C. Schulman, A. Zlotta, Dept. Urology, University Clinics of Brussels, Erasme Hospital, Brussels, Belgium

Many attempts have been made to develop a method for treating benign prostatic hyperplasia (BPH) that is minimally invasive, efficacious, and low cost. The transurethral needle ablation (TUNA) device has recently been developed to treat BPH by selectively ablating hyperplastic prostatic tissue. A special catheter incorporates needles that deliver low-level radiofrequency power directly to a very localized area of the prostate. The needles have adjustable shields to protect the urethra if desired or necessary. It is positioned via transrectal ultrasound or direct vision. A pilot study was performed in patients to evaluate TUNA feasibility via histopathological measurement of thermal lesion size and TUNA safety. Fifty patients have been treated, twenty-five using TUNA prior to scheduled retropubic prostatectomy. The surgical prostatic specimens were recovered from 1 day to 1 month after TUNA, stepsectioned, and examined histologically. Patients were 69 years old on average with prostate weight varying from 14 to 88 g. The TUNA procedure averaged 30 minutes, 4 lesion treatments per prostate, and 4-15 W of power applied for 3 to 5 minutes. Central lesion temperatures was about 110°C. Urethral temperature averaged 37-42°C and rectal temperature remained unchanged. Macroscopic and microscopic examination showed lesions of extensive coagulative necrosis up to 35 X 15 mm with destruction of all tissue components. Preservation of the urethra and capsule integrity were noted. Magnetic resonance imaging showed lesions in the prostate corresponding to the recovered surgical specimen. All patients were treated without anesthesia and tolerated the procedure well. Of the 9 patients treated for chronic retention, 6 recovered voiding within 48 hours. 3 month follow-up in 11 patients showed significant improvement in both objective and subjective parameters. This study demonstrated TUNA safety through no rectal temperature change and lesion localization; feasibility shown by the creation and sustainability of adequately sized lesions, and good tolerance for outpatient treatment. Clinical studies are ongoing to evaluate the sustained efficacy of the procedure.

P10

GM-CSF AND PROSTATE CANCER.

Shona Lang, William.R. Miller & Fouad.K. Habib, Departments of Surgery (WGH) and Clinical Oncology, Western General Hospital, Edinburgh.
Supported by the Imperial Cancer Research Fund and the Association of International Cancer Research.

GM-CSF has been implicated in the aetiology of several cancers (eg. Young et al. 1992, Int. J. Cancer:50,628-634). Its apparent role in tumour progression may be as an autocrine regulator. Our earlier studies have shown that medium supplemented with exogenous rGM-CSF can stimulate the growth of hormone insensitive (PC-3 and DU145) and hormone sensitive (LNCaP) prostate carcinoma cell lines. The aim of the present study was to investigate whether these same prostate cell lines could also produce GM-CSF. Immunohistochemical studies have identified material that was immuno reactive with an anti-human GM-CSF monoclonal antibody (mAB) in DU145 and PC-3 cell lines but not LNCaP. Furthermore media conditioned (CM) by the three prostate cell lines was analysed by ELISA and Western Blot. The ELISA assay demonstrated large concentrations of GM-CSF-like material in the conditioned medium of PC-3 and DU145 cultures but not LNCaP. Western Blot analysis revealed protein bands that were immunoreactive with the GM-CSF mAB and which co-migrated with recombinant GM-CSF, only from DU145 and PC-3 CM. These studies were also extended to examine the presence of GM-CSF gene transcripts in total RNA from the prostate cell lines. Employing RT-PCR, GM-CSF transcripts were detected in both the PC-3 and DU145 cells. Preliminary immunohistochemical studies with prostate tissue sections found GM-CSF-like material in poorly differentiated cancers but not well differentiated. These results indicate GM-CSF is produced by the hormone insensitive prostate cancer cell lines, DU145 and PC-3, but not the hormone sensitive cell line, LNCaP. Since rGM-CSF can stimulate the growth of all three cell lines it raises the possibility that GM-CSF may be acting as an autocrine growth factor in the hormone insensitive cell lines.

P11

LOSS OF KGF RECEPTOR EXPRESSION IN AGGRESSIVE TRANSITIONAL CELL CARCINOMA (TCC) OF THE BLADDER

François Radvanyi*, Sixtina Gil Diez**, Annie Delouvée*, Jean-Paul Thiery*, Claude Abbou** and Dominique Chopin**.
*URA CNRS 1337, Paris, **Hôpital Henri Mondor, Créteil - France

The study of known genes with potential relevance for cancer development is one approach used to characterized tumors at the molecular level. Recent studies have provided informations that alterations of expression of cell adhesion molecule genes such as E-cadherin may be useful to predict clinical aggressiveness. We have been investigating alteration in growth factor and growth factor receptors in tumor progression. As result on extensive screening of the FGF family genes, attention has been drawn on the KGF receptor (KGFr) known as a specific epithelial markers which is involved in the paracrine regulation of stromal epithelial interactions. We have developed a semi quantitative RT-PCR analysis with various internal controls to study expression of growth factor and growth factor receptor on human tumors. Total RNA has been extracted from cells scrapped of normal urothelium, lamina propria, muscle of normal bladder (N = 5) and various TCC (N = 46). A semi quantitative RT-PCR analysis was performed using TFIID as an internal standard. Amplification products were selected in the exponential part of the reaction and quantified using a phosphoimager. KGF was found predominantly in the stroma whereas its receptor was found in the urothelium. In two carcinomas, an overexpression of the receptor mRNA was found, but in most of the tumors analysed (44 cases), the level of KGF receptor mRNA was either unchanged or decreased. Results indicates that a threshold of 30% for KGFr/TFIID ratio has a potential prognostic value comparing the progression rate using the log rank test ($p = 0.0124$) comparing time event probability estimated by the Kaplan Meier method. These data suggest that lost of expression of KGFr is an important molecular event in tumor progression. This kind of approach will allow a better understanding of the different changes associated with onset or progression of bladder cancer.

P12

EVALUATION OF RANDOM BLADDER BIOPSIES IN PATIENTS WITH BLADDER CANCER.

J.Alfred Witjes, Rainy Umbas, Frans M.J.Debruyne and Jack A. Schalken, Nijmegen, The Netherlands

Introduction. Histopathology of mucosal biopsies gives little prognostic information in patients with transitional cell carcinoma (TCC) of the urinary bladder. We investigated mucosal biopsies with several molecular markers presumably associated with progression of TCC, in 6 groups of patients with an increasing malignancy of the primary tumor.

Patients and methods. Six groups of 10 patients were selected: (1) benign disease (e.g. BPH); (2) low risk superficial tumors, disease free without adjuvant treatment; (3) superficial tumors, disease free with adjuvant intravesical instillations; (4) recurrent superficial tumors in spite of additional therapy; (5) progression (muscle invasivion or metastasis) in spite of additional therapy; (6): invasive or disseminated disease at presentation. In all patients cold-cup mucosal biopsies were taken from normal mucosa and stored in liquid nitrogen. The technique was tested in a pilot study. We studied the expression of HLA I, HLA II, E-Cadherin (HECD), cytokeratin (CK18-2), Fibronectin, I-CAM and EGF-R.

Results. CK18-2 expression was used as an epithelial marker. HLA II, HECD, Fibronectin and I-CAM expression showed no significant changes in the different groups. HLA I and E-GFR expression became increasingly more positive with a higher malignancy of the tumor.

Discussion. A relation between several markers and bladder cancer (progression) is described. However, the expression of these markers in normal mucosa is unknown. Theoretically one could expect a rise in E-GFR and I-CAM expression and a fall in HLA, E-cadherin and Fibronectin expression with increasing malignant potential of the urothelium. The fact that most markers show no changes indicates that the mucosa adjacent to tumors reacts similar to normal mucosa, and this does not support the "condemned mucosa" theory. The increasing HLA I expression is even the opposite of what was expected. However, the increasing expression of EGF-R might indicate a malignant potential of the normal mucosa, and might be of prognostic value. On the other hand, since this increased EGF-R expression especially is found in patients with invasive lesions, this might also indicate that increased EGF-R is rather an epiphenomenon, more than specifically associated with TCC progression.

Conclusion. Increasing EGF-R expression in normal looking mucosa of patients with increasing stages of bladder tumors could be a prognostic factor, or might indicate that this increase in expression is not tumor specific, but seen in the whole bladder.

P13

CORRELATION OF TGFβ-3 EXPRESSION WITH CELL PROLIFERATION AND APOPTOSIS IN NORMAL AND NEOPLASTIC PROSTATE

V. Djonov[1,2], A. Arnold[1], A.-C. Andres[1], U. Studer[1,2] and V. W. Merz[1,2]
[1]Laboratory for Clinical and Experimental Research, Tiefenaustrasse 120, 3004 Bern, Switzerland and [2] Dept. of Urology, Inselspital, 3010 Bern, Switzerland

There is accumulating evidence that factors involved in the control of normal cellular growth and differentiation plays also a fundamental role in the neoplastic development of the prostate. TGFβ-3, involved in tissue remodeling and epithelial cell growth arrest, may therefore play an important role in the homeostasis of the prostate. We initiated a comparative analysis of the expression of TGFβ-3 in normal prostate, in benign prostate hyperplasias (BPH), in carcinoma in situ and in invasive carcinomas of the prostate by immunohistochemical analysis. The results were correlated *in situ* with cell proliferation using the proliferation specific antibody Ki67 and with cell death by apoptosis using radioactive labeling of fragmented DNA by terminal transferase. In the normal prostate expression of TGFβ-3 in basal epithelial cell layer corresponded with labeling of epithelial cells by terminal transferase. In contrast, regions of cell proliferation, indicated by Ki67 staining, did not show TGFβ-3 expression. Interestingly, co-expression of TGFβ-3 and the Ki67 antigen was found in the basal epithelial cell layer of BPH. The subsequent cell layers were negative for both markers and no apoptotic cells were detected in the hyperplasias. A similar result was found in the carcinomas in situ, however, cell proliferation was not limited to the basal cell layer. Invasive carcinomas were characterized by high cell proliferation and the complete absence of TGFβ-3 expression. Cell death by apoptosis could be observed occasionally in the center of tumors. These results suggest that the balance between the expression of TGβ-3 and epithelial growth arrest, is progrediently lost during neoplastic development and the complete absence of TGFβ-3 expression characterizes the invasive carcinomas of the prostate.

P14

'NERVE GROWTH FACTOR IN BPH AND PROSTATE CANCER.'

Alan B. Paul, Geoffrey D. Chisholm, Fouad K. Habib.

Dept of Surgery/Urology, University of Edinburgh, W.G.H. Crewe Road Sth. Edinburgh, U.K.

Supported by the Melville Trust for the Care and Cure of Cancer.

The peptide hormone β Nerve Growth Factor (βNGF) is trophic for autonomic neurones and mitogenic for prostate cancer cell lines. We have studied its expression in prostate cancer (CaP) and benign prostatic hyperplasia (BPH.)

βNGF concentrations were measured by enzyme linked immunosorbent assay. The mean concentration in BPH was 1980pgg^{-1} wet weight of tissue (n=15, SD=730.) The concentration in CaP was higher; x=3100pgg-1 (n=15, SD=1505, $p<0.05$, Mann-Whitney.) Reverse transcription-polymerase chain reaction was used to demonstrate βNGF mRNA. Primers in the open reading frame and spanning an intron of around 7000 bases, were designed. The expected product of 542bp was demonstrated in all BPH and CaP tissue examined. βNGF was localised to epithelial cells in both BPH and CaP by immunohistochemistry using a rabbit anti-mouse βNGF polyvalent serum. This finding contradicted previous publications where βNGF was localised to stroma. Therefore the specificity of this antiserum was tested by immunoblotting. Protein extracts from BPH and CaP were subjected to SDS-PAGE and blotted to nitrocellulose. The antiserum specifically detected a protein band at 30,000 daltons consistent with βNGF in its dimeric form. This band co-migrated with a band produced by recombinant human βNGF. We did not observe bands suggestive of other NGF-like neurotropins or of a heavier NGF-like protein described in cell culture studies. βNGF-receptor was localised to epithelium in CaP and BPH using a monoclonal anti-human βNGF receptor antibody. NGF-like immunoreactivity in the human prostate represents true βNGF - not other neurotropins known to react with βNGF antisera. βNGF is endogenously produced by epithelium in both CaP and BPH in biologically active concentrations. Concentrations are higher in CaP than in BPH. βNGF may act as an autocrine mitogen in CaP with both hormone and receptor localised to epithelial cells. It may mediate the growth of autonomic nerves, and so smooth muscle, secondary to epithelial growth. in BPH.

P15

PCNA, Ki-67 AND MA903 EXPRESSION IN BPH AND PROSTATIC CARCINOMA. Pieter J van den Broeke, Fibo J ten Kate, George van Andel, Karl-Heinz Kurth and Denis H Schamhart. Dept. Urology, Univ. Amsterdam, Amsterdam, The Netherlands.

Introduction. Discrimination between patients with benign prostatic hyperplasia (BPH), prostatic carcinoma (PCa) or prostatic intraepithelial neoplasma (PIN) is crucial to the management of the disease. In a ongoing study the proliferative activity or growth fraction in tissues originating from BPH, PCa, grade 1 and PIN, grade 3 were investigated immunohistochemically using MoAbs against PCNA (Proliferating Cell Nuclear Antigen) and Ki67. The additional value of an anti-keratin MoAb (MA903), specifically recognizing the basal cell layer (BCL) in BPH-tissue was explored for the diagnosis of either BPH or PCa.

Patients and methods. Tissue samples of a total of 25 patients were histopatologically diagnosed as BPH (10), PCa, grade 1 (10) and PIN, grade 3 (5). Immunostaining was performed on routinely, formalin fixed, dewaxed tissue slides. The MoAbs PC10, MIB1 and MA903 were used for staining of PCNA, Ki67 and keratin of the BCL, respectively. A semiquantitative scoring system expresses the labeling index (LI) as number of positive nuclei per total number of nuclei (500x power field).

Results. PCNA expression was very heterogeneously in carcinomatous tissue and no significant difference in proliferative activity was found between BPH and PCa, grade 1. The average PCNA score of 10 PCa pts was 7 (LI=0%), 1 (LI=2%), 1 (LI=5-20%), 1 (LI>20%) compared to 6 (LI=0%), 2 (LI=2%), 2 (LI=5-20%) and 0 (LI>20%) in 10 BPH pts. At present, the sample size of PIN pts prevent definite conclusions, although a trend for an increased proliferative activity compared to BPH was observed. In contrast, the significant ($p<0.009$; Fischer's exact test) increase of the average Ki67 score of 10 PCa pts was 2 (LI=0%), 1 (LI=1%), 2 (LI=2%), 3 (LI=3-5%) and 2 (LI>5%) compared to 4 (LI=0%), 4 (LI=1%), 2 (LI=2%), 0 (LI=3-5%) and 0 (LI>5%) in 10 BPH pts. Staining of the basal cell layer with MA903 was only observed in BPH (2/10) and PIN, grade 3.

Conclusions. In the absence of staining of the basal cell layer with MA903, prostatic carcinoma should be considered. Combination of MA903 negativety and a labeling index > 5% (Ki67) strongly suggest the presence of prostatic carcinoma. Determination of Ki67 score may be helpful for further evaluation of atypical cells or PIN's in BPH.

P16

DETERMINATION OF THE Ki67 DEFINED GROWTH FRACTION BY THE MONOCLONAL ANTIBODY MIB1 IN FORMALIN-FIXED, PARAFFIN EMBEDDED PROSTATIC CANCER TISSUES.

Marinus A. Noordzij; Theodorus H. van der Kwast*; Gert Jan van Steenbrugge; Wytske M. van Weerden; Maria H.A. Oomen; Fritz H. Schröder. Departments of Urology and *Pathology, Erasmus University Rotterdam, The Netherlands.

Determination of the proliferative activity has been used as prognostic factor in many tumors among which prostate cancer. In the present study, different modalities of determination of the Ki67 defined proliferative activity were studied in two androgen dependent prostatic tumor models: the in vivo PC82 model and the in vitro LNCaP model. The growth rate of both models was manipulated by growing the cell lines at various androgen concentrations. Previously, Ki67 was thought to be applicable only to frozen tissues (F-Ki67), but it has recently been shown that Ki67 (P-Ki67) and a new monoclonal antibody against the Ki67 antigen (MIB1) are applicable to paraffin-embedded tissues following antigen retrieval. BrdU-uptake was used as S-phase marker. The proliferative activities determined by the different methods clearly reflected the growth response of the tumors upon the different hormonal conditions. A statistically significant correlation was found in 15 acetone-fixed LNCaP cell cultures between Ki67 and MIB1 scores ($r=0.95$, $p<0.001$). Statistically significant correlations were found in PC82 tumors between MIB1 scores and P-Ki67 ($r=0.91$, $p<0.001$, $n=25$), F-Ki67 ($r=0.90$, $p<0.001$, $n=21$) and BrdU-uptake ($r=0.65$, $p<0.001$, $n=24$), respectively. Although P-Ki67 scores were two times as high as F-Ki67 scores (linear regression coefficient $\beta= 2.03$), a significant correlation was found between these measures ($r=0.79$, $p<0.001$). Strikingly, MIB1 scores determined on PC82 sections largely exceeded F-Ki67 and P-Ki67 scores ($\beta= 3.67$ and 1.57 respectively). In LNCaP slides MIB1 scores almost doubled the Ki67 scores ($\beta= 1.81$). The staining procedures were identical for MIB1 and P-Ki67 in PC82 tumors and for MIB1 and Ki67 in acetone-fixed LNCaP slides. MIB1 staining showed marked variation in intensity but was in general more pronounced than Ki67 staining and therefore a number of MIB1 positive cells might have been Ki67 negative. Other explanations for these findings are speculative, however. Although some points need to be clarified, it is concluded that MIB1 is a promising means of evaluating the presence of the Ki67 antigen in paraffin embedded human tumors, especially in relatively slowly growing tumors like prostate carcinomas.

P17

HEPARIN BINDING GROWTH FACTORS bFGF AND KGF IN PROSTATIC CARCINOMA : IN VITRO EFFECTS ON CELL GROWTH AND DETERMINATION OF bFGF SERUM LEVELS IN PATIENTS

Marcus V. Cronauer , Christian Radmayr , Alfred Hobisch, Zoran Culig, *Anton Hittmair, Andreas Reissigl , Georg Bartsch and Helmut Klocker, Department of Urology and Pathology*, University of Innsbruck, Austria

Prostatic carcinoma, a common cancer in males is among the leading causes of cancer death in elderly men. Cellular growth of prostatic cells is stimulated by androgens, the mechanisms of hormonal control however remain unclear. Recent studies demonstrated the importance of stromal-epithelial interaction in the development of prostatic malignancies. Heparin binding growth factors such as basic fibroblast growth factor (bFGF) and keratinocyte growth factor (KGF) seem to play a crucial role in stromal-epithelial interaction. Moreover, bFGF shows considerable angiogenic properties which is vital for tumorogenesis and metatstatic growth.

Primary cell cultures of prostatic epithelial cells were sensitive to a stimulation by bFGF and KGF. Addition of 10 ng/ml KGF aproximately doubled the proliferation rates whereas bFGF exhibited only small stimulatory effects in our culture system. Using an ELISA assay we detected bFGF secretion into the supernatant of epithelial and fibroblast cells of the prostate. Suspecting that bFGF levels may be altered in patients with prostatic proliferative disorders we evaluated serum bFGF levels in these patients. In 12 patients with benign prostatic hyperplasia (BPH) we measured 0 to 7.5 pg/ml bFGF (mean = 2.45), in 23 prostate cancer patients 0 to 23 pg/ml (mean = 8.06 pg/ml). Control sera from 9 healthy individulas had bFGF levels ranging from 0 - 2.4 pg/ml (mean = 0.63 pg/ml). There was no strict correlation between PSA and bFGF serum levels in cancer patients, however, patients with high bFGF serum concentrations also had higher PSA serum levels.

In view of the modulation of proliferation of prostatic cells in vitro by KGF and bFGF our present data suggest an important role of these two growth factors in the regulation of normal and malignant cellular growth of the prostate. The fact that bFGF is measurable in the supernatant of primary cell cultures and in the sera of patients makes it an interesting candidate for an additional complementary tumor marker in prostate cancer.

P18

IS APOPTOSIS IMPORTANT FOR THE CASTRATION INDUCED INHIBITION OF THE DUNNING R3327 PAP ADENOCARCINOMA

Anna. Brändström[1,3], Anders Bergh[2], Stefan Cajander[2], Patrick Westin[2] and Jan-Erik Damber[1]. Departments of Urology and Andrology (1), Pathology (2) and Physiology (3), Umeå, Sweden.

The idea that programmed cell death (apoptosis) is important for the effect of castration on prostatic carcinoma is supported by findings in the normal prostate. In the present study we investigated the effects of castration on the androgen-sensitive prostatic Dunning R3327 PAP adenocarcinoma in comparison with the ventral prostate.

Methods: Tumor tissue and the ventral prostate were collected from intact and castrated animals at 1, 2, 3, 5, 7, 10 and 14 days of castration. Both tissues were examined immunohistochemically for bromodeoxyuridine (BrdU) incorporation and the expression of sulphated glycoprotein-2 (SGP-2, TRPM-2) and tissue type plasminogen activator (t-PA). Testosterone repressed prostatic message-2 (TRPM-2) and t-PA were also analysed at the transcriptional level with Northern blot and hybridization experiments. Genomic DNA integrity was gelelectrophoretically examined and apoptotic tumor epithelial cells were detected with *in situ* End Labeling (ISEL).

Results: Androgen ablation resulted in an induction of TRPM-2 and t-PA on both RNA and protein level in the ventral prostate, but not in the Dunning tumors. ISEL-index for the Dunning tumors as well as the DNA-gelelectrophoretically analysis confirmed that there were no increase of castration induced apoptosis in the tumors compared to the elevated active cell death observed in the ventral prostate. Immunostaining for BrdU showed that castration induced an initial increase in the number of BrdU-positive cells in the ventral prostate, followed by a decline in the number of stained cells (cells in s-phase). In the tumors castration resulted in a decrease in BrdU-positive cells.

Conclusion: The present study demonstrate major difference in the response to castration between the ventral prostate and the prostatic tumor in the same animals. Both types of tissues undergo regressive changes, but an induction of apoptosis were only observed in the ventral prostate. The reasons to these differences are unknown, but they may indicate differences in the androgenic regulation between normal and malignant prostatic tissue.

P19

IS DECREASED APOPTOTIC RATE ONE MECHANISM BEHIND HORMONE-INDEPENDENT TUMOR REGROWTH, IN PROSTATIC CANCER AFTER CASTRATION?

M. Landström †, J.-E. Damber‡, A. Bergh†. † Departments of Pathology and ‡Urology, University of Umeå, S 901 87 Umeå Sweden

INTRODUCTION: We have developed a new experimental model to study hormone-refractory tumor growth in the Dunning rat prostatic system. Castration of rats transplanted with the hormone-sensitive Dunning R3327 PAP tumor results initially in a reduction of tumor growth, but after approximately 3 months some of the tumors started to grow again (1). Apparently, castration induces an alteration of the androgen-sensitive R3327 PAP tumor phenotype, to an androgen-insensitive tumor with an altered morphology. During the last decade apoptosis has been shown to be of fundamental importance for tissue growth and regression both in normal organs and in tumors. It has been suggested that an increase in tumor volume is not nessecerily the result of a higher proliferation rate but of a decrease in the number of cells being depleted by apoptosis. The present study was performed in order to examine whether the hormone-refractory tumor regrowth in the R3327 PAP model may be explained by decreased cell death rate.

MATERIAL & METHODS: Rats transplanted with R3327 PAP tumors were castrated and followed for 16 to 20 weeks. During this time some of the tumors (N=6) started to grow again after an initial inhibition in tumor growth but others remained stable (N=12), making it possible to investigate differences between the relapsing and non-relapsing tumors. Pieces of tumors were fixed for morphological and immunohistochemical investigations. Morphology was examined in 1 micron thick sections of 1000 X magnification and the number of mitotic and apoptotic cells were counted. ISEL (in situ end-labelling) was also used to labell apoptotic cells. To investigate the proliferation rate of the tumor cells, immunostaining for PCNA was also performed.

RESULTS: Tumor growth rate was negatively correlated to the number of apoptotic cells showing a rank-correlation (RS) of -0.76 ($p<0.002$, N=18) for light-microscopic identified cells and RS -0.75 ($p<0.002$) for ISEL positive cells. The mitotic index was not correlated RS=0.33 ($p<0.25$) to tumor growth rate. The number of tumor cells stained by the PCNA-antibody did not correlate with tumor growth rate, RS=-0.34 ($p=0.23$).

CONCLUSION: Hormone refractory tumor regrowth in the R3327 PAP model depends on a reduction of the number of tumor cells being depleted by apoptosis, rather than on an increase in proliferation rate. This suggest that relapse could be caused not by local secretion of growth factors but induction of survival factors!

1, Landström M, Damber J-E, Bergh A: Estrogen treatment postpones castration-induced dedifferentiation of the Dunning R33327-PAP prostatic adenocarcinoma. The Prostate, in press 1993.

P20

ANDROGEN RECEPTOR GENE MUTATIONS IN HUMAN PROSTATIC TUMOURS

Maureen E. Harper, Bronwen A.J. Evans, Claire E. Daniells & Keith Griffiths. Tenovus Cancer Research Centre and Dept. of Child Health, University of Wales College of Medicine, Cardiff, Wales, UK.

The majority of prostatic carcinomas are initially androgen responsive but these eventually relapse to an androgen independent stage. A key component of the androgen signal transduction cascade in responsive tissues is the androgen receptor (AR) and changes in this moiety may be responsible for the development of androgen insensitivity.

We initially extracted DNA from 15 prostatic carcinoma specimens and 10 BPH specimens, the 3 cell lines PC3, DU145, and LNCaP and the transplantable prostatic tumours TEN 12 and Ten15 for somatic mutations in the AR gene. All the primary prostate carcinoma samples were collected from patients prior to therapy.

The coding regions of the gene were amplified by the polymerase chain reaction (PCR) using oligonucleotide primers spanning the 8 exons then screened for single strand polymorphisms (SSCP) to identify mutations. The presence of a mutation was indicated by a shift in the mobility of the denatured PCR fragments, when run on non-denaturing gels, compared to fragments prepared from normal DNA. If a band shift was evident that sample was then sequenced to determine the position and precise nucleotide change in the gene. In one well differentiated carcinoma we found a point mutation (C to G) in exon F which leads to substitution of glutamine with glutamic acid at position 798 of the AR protein. This mutation was also found in the patient's peripheral blood lymphocytes DNA. In two BPH samples intronic mutations have been detected, one (G to A) at position 3746 of intron 6 and the other (C to T) at position 2410 in intron 3. The latter mutation was also present in the patient's blood. Polymorphisms in the A exon has also been detected in the 5 prostate samples and the DU145 cell line. Prostate carcinoma specimens from patients with advanced disease and a few specimens from androgen relapsed patients are currently being screened for mutations. The possibility that AR mutations are important factors in the development of androgen resistance should be indicated by an increased frequency of somatic mutations in this latter group of specimens. Significant mutations should alter one or more of the receptor protein's functional binding activities ie to HRE sites on DNA, heat shock protein 90 and to steroids in terms of affinities and/or specificity .

P21

DO NEUROENDOCRINE CELLS IN HUMAN PROSTATE CANCER EXPRESS ANDROGEN RECEPTOR?

Th.H. van der Kwast, J.L.M. Krijnen, P.J.A. Janssen, J.A. Ruizeveld de Winter, H. van Krimpen, F.H. Schröder+. Departments of Pathology and Urology+, Erasmus University, Rotterdam, The Netherlands.

The presence of androgen receptors (AR) in neuroendocrine (NE) cells was investigated in benign tissue of ten prostatectomy specimens, in twelve prostatic adenocarcinomas with focal NE different-iation and in one case of a pure NE small cell carcinoma of the prostate. NE cells were defined by their reactivity with an antibody to chromogranin A. Monoclonal antibody F39.4 directed against the N-terminal domain of the AR molecule was used to detect AR. AR and chromogranin A were simultaneously visualized with a double immunofluorescence technique.

The results indicate that chromogranin positive cells in both benign and malignant prostatic tissue lack detectable expression of AR in contrast to the surrounding tumour cells. No effect of endocrine therapy was noted. The pure NE small cell carcinoma also lacked AR expression. These results are in line with the hypothesis that prostatic NE tumour cells represent an androgen insensitive cell population that incidentally may expand to replace the androgen sensitive tumour cell population during androgen ablation therapy.

P22

MODULATION OF ANDROGEN RECEPTOR, EPIDERMAL GROWTH FACTOR, bcl2 EXPRESSION BEFORE AND AFTER ANDROGENIC DEPRIVATION IN HUMAN PROSTATIC CARCINOMA (CaP)

Sixtina Gil Diez*, Marc C. Colombel*, Theodorus H. Van der Kwast, Claude C. Abbou*, Dominique K. Chopin*.**

* Service d'Urologie, Hôpital Henri Mondor, Créteil, France

** Erasmus University, Rotterdam, The Netherlands

There is a need to better understand mechanisms involved in cell growth after androgenic deprivation in human prostatic carcinoma. We studied by mean of immunohistochemical techniques the expression of androgenic receptor (AR), epidermal growth factor receptor (EGFr) and bcl2 in various prostatic tissues. To precisely phenotype changes occuring before and during hormonal deprivation and after hormonal failure we used a panel of antibody-markers: F39-4-1 (androgen receptor), RGE53 (cytokeratin 8-18), RCK 103 (cytokeratin 5 and others), EGFr (ab1, Oncogene Science), bcl2 (Dako), prostatic specific antigen (PSA-ER-PR8). This panel of antibody-markers was used on the same step sections of fresh frozen specimen on 4 fetal prostates, 10 normal prostates, 17 localised CaP treated by radical prostatectomy only (group 1), 20 CaP after androgenic deprivation (group 2), 9 CaP after clinical patent hormonal failure (group 3). Combination of immunostaining allowed a precise localization and description of prostatic carcinoma on fresh frozen sections. The expression of antibody markers on tumor cells is as follow: group 1 expressed AR on more than 80% of malignant cells the androgen receptor (6/7); in group 2 the expression was decreased and heterogenous (12/14); in group 3 the expression was heterogenous but increased, nuclear and cytoplasmic staining was observed in all specimens. EGFr was seldom expressed (3/17) on a subset of malignant cells in group 1 but increased after androgenic deprivation (14/20) and hormonal failure (8/9). A shift in the pattern of cytokeratin was also observed with expression of luminal cell type and basal cell type keratins in the same malignant cells in groups 2 and 3. Expression of bcl2 was noted on a subset of malignant cells in group 1 and increased after hormonal deprivation and hormonal failure. These results allow a characterization of malignant cells surviving after androgenic deprivation and marker for phenotyping this subpopulation of cells in prostatic carcinoma. These results may be useful for the search for antigrowth factor therapy and a better understanding of cell death-resistance mechanisms after conventional hormonal therapy in prostatic carcinoma.

P23

PRODUCTS OF DIHYDROTESTOSTERONE METABOLISM ACTIVATE A MUTANT ANDROGEN RECEPTOR DETECTED IN A LATE STAGE PROSTATIC CARCINOMA.

Alfred Hobisch, Zoran Culig, #Andrew C.B.Cato, #Anne Denninger, *Jan Trapman, Christian Radmayr, Georg Bartsch, and Helmut Klocker, Department of Urology, University of Innsbruck, Austria, #Institute of Genetics, Kernforschungszentrum Karlsruhe, Germany, and *Department of Pathology, Erasmus University Rotterdam, The Netherlands

We have recently demonstrated activation of the mutant androgen receptor AR^{715}met, which was detected in a sample obtained from a metastatic prostatic carcinoma, by adrenal androgens dehydroepiandrosterone and androstenedione, and by progesterone (Culig et al: Mol. Endocrinol, in press). This aberrant receptor activation occurs although binding affinities of the mutant receptor for these hormones remain unchanged. We extended these studies and investigated effects of products of dihydrotestosterone (DHT) metabolism on the activation of AR^{715}met.

Either wild-type or mutant AR expression vectors, together with reporter plasmids, were introduced into CV-1 cells, which are devoid of endogenous steroid receptors. The reporter gene was the chloramphenicol acetyl-transferase (CAT) gene, which was driven either by a thymidine kinase promoter consisting of two androgen-responsive elements (ARE2 tk CAT construct), or by the naturally ocurring prostate-specific antigen (PSA) promoter (PA-CAT). 24 hours after transfection, DHT, synthetic androgen mibolerone, and DHT metabolites androsterone and androstandiol were added to the transfected cells. DHT metabolites were applied in concentrations of 50-100 nM. CAT activity was determined in cell extracts 30 hours after addition of hormones. Androsterone and androstandiol were more effective in transactivation of the androgen-responsive reporter genes when added to cells transfected with the mutant AR expressioin vector. These differences were more pronounced with ARE2 tk CAT than with PA CAT. Androsterone and androstandiol, at concentration of 100 nM induced 80% of the maximal reporter gene expression in the presence of the mutant receptor whereas CAT activity mediated by the wild type receptor was only 20% in response to these substances.

These results provide further evidence for a broad steroid specificity of AR^{715}met. Taken together, our data imply that the activation of the mutant AR^{715}met by precursors and metabolites of DHT may reflect growth advantage of prostatic cells bearing this mutant receptor.

P24

PRIMARY CULTURE OF CANCER OF PROSTATE EPITHELIAL CELLS: THE LEICESTER EXPERIENCE

Elijah O Kehinde, Timothy R Terry, Natu Mistry, Terence Horsburgh, S Javid Iqbal, Kevin O'Reilley, Peter R Bell.
University of Leicester, Leicester, United Kingdom

Cell lines are useful in the evaluation of the biological defects involved in carcinogenesis. However, serial passaging of cell lines can lead to loss of tumour characteristics,thus a reliable method for short term culture of primary tumour tissue might provide a more relevant *in vitro* cancer model. We used WAJC 404 medium supplemented with insulin, Dexamethasone, Epidermal growth factor, Penicillin and Streptomycin, HEPES and $NaHCO_3$ (Primary Epithelial Growth Medium: PEGM), to culture epithelial cells from benign prostatic hyperplastic glands (BPH) and Cancer of prostate (CaP) specimens. Specimens were obtained from Transurethral resection of prostate gland (TURP) chips, radical prostatectomy specimens (RPS) or Bard biopty gun biopsy for CaP. All samples were checked for the presence of tumour, sterility and the presence of keratin. Growth of primary and secondary cultures was assessed using ^3H thymidine incorporation, with total and prostatic acid phosphatase measured in the supernatant of confluent cell cultures. In PEGM the rate of establishment of primary cultures without fibroblasts was: 5/6 of BPH from TURP (control), 2/6 CaP from TURP, 3/3 CaP from wedge resection of RPS, 2/3 CaP from Biopty gun biopsy. The total and prostatic acid phosphatase levels were proportional to the cell density of the CaP cultures tested. The cells tested positive for keratin 8 and 18. Primary culture of tissues in 10% FCS yielded both epithelial cells and fibroblasts, but secondary culture of these cells produced mostly fibroblasts. Summary: a) WAJC 404 with supplements (PEGM) supports the serial passage and clonal growth of human CaP epithelial cells and suppresses the proliferation of fibroblasts. b) The best clonal growth is obtained by using specimens obtained from radical prostatectomy. c) The ability to derive cultures from Biopty gun specimens allows the characterisation of cells from primary CaP of high malignant grade, or those of stage D_3 CaP specimens.

P25

NEW METHODS OF PRIMARY CULTURE OF EPITHELIAL CELLS AND FIBROBLASTS DERIVED FROM HUMAN NORMAL AND MALIGNANT UROTHELIUM

Iris Eder, Arnulf Stenzl, Hermann Draxl, Marcus Cronauer, Georg Bartsch and Helmut Klocker. Department of Urology, University of Innsbruck, Austria.

Primary cell culture has become a valuable tool as an *in-vitro* model in cancer research. Primary cultures of non-transformed urothelial cells are difficult to obtain and to handle. Therefore mainly permanent cell lines were established and used in bladder cancer investigations. However, such cell lines often change cellular characteristics, chromosomal patterns or growth properties in long-term culture. Therefore we have attempted to cultivate freshly established cells that are more likely to resemble the properties of the original tissue. So far we tasted various culture media and cultivation techniques. Often used methods such as DMEM in combination with tissue disaggregation by trypsin in our hands resulted in cultivation of fibroblasts. For that reason we used an alternative mechanical disaggregation method and digestion with collagenase/dispase, respectively. The medium was supplemented with insulin, transferrin, and non essential amino acids and antibiotic concentration was reduced in order to improve initial cell growth. Considerable advances in the cultivation of epithelial cells where achieved when a modified Keratinocyte serum-free medium (KSFM) was used. This medium was shown to be selective for epithelial cells. Due to a low calcium concentration this medium selectively supports growth of epithelial cells. Supplementation with 1% of non essential amino acids further improved proliferation rates of bladder epithelial cells. Subcultivation of these cells was made by digestion with collagenase/dispase and seeding with ECL (entactin - collagen IV - laminin). Our modified medium and the very gentle form of subcultivation enabled us to increase the cell doubling time of epithelial cells and to reduce the overgrowth with fibroblasts. The latter cell type was cultivated with DMEM + 10% FCS and passage with trypsin. So far 15/18 normal and 25/35 malignant tissue specimens could be maintained in short term culture and used for investigations. We managed to grow primary epithelial cells for 3-5 passages using this culture system.

P26

SUBLINE ISOLATION AND CHARACTERIZATION OF BIOLOGICAL BEHAVIOR OF THE METASTATIC HUMAN PROSTATE CANCER CELL LINE TSU-PR1

Klaus Rembrink* Theo H. van der Kwast#, Johannes C. Romijn, Fritz H. Schröder; Depts. of Urology and Pathology#, Erasmus University Rotterdam, The Netherlands

With the technique of orthotopic transplantation of human prostate cancer cell lines we have been able to reproducibly induce the formation of iliac lymph node metastases in NMRI nude mice. After removal and retransplantation of a metastatic lymph node we succeeded in isolating an in vitro subline of the human prostate cancer cell line TSU-PR1. We investigated the biologic behavior of the parental and the subline with the following in vivo and in vitro experiments.

In vivo we determined the rate of tumorigenicity and metastasis of the parental TSU-PR1 and the derived subline TSU-PR1N (considered to be more metastatic). We injected 1.25×10^5 cells per cell line into the dorsal prostate of 9-12 weeks old NMRI nude mice (n=12). After sacrifice (24-27 days post-OP) of the animals, 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. In vitro we compared the growth capabilities of both cell lines using the MTT-assay. The motility, as one of the parameters in the metastatic cascade, was evaluated by the Boyden Chamber (BC) assay. Fibronectin, added to the lower compartment of the BC in various concentrations (0-15 μg/ml), was used as a chemoattractant. Cells were seeded in the upper compartment at a density of 2.5×10^4 cells per well. A filter barrier of 8 μm pore size was placed between both compartments. Motility was quantitated by using an image analysing system (IBAS/Zeiss).

In vivo both cell lines showed a tumorigenicity rate of 100 % but the mice with the injected subline had much bigger tumors and more side effects, resulting in urinary obstruction in 40 % of the animals. Metastases occurred in 33 % of the animals inoculated with the parental line whereas the subline resulted in 80 % metastases. This is in contrast to the growth capabilities in vitro where the parental line had a faster growth rate than the subline. In the motility experiments the more metastatic subline TSU-PR1N showed 50 % less migration than the parental TSU-PR1.

We conclude that the newly isolated subline is biologically different from the parental line. There is no correlation between the in vitro capabilities of growth and motility and the in vivo aggressiveness expressed by the number of metastases.

* Recipient of a research grant of the Deutsche Krebshilfe

P27

CHARACTERIZATION OF SEVEN HUMAN PROSTATE TUMOR MODELS ESTABLISHED IN NMRI NUDE MICE.

Wytske M van Weerden, Corrina MA de Ridder, Gert Jan van Steenbrugge, Johannes C Romijn, Theo H van der Kwast and Fritz H Schröder. Erasmus University Rotterdam, The Netherlands.

The take rate of human prostate tumor tissue subcutaneously transplanted in athymic nude mice has always been considered to be very low. In our laboratory one hormone-dependent prostate tumor line (PC-82) and two androgen-independent tumor lines (PC-133 and PC-135) were developed from over 100 human prostate cancer specimens implanted in Balb/c athymic nude mice. Recently, however, as much as 7 human prostatic tumor lines could be established from subcutaneous transplantation of 19 human prostate tumor specimens in NMRI nude mice. The reason for the relatively high take rate in NMRI nude mice is not yet clear, but a typical very active (murine) stromal infiltration into the human prostate tissue can be observed in these tissues transplanted in NMRI mice.

Two tumor lines are derived from primary prostate tumor tissue (PC-310 and PC-329), 3 tumors originate from transurethral resection material (PC-324, PC-339 and PC-346) and 2 lines are established from metastasic lesions (lymph node (PC-295) and skin (PC-374)). This panel of in vivo human prostate tumor models comprises tissue of various degrees of differentiation and with different growth patterns. Tumors were confirmed to be human by bisbenzimid DNA staining and are routinely checked with every mouse passage. Immuno-histochemical analysis revealed that all tumor lines, except the PC-339, secrete prostatic acid phosphatase (PAP), whereas none expressed the prostate specific antigen (PSA). The PC-310 tumor line expresses the androgen receptor and is androgen dependent. The PC-295 and PC-329 tumor lines also contain the androgen receptor, but their androgen responsiveness is not yet definite. The PC-346 and PC-374 tumor lines show a heterogeneous staining of the androgen receptor and seem to be sensitive but not dependent of androgens. The PC-324 and PC-339 tumor lines lack the androgen receptor and are androgen independent.

This panel of prostatic xenografts offers the unique opportunity to study and compare various biological aspects of human prostatic cancer in different stages of tumor progression.

P28

EFFECT OF THE TRANSFER OF THE HUMAN CHROMOSOME 9 IN BLADDER CANCER CELL LINES

Laura Possati*,Wu Chang°,Giuseppe Barbanti-Brodano°, Marco Montesi*,Valentina Sparacciari*,Manuela Malatesta^,Franco Marcheggiani^ and Giancarlo Gazzanelli^

*Institute of Biomedical Sciences,University of Ancona,°Institute of Microbiology,University of Ferrara, ^Institute of Histology and Laboratory Analysis,University of Urbino,Italy

Deletions of chromosome 9 are described in 65-70% human bladder cancers, even in superficial forms, where analysis of loss of heterozygosis show deletions of the whole chromosome 9 or 9q or 9p12-q34. We transfected five different bladder cancer cell lines (RT4, 5637, SCABER, HT1376 and RT112) with human chromosome 9, after testing these cells for tumourigenicity in nude mice. After transfection we obtained several clones, which were tested for tumourigenicity in nude mice in the same conditions as the parental not transfected cells. The results so far obtained concern a number of clones of RT4 and 5637 cell lines: 7 out of 15 RT4 transfected clones and 10 out of 13 5637 transfected clones appeared not tumourigenic for nude mice; the nude mice tumourigenicity resulted partially suppressed in 7 out of 15 RT4 transfected clones and in 3 out of 13 5637 transfected clones; only 1 RT4 transfected clone showed the same tumourigenicity as the parental cell line. The cells of the transfected clones and of the tumours obtained in nude mice are studied now with molecular analysis for chromosome 9.

This work was supported by a grant of AIRC, Italy

P29

INVASION OF T24 CELLS OCCURS ONLY ON TRAUMATIZED UROTHELIUM IN PRIMARY CULTURES

Johanna MJ Rebel, Cornelia DEM Thijssen, Marcel Vermey, Ellen C Zwarthoff and Theo H Van Der Kwast. Dept. of Pathology, Erasmus University, Rotterdam, The Netherlands.

The frequency of tumour recurrence of human bladder carcinoma after local resection of superficial transitional cell carcinomas is very high. Clinical and experimental data indicate that the tumour recurrences could be a consequence of seeding of tumour cells on traumatized bladder mucosa. The aim of this study is to investigate if tumour cells can attach and invade intact urothelium.

We used explant cultures of adult mouse bladder mucosa on transparent membranes as an in vitro model for the in vivo situation. The outgrowths of the primary murine bladder explants closely mimic the in vivo situation with respect to multilayering and maturation into umbrella cells.

A suspension of the human bladder carcinoma cell line T24 was applied on the intact expanding bladder culture. After 24 hr the culture was stained with an antibody specific for human cytokeratin 19 which is specific for the T24 cells. T24 cells were only found on the part of the membrane which was not covered with urothelium. Even when the number of cell layers or the maturation into umbrella cells was influenced (with laminin, collagen type IV or epidermal growth factor) T 24 cells were not able to attach and invade into the urothelium. However, when the bladder outgrowth was damaged the T24 cells were able to attach in the wounded area and invaded the surrounding normal urothelium, even though the proliferative activity of this urothelium was very high. The damaged area of the cultures without T24 cells were covered with basal cells within 12 hr.

In conclusion T24 cells can not invade in intact urothelium. Attachment and invasion of T24 cells was also prevented when the bladder had only one cell layer or no umbrella cells.

P30

CHANGES IN CYTOKERATIN EXPRESSION PATTERN DURING TUMOR PROGRESSION IN A RAT BLADDER TUMOR MODEL SYSTEM

Reindert JA van Moorselaar, H. Ewout Schaafsma, Frans C.S. Ramaekers, Frans MJ Debruijne, Dirk J. Ruiter, Jack A Schalken. Urological Research Lab., Department of Urology, University Hospital Nijmegen, The Netherlands.

The expression of cytokeratins (CK), the epithelial specific intermediate filaments, can change during tumor progression. In order to find a CK expression pattern related to tumor progression in bladder cancer, we have examined the expression of various CK in the spontaneously arisen rat bladder tumor, RBT323, that progressed during serial transplantation. The initial tumor was a grade II transitional cell carcinoma, which progressed to a grade III tumor in the third transplant generation. The tumor doubling time decreased from 13 days to 3.5 days. Most importantly, in the 5th passage the tumor acquired a high metastatic potential, i.e. more than 90% of the animals show lung metastases and occasionally lymph node metastases. In the study presented here frozen sections of the original tumor and 11 subsequent passages have been stained with 22 monoclonal antibodies against different CK.

The original tumor showed, like the normal bladder, no expression of CK 10, 13 and 16. In subsequent tumor passages the tumor gained expression for these CK. CK 14 is specific for the basal and parabasal cell layers, however, upon tumor progression the superficial cells of the tumors also became positive. In the original tumor all superficial cells showed expression of CK 18, in the subsequent passages the amount of positive cells decreased.

Generally the CK expression profile of lung and lymph node metastases was similar to that of the primary tumors. CK 4 expression, however, increased in the metastases.

Conclusion: A differential cytokeratin expression pattern between the normal bladder and tumors and among tumor passages has been found. This tumor model can be helpful in the identification of markers associated with the progression of superficial bladder cancer.

P31

CHARACTERIZATION OF THE HUMAN WILM'S TUMOR MODEL AC-Nbl-2

D. Rohde*, D. Malin*, U. Cremer°, M. Valdor^X, R. Mertens^Y, G. Jakse*. * Dept. of Urology, ° Dept. of Legal Medicine, ^X Dept. of Pathology, ^Y Dept. of Pediatrics, RWTH Aachen, Germany

Tumor models of nephroblastoma (WT) are rare. In this study we present the recently established tumor model AC-Nbl-2.

8-9 weeks after injection of tissue from a lymph node metastasis of a WT, that occured after polychemotherapy, nude mice developed a semisolid subcutanous tumor. The resulting xenografts (AC-KLxe-X) could be serially transplanted in different strains, and mimic the blastema of the WT. They contain exactly the same DNA finger prints as the original. Only vimentin was found to be distributed in a heterogenous fashion while tumor cell did never express cytokeratin, desmin, ß-HCG or CEA. Cell culture cells from the first xenograft grew as biphasic short term cultures in the beginning; the resulting cell line AC-Nbl-2.2 expressed a fibroblastoid malignant phenotyp. Doxorubicin, etoposide, vincristin and ifosphamide inhibited tumor growth of these aneuploid (FACS-scan; DI: 1,94) cells in-vitro 70 %, 40 %, 40 % and < 10 % respectively. The cells did not express P170-glycoprotein but contain an elevated level of gluthation-S-transferase. Reinjection of AC-Nbl-2.2 into nude mice effected high proliferating sarkomatoid xenografts (AC-Nbl-2.2xe) that expressed an alterated human DNA-pattern.

Conclusion: AC-Nbl-2 represents a new, easy reproducible human test-system for research about tumorbiology, phenotypical differentiation and treatment of a chemotherapy resistant metastasis from a Wilm's tumor.

P32

TUMOR MARKER EXPRESSION IN PRIMARY CULTURE OF 51 GERM CELL TUMORS - A NEW TESTSYSTEM ?

Thomas Otto [1], Claudia Fuhrmann [2], Friedhelm Noll [1], Mark Goepel [1], Herbert Rübben [1]

1 Department of Urology, University of Essen, Medical School, Hufelandstr. 55, 45122 Essen, FRG
2 Institute of Radiobiology, University of Essen, Medical School, Hufelandstr. 55, 45122 Essen

From 1991 - 1993 we performed cell culture analysis of 51 different germ cell tumors.

We investigated 8 seminomas and 43 non seminomatous germ cell tumors, 20 tumors were primary cancers and 21 were metastatic retroperitoneal lesions after chemotherapy.

Primary culture was successful in 30/51 tumors (59 %). We revealed increased human choriongonadotropine (HCG) levels in the supernatant of primary germ cell culture in 70 %; alpha fetoprotein was increased in 37 %. DNA measurement was polyploid in 62 %.

Cell culture technique yielded information in addition to standard histopathology in 20 % of the metastatic lesions. 3/4 patients with positive cell culture assay and the histopathological finding of necrosis in the retroperitoneal specimen developed early tumor progression.

Primary culture assays, karyotype- and DNA-analysis are powerful additional techniques in the examination of germ cell tumors.

Oral presentations: Apoptosis

O33

ANALYSIS OF PROLIFERATION, CELL DEATH, AND RELATED GENES IN NORMAL PROSTATE AND BENIGN PROSTATIC HYPERPLASIA (BPH)

Marc Colombel, Zivko Popov, Sixtina Gil Diez, Claude Abbou and Dominique Chopin, Service d'Urologie, Hôpital Henri Mondor, Créteil, France.

Tissue growth, such as occurs in BPH, is the end result of an imbalance between the cellular proliferation rate and the cell death (apoptosis). In a prior study, we have already demonstrated an increased proliferative activity of basal cells areas of BPH, corresponding with an increased expression of EGF receptors on these cells. In this report, we examined and quantitated proliferative (BrdU incorporation into nuclear DNA and Mib1 immunostaining) as well as cell death (by in situ end labeling of fragmented nuclear DNA) markers to determine the extend to which the rate of these opposing processes are altered in BPH. In addition, we evaluated by immunostaining whether expression of the anti-apoptosis oncoprotein, bcl-2, is altered in BPH relative to normal prostate tissues. Ten normal young adult glands were sampled according to McNeal's zonal anatomy in addition to 20 BPH obtained during open surgery. Transitional (TZ), Peripheral (PZ) and Central zones (CZ) and BPH samples were obtained and were partly incubated in a BrdU solution and fixed in formalin and partly snap frozen. Quantitative results of proliferation and cell death (positive cells per champ X40) are summarized in this table:

Tissu prostatique	Mib1	BrdU	Apoptosis
TZ	2 ± 1.2	8 ± 4.2	4.5 ± 3.2
PZ	2.5 ± 2	9 ± 6.1	3.5 ± 2.1
CZ	3 ± 2.1	15 ± 6.3	5.2 ± 2.1
HBP	12 ± 3.5	37 ± 12.5	4.8 ± 1.5

In normal prostate bcl-2 was clearly enhanced in glandular cells of peripheral zone, all basal cells and also luminal cells were positive. In the central zone, Bcl2 was regularly expressed in basal cells and in most of glandular cells of the intraluminal ridges. Bcl-2 expression in transitional zone was limited to some dispersed basal cells. In adenoma, bcl-2 was strongly expressed in basal cells in glands and in most of the cells in small nodules. These results suggest that BPH is the result of an increased activity of proliferation in basal cells that is not counterbalanced by an increase of cell death (apoptosis). Although normal transitional zone expressed low level of bcl2, increase expression of this anti-death protein is a possible factor of dysregulation of prostate epithelium homeostasia that lead to BPH.

O34

COMBINED CASTRATION AND ESTROGEN TREATMENTS INDUCE-WHEREAS CASTRATION ALONE SUPRESSES EPITHELIAL-CELL APOPTOSIS IN THE DUNNING R-3327PAP PROSTATIC ADENO-CARCINOMA

Patrick Westin[1], Anna Brändström[2,3], Anders Bergh[1], Jan-Erik Damber[3]
Departments of Pathology (1), Physiology (2) and Urology and Andrology (3) University of Umeå , Umeå , Sweden.

The positive effect of castration treatment of prostatic cancer patients is considered to be related to the induction of apoptosis in the androgen-dependent tumor cells. However, castration treatment alone does not induce apoptosis in the highly differentiated, androgen-sensitive Dunning R-3327PAP prostatic adenocarcinoma. To elucidate the possibilities and potential mechanisms of apoptotic induction in this tumor, Rats with subcutaneously implanted Dunning R-3327PAP tumors were treated with vehicle (I), castration +vehicle (C) or castration + 50 ug of oestradiol benzoate per day (C+E2). At different times after treatment, the effects on tumors and ventral prostates were examined morphometrically, by *in situ* end labelling (ISEL) of apoptotic cells, immunohistochemically with monoclonal antibodies to Proliferating Cell Nuclear Antigen (PCNA) and for the expression of Testosterone Repressed Prostatic message-2 (TRPM-2) mRNA. 3 days after castration, massive epithlial-cell apoptosis was detected in the ventral prostates. In tumors from castrated (C) animals the epithelial mitotic index was reduced but tumor volume was unaffected. At this time and at 7 days after treatment, apoptotic index for epithelial cells was significantly decreased as compared to the I-and C+E2 groups. At 3 days, rats treated with C+E2 displayed a decrease in tumor volume and in epithelial volume density which prompted us to examine the treatment groups after 24 hours. In the C+E2-group, there was a 100% increase in epithelial apoptotic- and ISEL indexes as well as an increase of the expression of TRPM-2. This elevation coincided with an increased stromal mitotic and PCNA-labelling index. In this group at 12 hours, there was a stromal proliferation but no increase in epithelial morphologic and ISEL indexes. At 12 and 24 hours in the C-group epithelial-cell apoptotic- and ISEL indexes as well as the expression of TRPM-2 was significantly decreased as compared to the I-and C groups. We conclude that in this Dunning tumor, castration treatment rapidly induces suppression of epithelial-cell apoptosis. In contrast, combined castration and estrogen treatments induces an elevated level of epithelial-cell apoptosis. This induction may be stroma-mediated .

O35

PROGRAMMED CELL DEATH IN THE PROSTATE FOLLOWING CASTRATION: CLONING OF A TESTOSTERONE-REGULATED NADH DEHYDRO-GENASEGENE

R. Friis, Guo Ke, R. Ball, W. Bielke, S. Saurer and V. Merz*
Univ. Berne, Lab. for clin.-exp. Res., Tiefenaustr. 120, CH-3004 Bern and *Urology Clinic, Inselspital, CH-3010 Bern, Switzerland

Prostate involution occurs after castration in rats and is associated with the death by apoptosis of a large fraction of the epithelial cells. We have isolated several genes from a prostate involution bacteriophage lambda cDNA library using differential screening methods. Among these clones, one demonstrated an especially strong signal when used as a probe against Northern blots of prostate mRNA obtained before, and at different times after, castration. This gene is down-regulated after castration by 40-fold within 5 days. Intramuscular injection of a testosterone depot resulted in complete restoration of expression within 24 hours. Upon sequencing it became apparent that this clone has a high degree of homology to an NADH dehydrogenase encoded in mitochondrial DNA, which has not previously been studied for its expression. The genomic 5' flanking sequence contains 2 testosterone response elements in tandem. The clone failed to hybridize to any transcripts from rat organs other than prostate. We are now in the process of isolating the human homolog to this gene for use as a biomarker in study of benign hyperplasia and developing carcinoma. This gene is a possibly useful indicator of cells lacking functional testosterone receptor.

Oral presentations: Tumor suppressor genes, growth factors

O36

ANTI-APOPTOSIS GENES AND PROSTATE CANCER

Ralph Buttyan, Anthony Raffo, Harris Perlman, Min Wei Chen
Columbia University, New York, NY U.S.A.

Castration eliminates prostate cancer cells by activating the cell death process referred to as apoptosis. Previously, two oncogenic proteins, bcl-2 and mutated/defective tumor suppressor protein p53, were shown to be potent apoptosis-suppressor genes in hematopoietic cancer cells. Both of these anti-apoptosis proteins have also been found in hormone-refractory human prostate cancers. In order to test whether bcl-2 or mutated p53 can effectively suppress apoptosis of prostate cancer cells, we developed derivatives of the prostate cancer cell line, LNCaP. LNCaP cells cultured in our laboratory express very low amounts of bcl-2 protein and maintain the wildtype p53 gene. These cells were transfected with neomycin-resistant expression vectors containing cDNA for bcl-2 (LNCaP-bcl) or mutated (143 val→ala) p53 (LNCaP-p53). Control cells transfected with the neomycin-resistant expression vector alone (LNCaP/neo) were also obtained. Similar to the parental LNCaP cells, LNCaP/neo cells undergo apoptosis over a 72 hr period when they are placed in a serum-free medium or when phorbol ester (PMA, 10 nM) is added to serum-containing medium. The control lines show a prominent pattern of internucleosomal DNA fragmentation by 12-24 hrs after the apogenic stimulus. In contrast, two lines of LNCaP-bcl cells were highly resistant to these treatments as were LNCaP-p53 lines. Expression of bcl-2 or mutated p53 eliminated apoptotic DNA fragmentation at these same time periods. These experiments establish that expression of the bcl-2 oncoprotein or expression of mutated p53 protein can confer apoptotic resistance to prostate cancer cells. Our continuing studies have also enabled us to identify a new anti-apoptosis gene. LNCaP cell lines transfected with sulfated glycoprotein-2 (SGP-2) cDNA were also found to be highly resistant to apoptotic stimuli. SGP-2, previously found to be highly induced during prostate cell apoptosis, has never before been described as an anti-apoptotic gene. This *in vitro* system for prostate cancer apoptosis can thus be used to identify novel anti-apoptosis genes in prostate cancer. **(Supported by the U.S.P.H.S./National Cancer Institute)**

O37

BCL-2 PROTEIN EXPRESSION IN PRIMARY HUMAN PROSTATIC ADENOCARCINOMA. Freddie C. Hamdy, O. Olasimbo, Derek J. Rosario, Paul B. Silcocks, Clare E. Fuller and Janice A. Royds, Departments of Urology and Pathology, Royal Hallamshire Hospital and the University of Sheffield Medical School, Sheffield, England.

Hormonal manipulation in the form of androgen ablation remains the gold standard in the management of advanced prostate cancer. However, a proportion of patients will not respond to treatment, and hormonal escape after an initial good response is a well recognised phenomenon, which remains largely unexplained and unpredictable by current investigative methods.

Succesful treatment with androgen ablation is thought to rely on the presence of androgen sensitive clones of tumour cells, involving programmed cell death or apoptosis. Any factors blocking tumour cell death, including the absence of androgen receptors or the presence of oncogenes preventing apoptosis will lead to hormone resistant disease.

The Bcl-2 proto-oncogene is located on chromosome 18q21.3 and is known to protect cells from apoptosis. Bcl-2 gene products have been demonstrated in cells with an extended life-span, and overexpression has been linked with androgen resistant prostate cancer in the rat. The aim of this study was to investigate Bcl-2 protein expression by immunohistochemistry in primary human prostatic adenocarcinoma.

35 men with histologically proven prostate cancer were studied. 14 (40%) had evidence of metastatic disease confirmed by a positive bone scan. Treatment consisted of androgen suppression for advanced disease and external beam radiotherapy for tumours confined to the prostate. 3 patients had an incidental (To) cancer and were not treated. 4 of 35 patients (11%) failed to respond to hormonal manipulation and died within 6 months of diagnosis. 13 of 35 patients (37%) relapsed within an average of 20 months (range 14-26 months) following treatment. The specimens analysed were collected from paraffin-embedded transurethral resection samples. A monoclonal antibody against Bcl-2 (Dako, UK Ltd), was used for immunohistochemical staining.

15 of the 35 patients studied (43%) were found to express Bcl-2 in their primary prostatic tumour. There was no statistically significant differences between patients with positive or negative Bcl-2 expression in terms of serum prostate specific antigen levels and Gleason scores. 9 of the 17 patients (60%) who escaped hormonal control or failed to respond to initial treatment expressed Bcl-2. Of the 7 Bcl-2 positive patients treated with androgen suppression, 1 only (14%) had a sustained satisfactory response.

These findings suggest a correlation between Bcl-2 expression and poor response to treatment, and warrant further investigation into the possible role of Bcl-2 in the hormone resistance mechanisms of advanced prostate cancer.

O38

DETECTION OF ONCOGENE AMPLIFICATION AND P53 DELETIONS IN BLADDER CANCER USING FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Guido Sauter, Holger Moch, Michael J. Mihatsch, Thomas Gasser, Gianfranco Mattarelli, Frederic M. Waldman*, Departments of Pathology and Urology, University of Basel, Switzerland and Division of Molecular Cytometry, University of California, San Francisco*

Development and progression of tumors is thought to be driven by an accumulation of genomic alterations in tumor cells. Genomic alterations include deletions of tumor suppressor genes or amplification of oncogenes. Fluorescence in situ hybridization allows visualization and enumeration of chromosomes and genes on interphase nuclei on a cell by cell basis.

In order to examine the relationship of genomic alterations with tumor phenotype, we examined 170 bladder tumor specimens (50 unfixed imprint preparations, 120 formalin fixed paraffin embedded samples) with probes for genes which have been implicated in bladder cancer biology. This included p53 (17p), erbB-2 (17q21), epidermal growth factor receptor (EGF-r; 7p13), and c-myc (8q24). Probes for the target gene and for a centromeric region of the respective chromosome were applied simultaneously. Deletion was defined as less gene signals than centromere signals in greater than 40% of cells. Amplification was assumed if a significant number of cells contained a clearly increased number of gene signals as compared to centromere signals.

A 17p deletion was found in 52 of 143 bladder tumors. Prevalence of 17p deletion (p53) was lower in pTa (4/43), than in pT1 (20/42) or pT2-4 tumors (28/58; p<0.0001). There was also a strong correlation of 17p deletions with tumor grade (p=0.0001). Amplifications of erbB-2 (10 of 122 cases) and EGF-r (5/106) were less frequent and preferably found in pT2-4 tumors. The distribution of amplified EGF-r signals allowed a distinction of intrachromosomal (amplified signals in clusters) and extrachromosomal amplification (diffuse distribution of amplified signals). A clear cut c-myc amplification was found in 3 of 87 cases. Surprisingly there were a few extra c-myc signals in more than 10% of cells of 32 of the 84 remaining cases. The presence of c-myc extra copies was strongly associated with tumor stage. Extra c-myc signals were seen in 2 of 25 pTa tumors, 7 of 22 pT1 tumors and 20 of 32 pT2-4 tumors (p<0.0001). Presence of increased c-myc copy number was also associated with tumor grade, p53 immunostaining, and polysomy of the chromosomes 7, 8 and 17 (p<0.0001 each).

In summary, copy number alterations of tumor genes are frequent in bladder cancer. The association of p53 deletions and oncogene copy number alterations with phenotype is indication for their biologic relevance. FISH is an ideal tool for investigation of subtle gene copy number alterations in bladder cancer.

O39

EVALUATION OF PROLIFERATION AND p53 OVEREXPRESSION IN BLADDER CANCER. Zivko Popov, Vincent Ravery, Marc Colombel, Catherine Mazerolles*, Jean-Jacques Patard, Claude Abbou, Jacqueline Bellot, Dominique Chopin. Hôpital Henri Mondor, Créteil and *Hôpital Purpan, Toulouse - France.

Management of Transitional Cell Carcinoma (TCC) of the bladder depends on clinicopathological parameters at initial presentation. Evidence has accumulated that biological markers may provide significant information to predict the potential aggressiveness of a given tumor and chance to respond to therapy. The assessment of proliferation has been helpful in the prediction of bladder tumor reccurence and progression. p53 suppressor gene mutation is frequent in superficial and invasive bladder tumors. Since wild type p53 acts as a suppressor of proliferation, we investigated the relationship between abnormal expression of p53 and the percentage of proliferating tumor cells. Samples from 80 TCC and 8 normal bladders were immunostained with Mib1 monoclonal antibody that specifically identified proliferating cells, and by 1801 (ab2 oncogene science) and DO7 (Dako) which recognized two different epitopes for mutated or wild type p53 protein. Quantification of nuclei stained by Mib1 and p53 was done using a procedure described by Lipponen (1992). Briefly the estimation was calculated on 500 nuclei per slide using 3-5 fields (magnification x40). Relationship between stage, grade and percentage of nuclei expressing Mib1 and p53 are as followed:

		Mib1 < 20%	Mib1 > 20%	p53 < 20%	p53 > 20%
Stade	T1	63%	37%	73%	27%
	T2 T3	6%	94%	12%	88%
	T4	0%	100%	0%	100%
Grade	G1	68%	42%	77%	23%
	G2	30%	70%	46%	54%
	G3	7%	63%	7%	93%

There is significative correlation between Mib1 proliferation marker and p53 overexpression by mean of immunohistochemistry. Progression rate survival using Kaplan Meier curves indicates (log rank test) a prognostic significant value for p53 staining (cut off 18%) and Mib1 staining (cut off 20%), p = 0.0059 and p = 0.0124, respectively. These data indicates that determination of these markers have a strong prognostic significance. In addition the possibility of their utilisation on routine paraffin embeded material suggest that they may have clinical relevance

O40

AMPLIFICATION OF THE CHROMOSOME 11q13 REGION IN BLADDER TUMORS. Pierre-paul BRINGUIER, Yahya TAMIMI, Ed SCHUURING, Jack SCHALKEN. Urological Research Laboratory. University Hospital Nijmegen, Nijmegen, the Netherlands

11q13 amplification has been found in several cancers and is frequent in breast cancer and head and neck carcinoma. In bladder the incidence is of 15%, according to the only study with sufficient number of patients. Two oncogenes (int2=FGF3 and hst=FGF4) lie in the amplicon but do not seem to be activated during amplification since most of the time no expression can be detected. Recently, 2 other genes that might confer a selective advantage upon amplification have been cloned: Prad 1 encodes the cyclin D1 which regulates the G1 to S phase transition of the cell cycle. Ems 1 encodes a protein that might affect the cytoskeleton and cell adhesion structures.

To get more insight into the role of 11q13 amplification in bladder tumor development, we have studied the amplification and the expression of those 2 genes and of int 2. Co-amplification (2 to 25 folds) have been found in 6/44 (≈14%) tumors analysed. Only one of these tumors was infiltrative.

To assess prad1 and ems1 expression in normal urothelium we used *in situ* hybridization to discriminate truly epithelial expression from stromal contribution. A low expression was found in normal urothelium. Northern analysis of 37 tumors shows that tumors with amplification have a higher mRNA for prad1 and ems1 (6.8 ± 1.2 and 7.2 ± 1.1 respectively) than tumors without amplification (2.5 ± 0.6, p<5% and 2.3 ± 0.1, p<1%). In contrast, we were unable to detect any int2 expression in 18 tumors tested. Thus prad1 and ems1 are likely to play a pathogenic role in the 11q13 amplification.

However, amplification is not the unique way of activation of these genes since most bladder tumors have a high expression of prad1 and ems1. Interestingly a higher expression seems to occur in superficial versus invasive tumors (6.6 ± 0.6 versus 2.1 ± 0.1; p≈10% for prad1 and 5.6 ± 0.4 versus 2.0 ± 0.1 p≈5% for ems1). Moreover, the 9 tumors with low expression are all very aggressive, leading to the hypothesis that these tumors develop through a prad1/ems1 independent pathway. This pathway would produce aggressive tumors.

O41

KERATINOCYTE GROWTH FACTOR AND INSULIN-LIKE GROWTH FACTOR-I ACTIVATE ANDROGEN RECEPTOR IN DU-145 HUMAN PROSTATIC CARCINOMA AND IN CV-1 MONKEY KIDNEY CELLS.
Zoran Culig, Marcus Cronauer, Alfred Hobisch, Johannes Eberle, Georg Bartsch, and Helmut Klocker, Department of Urology, University of Innsbruck, Austria

The majority of late stages prostatic carcinomas do not respond to androgen ablation therapy. The molecular mechanism responsible for development of androgen indepedence is currently unknown. Besides alterations in the androgen-signal transduction cascade an aberrant interaction of growth factors and the androgen receptor (AR) may play a crucial role in this process. Tumor cell line DU-145, derived from a brain metastasis of prostatic cancer, is a model for an advanced progressive prostatic carcinoma. We investigated the effect of keratinocyte growth factor (KGF), which belongs to the group of heparin-binding growth factors, and insulin-like growth factors (IGFs) on AR activation in DU-145 and in CV-1 cells.

DU-145 as well as CV-1 cells do not express endogenous steroid receptors. These two cell lines were transiently cotransfected with an androgen-inducible chloramphenicol-acethyl transferase (CAT) reporter gene and AR expression vectors. Two types of AR expression vectors were used: HAOa and HAOa-^{715}met. HAOa contains the wild-type AR cDNA, while HAOa-^{715}met contains the cDNA encoding the mutant AR^{715}met, which was detected in a sample of an advanced prostatic cancer. One day after transfection the cells were stimulated with either synthetic androgens (mibolerone, methyltrienolone) or with growth factors (KGF, IGF-I, IGF-II). Reporter gene activity was measured in cell extracts 30 h later. KGF and IGF-I induced CAT activity in both cell lines. At concentration of 50 ng/ml both growth factors induced CAT activity at the same level as synthetic androgens. Reporter gene expression was inhibited by 1 μM of the nonsteroidal antiandrogen Casodex. This fact, as well as the inability of growth factors to stimulate reporter gene activity in cells transfected with the reporter plasmid and an empty expression vector indicate that the effect of KGF and IGF-I is mediated by the AR. The pattern of stimulation of AR^{715}met was indistinguishable from the wild-type receptor. IGF-II, applied in concentrations up to 50 ng/ml, did not display any effect on reporter gene transcription.

Our results show that IGF-I and KGF activate the AR in the absence of androgens and thus demonstrate a link of growth factors and the androgen transduction cascade. These findings may have further implications on growth of prostatic cells in an androgen-depleted environment.

Poster session: Genetic alterations, tumor suppressor genes, prognostic factors

P42

IDENTIFICATION OF GENES ASSOCIATED WITH PROSTATE CANCER DEVELOPMENT.

Marion J. Bussemakers and William B. Isaacs. James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD, 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, the technique of differential display has been proven to be a powerful tool to identify and clone differentially expressed genes. The technique involves the reverse transcripiton of messenger RNA using an anchored oligo-dT primer, followed by a PCR amplification reaction using several different, arbitrarily chosen primers and subsequent seperation of the products on a denaturing gel. In the study presented here, messenger RNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients was extracted and used for differential display analysis. Twenty different combinations of primers were tested. Twelve apparently differentially expressed mRNAs (overexpressed in tumor or in normal tissue) were identified this way. The complementary DNA fragments were recovered from gel, reamplified and used as probes for Northern analysis. One of the probes (DD3) detected two transcripts (2.2 and 4.0 kb) that are specifically expressed in human prostatic tumors (9 out of 12 tumors studied) whereas no expression of these transcripts was found in normal or benign hyperplastic prostate tissue. Nucleotide sequence analysis showed no homology to any known gene. Currently, more sequence data are being obtained as well as studies are being performed to analyse the tissue-specificity of DD3 expression and expression in other tumors.
In conclusion, differential display is a useful approach to identify differentially expressed genes in prostate tumor development and DD3 might be useful in prostate cancer detection.

P43

GENETIC ALTERATIONS IN LOCALIZED PROSTATE CANCER: IDENTIFICATION A COMMON REGION OF DELETION ON CHROMOSOME 18q.

A. Latil[1], JC. Baron[2], O. Cussenot[3], G. Fournier[4], T. Soussi[5], L. Boccon-Gibod[2], A. Le Duc[3] J. Rouëssé[1] and R. Lidereau[1]. [1]Laboratory of Oncogenetic, Centre René Huguenin, F-92211 St-Cloud, France; [2]Department of Urology, CHU Bichat, F-75018 Paris, France; [3]Department of Urology, CHU Saint-Louis, F-75010 Paris, France; [4]Department of Urology, CHU A. Morvan, F-29609 Brest, France; [5]U 301 INSERM, Institut de Génétique Moléculaire, F-75010 Paris, France.

Accumulation of mutations in oncogenes and tumor suppressor genes transforms a normal cell into a malignant cell by allowing it to escape from normal control of growth. For prostate tumorigenesis, the current model defines specific mutations of the TP53 tumor suppressor gene and loss of heterozygosity (LOH) for loci on chromosomes 8p, 10q, 16q and 18q. In order to determine if alterations frequently found in other adenocarcinomas (breast, ovarian, gastric, colorectal) including losses of heterozygosity on chromosomes 1p, 3p, 7q, 11p, 17p, 17q and 18q, and amplification of c-myc, c-erbB-2/neu oncogenes and the 11q13 region are also involved in prostate cancer, we examined 21 localized early stage prostate tumors. We detected no amplification of the c-myc, c-erbB-2/neu proto-oncogenes and 11q13 region (int2/FGF3), or mutations of the TP53 gene. Allelic losses were found in chromosomal regions 10q (20%), 7q (33%) and 18q (33%). Furthermore, as the first step toward isolation of tumor suppressor genes on 18q, we used six polymorphic markers and identified a common region of deletion between the 18q centromere and the D18S19 locus.

P44

DELETION MAPPING OF CHROMOSOME 18q IN PROSTATE CANCER (PC) AND TRANSITIONAL CELL CARCINOMA OF THE BLADDER (TCC).

Simon F Brewster, Keith W Brown and J. Clive Gingell
Dept. of Urology, Southmead Hospital, Bristol and Dept. of Pathology, School of Medical Sciences, University of Bristol, UK.

Somatic allelic loss is a hallmark of tumour suppressor gene (TSG) inactivation. To investigate the potential role of the TSG DCC [deleted in colorectal carcinoma] in two common urological cancers, 30 PCs and 31 TCCs were allelotyped at 5 chromosome 18q loci using polymorphic DNA probes: pL2.7 (18q12); pOLVIIA8 (18q12.1-21); p15-65, SAM 1.1, JOSH 4.4, DCC 1.9 (all within DCC, 18q21.3) - B.Vogelstein, Baltimore; pOS-4 (18q22) and pL159-I (18q23).

7/26 (27%) of PCs and 8/25 (32%) of TCCs exhibited loss of heterozygosity (LOH) on 18q, including DCC in 6 PCs and all 8 TCCs. LOH correlated with advanced stage in PC and with TCC recurrence or muscle-invasion (both $p<0.05$). In one PC and 5 TCCs, centromeric markers also exhibited LOH; one PC and 2 TCCs retained centromeric alleles. 4 PCs and 7 TCCs also exhibited LOH at telomeric loci. One PC exhibited LOH only telomeric to DCC. No TCC retaining heterozygosity at DCC exhibited LOH elsewhere. The regions of commonest deletion (RCD) were 18q22-23 in PC and 18q21.3-23 in TCC.

These results suggest the presence of a late-acting TSG on 18q in both cancers. DCC is implicated as the target TSG in TCC, located within the RCD; that for PC may be telomeric to DCC. (Supported by a SWRHA research grant).

P45

ANGIOGENIC ACTIVITY OF TWO HUMAN PROSTATE TRANSPLANTABLE CELL LINES. Peter J. Hepburn, Maureen E. Harper & Keith Griffiths. Tenovus Cancer Research Centre, University of Wales College of Medicine, Cardiff, Wales, UK.

Angiogenesis is the generation of new capillary blood vessels which occurs by a process involving the proliferation, migration and maturation of endothelial cells, a prerequisite for tumour growth and metastases. In 1985 a transplantable cell line (Ten12) was established in nude mice from a primary human prostatic cancer. The particular feature of this patient's tumour was its high degree of vascularisation, a characteristic that has been maintained in the mouse xenograft, presumably due to the release of one or more angiogenic factors.

Initial studies using serum-free conditioned medium from primary *in vitro* cultures of Ten12 on logarithmic cultures of bovine aortic endothelial (BAE) cells did not show a significant increase in the population doubling time. Morphological transformation of BAE cells however was observed, from the normal cobblestone growth to cells exhibiting a more elongated shape and with the appearance of sprouting cells self-associating into a complex network of tube-like structures. Serum-free conditioned medium from LNCaP, an established human prostate cancer cell line, did not affect the morphology of BAE cells to the same extent.

The effect of primary *in vitro* cultures of Ten12 were compared with those of Ten15 (another transplantable human prostatic cancer cell line established from a metastatic lesion which exhibits a lower degree of vascularisation) on both logarithmic and quiescent BAE cells using an insert co-culture system. Growth curves for both lines were consistently higher than controls and those observed with conditioned medium, but were not statistically significant. Greater morphological changes to BAE cells were however observed with co-cultures of Ten12 cells than those shown with Ten15 cells.

Angiogenic effects on macrovascular and microvascular endothelial cells are reported to differ. We therefore recently assessed the effects of conditioned medium and primary *in vitro* co-cultures of Ten12 and Ten15 cells on microvascular endothelial cells derived from bovine adrenal capillaries (BAC). Initial identification of which angiogenic factors are important for the vascularisation of Ten12 tumours will be attempted using a panel of neutralising antibodies and electrophoretic analysis.

P46

CHROMOSOME 8p DELETION IN PROSTATE CANCER DETECTED BY *IN SITU* HYBRIDIZATION. Hideyasu Matsuyama, Irja Nilsson, Pan Yee, Bernhard Tribukait, Lambert Skoog, Peter Ekman, Peter Lichter, Ulf S.R. Bergerheim, Karolinska Hospital, Stockholm Sweden; and DKFZ, Heidelberg, Germany (Presentation by Dr. Matsuyama)

Fluorescent *In Situ* hybridization (FISH) technique was applied to 39 cases of prostate cancer and 8 cases of histologically benign prostate for the detection of structural aberrations for chromosome 8. The centromeric DNA probes for chromosome 8 were labelled with biotin and the cosmid probes for two chromosome 8p loci (LPL/8p22 and D8S7/8p23) with digoxigenin-11-dUTP by nick translation, respectively. Thirty-one cases of touch biopsy samples (15 prostates, 9 lymphnodes, 6 bone metastases and 1 bladder invasion) were analysed. In eight cases obtained from aspiration biopsy of the prostate, only the D8S7 locus was analysed. More than 150 nuclei were counted by two observers in each case. Deletion was defined when the fractions with decreased spot number of cosmid signal compared with centromer signal (deleted fraction) exceeded 35% of all nuclei observed. Hybridizing efficiency was evaluated by counting nuclei with zero spot for cosmid signal. Cases with more than 20% of zero spot fraction for cosmid signal were regarded as inconclusive.

Twenty-nine of the 39 specimens (74.3%) were regarded as having any types of 8p deletions. Deletion of the terminal part was detected in 51.6% (both LPL and D8S7 in 32.2%, D8S7 but retained LPL in 19.4%). Interstitial deletion (only LPL deleted) was found in 22.6%. There was a significant correlation between different deletion patterns: 1) no deletion (neither D8S7 nor LPL deleted), 2) partial deletion (either D8S7 or LPL deleted) and 3) both deletion (both D8S7 and LPL deleted), with tumor grade (P=0.021, Chi Square test) and tumor stage (p=0.013) These data support the hypothesis that tumor suppressor gene function is inactivated by 8p deletion in prostate cancer. The variable non-overlapping deletion pattern with might indicate the presence of two tumor suppressor gene loci on chromosome 8p.

P47

Y CHROMOSOME LOSS IN BLADDER CANCER: DETECTION BY FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Gianfranco Mattarelli, Guido Sauter, Holger Moch, Michael J. Mihatsch, Urs Wagner, Hedwika Novotna, Thomas Gasser, Frederic M. Waldman*, Departments of Pathology and Urology, University of Basel, Switzerland and Division of Molecular Cytometry, University of California, San Francisco*

Y chromosome losses (-Y) have repeatedly been reported for various tumors. A recent study has suggested a prognostic relevance of -Y in bladder cancer. In order to examine the meaning of -Y in bladder cancer fluorescence in situ hybridization (FISH) was used to determine the prevalence of -Y , and to compare presence of -Y with known parameters of malignancy.
Cells from 80 formalin fixed paraffin embedded bladder tumors of male patients were examined by FISH using centromeric probes for chromosome 7 (p7alphaTET), 17 (p17HU8), X (pBAMX7), and Y (pY). Tumors were categorized according to their dominant population (largest non eusomic population exceeding 20%). Expression of the proliferation associated antigen Ki67 (MIB1) and p53 (CM1) was determined by immunohistochemistry.
The following results were obtained by FISH: for the Y chromosome: nullisomy in 33% of cases, monosomy in 44% and polysomy in 23%; for the X chromosome: nullisomy in 0%, monosomy in 54%, and polysomy in 46%; for chromosome 7: disomy in 44% and polysomy in 56%; for chromosome 17: disomy in 45% and polysomy in 55%. Nullisomic and monosomic tumors were not seen for the chromosomes 7 and 17. A relative Y chromosome loss was seen in 39% of cases (defined as presence of less Y signals than X signals, and less than half as many Y signals than centromere 7 and 17 signals). Patient age was higher for tumors with a relative Y loss (73.1±10.5 years) than for tumors without Y loss (67.3±11.2 years; p=0.0212). A relative Y chromosome loss was seen in 6 of 24 pTa tumors, 9 of 23 pT1 tumors, and 16 of 32 pT2-4 tumors (not significant). Similarily there was no association between relative Y loss and tumor grade. There was a strong association of polysomies for all chromosomes with p53 protein expression (p<0.005). However, neither Y chromosome nullisomy nor relative Y loss was associated with p53 expression. For all chromosomes analyzed, Ki67 LI was higher in polysomic tumors than in eusomic tumors (p<0.05). Interestingly there was also an increased Ki67 LI in Y nullisomic tumors as compared to Y monosomic tumors. Our data are consistant with different mechanisms for chromosomal gains (p53 associated) and losses (not p53 associated) in bladder cancer. The biological significance of -Y can not be finally assessed. The finding of an increased tumor cell proliferation in tumors with a Y nullisomy could reflect a growth advantage of cells with -Y. Arguments against a role of -Y for bladder cancer progression are however the lack of a clear association with tumor stage and grade as well as the association with increased patient age, which is analogous to previous observations in normal tissues.

P48

CHROMOSOME ANALYSIS OF TRANSITIONAL CELL CARCINOMA OF THE BLADDER.

HENRY C*, CIPOLLA B°, CORBEL L°, LE MEE F*, LOBEL B°, PICARD F*
* Laboratoire de Cytogénétique,
° Service d'Urologie,
C.H.R.U. Pontchaillou - 35033 RENNES - FRANCE -

The management of bladder cancer remains difficult and the choice of therapy is still based on the pathological classification. The aim of this study was to try to find chromosome aberrations in bladder cancer and a correlation between these eventual aberrations and prognosis.
Patients and Methods : Nine cases of transitional cell carcinoma of the bladder (eight men and one woman) were studied : pTaG2, n=2 - pT1G3, n=1 - pT2G2, n=3 - pT2G3, n=1 - pT3aG3, n=2). The bladder specimens were obtained during trans-urethral resection or cystectomy. After mincing and collagenase digestion of the fragments, the cells were cultured in RPMI 1640 medium added with foetal calf serum, glutamine and antibiotics. Metaphases were observed after colcemid treatment and then subjected to detailed cytogenetic analysis with a R-banding method.
RESULTS : Two specimens were not studied because of failure of culture. Chromosome aberrations were found in all remaining cases : Trisomy 7, usually considered as a primary event, was seen, in mosaicism, in three tumors of intermediate grade 2.
As for the loss of chromosome Y, found in 5 of the 6 male cases, it is associated with high-grade infiltrating tumors and may be correlated to tumor aggressiveness.
Anomalies involving the chromosome 1 (deletion of the long arm, polysomy 1), trisomy 5, polyploidy and marker chromosomes were associated with tumors of high grade.
These results tend to show a linkage between specific aberrations and histological grade. The prognosis value of this study is under evaluation.

P49

COMPARISON BETWEEN RT4 BLADDER CANCER CELL CLONES BEFORE AND AFTER TRANSFECTION WITH MOUSE gas-1 GENE

Giancarlo Gazzanelli^,Ferdinando Mannello^,Manuela Malatesta^,Valentina Sparacciari*,Marco Montesi*, Giuseppe Barbanti-Brodano° and Laura Possati*

^Institute of Histology and Laboratory Analysis,University of Urbino,*Institute of Biomedical Sciences, University of Ancona,°Institute of Microbiology,University of Ferrara,Italy

The gas-1 gene is located on chromosome 9q22.1 and it seems to arrest the cell growth in vitro. With the aim of investigating if gas-1 is an oncosuppressor gene we transfected a clone of RT4 bladder cancer cell line with either human or mouse gas-1 and assayed the subclones obtained after transfection for tumourigenicity in nude mice. All 18 H gas-1 transfected subclones appeared tumourigenic, while 4 out of 7 M gas-1 transfected subclones resulted tumour-suppressed. One suppressed clone and the parental cell line were studied morphologically and biochemically using electromicroscopic and electrophoretic techniques and the results were compared. As far as the ultrastructural aspect is concerned, a major difference consisted of large glicogen deposits in M gas-1 transfected cells, probably indicating cellular senescence. As far as the biochemical status is concerned, not transfected RT4 cells showed greater presence of cytoskeletal proteins and more evident collagenase activity in serum-free media collected at daily intervals, all properties which well correlate to the tumourigenic and metastatic ability of the cells.
This work was supported by a grant of AIRC, Italy

P50

CHARACTERIZATION OF THE P53 TUMOR SUPPRESSOR GENE IN UROTHELIAL CANCER CELL LINES

M.-Oliver Grimm[1], Despina Makri[1], Wolfgang A. Schulz[2], Klaus Decken[1], Bernd J. Schmitz-Dräger[1]

1: Urologische Klinik, 2: Physiologische Chemie I, Heinrich-Heine-Universität Düsseldorf, Germany

Inactivation of the p53 tumor suppressor gene has been found in about 60-70% of invasive urothelial cancers. More recently, immunohistochemical studies showed overexpression of p53 in 30-40% of superficial urothelial cancer and even in dysplasia, suggesting a potential role of p53 in the development of this malignancy [Benni et al., Int. J. Oncol. 3:817-821 (1993), unpublished data]. However, little is known about the impact of p53-inactivation and its interaction with other genes. Therefore, this study was designed to establish an in vitro model to further elucidate the role of p53. 14 urothelial cancer cell lines were analysed for expression and mutation of p53 by immunocytochemistry, Western blot, Polymerase Chain Reaction-Single Strand Conformation Polymorphism analysis (PCR-SSCP) and direct sequencing of double stranded PCR-products of genomic DNA. Using monoclonal antibody PAb 1801 seven of 14 cell lines showed immuncytochemically p53 overexpression which was confirmed by Western blot analysis. Sequencing analysis revealed missense point mutations in 11 of 14 cell lines. Four cell lines carrying p53-missense point mutations within the highly conserved regions and cell line T24, which does not express p53 due to a mutation leading to a stop codon, were choosen for further analysis. Luciferase assay and X-gal staining was carried out as an indicator of transfection efficiency. VM Cub 1 (175 His) showed high, VM Cub 3 (278 Leu), 639 V (248 Gln) and HT 1376 (250 Leu) moderate and T24 (126 Stop) poor luciferase activity using the calcium phosphate transfection method. X-gal staining confirmed these results for the cell lines analyzed. T24, showing poor luciferase activity using calcium-phosphate, was co-transfected by electroporation with RSVneo and a plasmid carrying human wildtype (hup53) or mutant (273His) p53 driven by the CMV-promoter, respectively. Co-transfection of T24 with RSVneo and CMVhup53 or CMV273His resulted in G418 resistant clones 12 days after transfection using electroporation. Using PCR technology primary cultures of the transfected cells were shown to contain either wildtype or mutant p53 constructs. However, no p53 plasmids were found in later passages. This observation is consistent with previous reports of p53 beeing only transiently expressed in HeLa cells [Ridgeway et al., Oncogene 8:1069-1074 (1993)]. Since this negative selection against p53 may be due to negative regulation of p53 to various promoters (e.g. RSV) we constructed inducible MTp53-constructs. Further experiments co-transfecting CMVp53 or MTp53 with either RSVneo (stable clones) or RSVß-gal (transient expression) are in progress to examine the effect of p53 in these cell lines.

P51

NUCLEAR AND CYTOPLASMIC DETECTION OF p53 OVEREXPRESSION IN BLADDER CARCINOMA: PRELIMINARY RESULTS.

E.Alcini, A.Sgambato (*), C.Ratto, G.Flamini (*), M.Giustacchini, A.Di Pinto, A.Alcini, F.Crucitti, A.Cittadini (*).

Dept. of Surgery (Urology Division) and (*) Oncologic Research Center "Giovanni XXIII" - Catholic University - Rome - Italy

INTRODUCTION: Alterations of p53, a tumor suppressor gene located on chromosome 17p, are suggested having a role in bladder cancer pathogenesis. Its overexpression would be related to a poorer prognosis. Immunohistochemically, the incidence of p53 positivity ranges from 25% to 54%. In the present ongoing study, expression of p53 protein, its localization in the cell compartments and relationships with other prognostic factors are evaluated. **METHODS:** At the moment, 30 patients with bladder carcinoma have been studied (25 males, 5 females). In 13 cases size of neoplasm was ≤1 cm, in 17 cases >1 cm. In 17 patients carcinomas were plurifocal. Fresh tissue samples were obtained from the surgically resected specimens and immediately frozen (-80°C). Five micron sections were obtained and submitted to immunohistochemical analysis utilizing D07 monoclonal anti-p53 antibody. The Vectastain ABC kit was used and reaction was revealed with diaminobenzidine. Tumor stage was Ta or T1 in 22 cases and T2, T3 or T4 in 8 cases. Grading of the neoplasms was G1 or G2 in 17 cases, G3 in 13 cases. Local recurrence was diagnosed in 19 patients. **RESULTS:** Immunohistochemical detection of p53 was observed in 13 specimens (43.3%). Intensity of staining was high in 10 cases, low in 3 cases. Cytoplasmic localization of p53 was detected in 2 samples. No significant correlations with patients' sex and age, size, plurifocality, stage, grading and local recurrence of the tumor were found evaluating this small number of cases. **CONCLUSIONS:** Phenotypic overexpression of p53 is a common event in bladder carcinomas, and immunohistochemical analysis seems to be a reliable method for its evaluation. Our study is still in progress: further data on a larger number of cases will highlight the value of p53 alterations in outcome prediction, with a special attention to the cytoplasmic positivity pattern.

P52

P53 ACCUMULATION AS AN EARLY EVENT IN THE DEVELOPMENT OF BLADDER CANCER

Claudia R.C. van Roeyen[1], Claus D. Gerharz[2], M.-Oliver Grimm[1], Wolfgang A. Schulz[3], Klaus Decken[1], Bernd J. Schmitz-Dräger[1]

1: Urologische Klinik, 2: Pathologisches Institut, 3: Institut für Physiologische Chemie I Heinrich-Heine-Universität, Düsseldorf, Germany

Accumulation of the p53 gene product has been observed in a wide variety of human tumors. Several investigators have reported p53 inactivation in 50-100% of advanced bladder cancer cases. Considerations by Lane (Nature 358, 1992, 15-16), however, suggest a potential role of p53 inactivation during carcinogenesis. To further elucidate the impact of p53 in the development of transitional cell carcinoma a variety of specimens representing the different steps of bladder cancer tumorigenesis from premalignant lesions to advanced tumors was examined for p53 accumulation.

Using monoclonal antibody DO-1, 50 paraffin embedded specimens from 46 patients (7 dysplasias, 14 pTis, 9 pTa, 6 pT1, and 14 ≥ pT2 specimens) were immunohistochemically analyzed. Positive staining was demonstrated in 2 out of 7 dysplasias (29%), 6/14 (43%) of pTis, 3/9 (33%) of pTa, 2/6 (33%) of pT1, and 5/14 (36%) of the advanced tumor specimens. While G1 tumors showed no p53 accumulation, positive staining was observed in 35% (7/20) of G2, and 41% (9/22) of the G3/4 grade tumors, respectively.

These results indicate, that p53 accumulation can be found in a subgroup of urothelial tumors, suggesting a potential role of this tumor suppressor gene in only a limited number of tumors. Furthermore, the observation of p53 accumulation in a considerable number of pTis and even in premalignant lesions is in concordance with considerations by Lane, suggesting a role of this gene product in bladder cancer development. In contrast to other investigators we found no significant increase of p53 accumulation during tumor progression, indicating that p53 alterations may be involved in the development, but not necessarily in the progression of a (highly malignant ?) subgroup of urothelial tumors. As a consequence from this study a further analysis of the molecular alterations caused by mutational p53 is required.

P53

p53 gene alterations are associated with genomic instability and rapid tumor cell proliferation in bladder cancer

Holger Moch, Guido Sauter, Michael J. Mihatsch, Thomas Gasser, Gianfranco Mattarelli, Fred Gudat, Frederic M. Waldman*,

Departments of Pathology and Urology, University of Basel, Switzerland and Division of Molecular Cytometry, University of California, San Francisco*

The p53 tumor suppressor gene is thought to be involved in preservation of genomic stability of cells. p53 gene alterations have been shown to be relevant for prognosis in bladder cancer. This may be due to an increased genetic instability in tumors with p53 alteration.

It was the goal of this study to investigate the relationship of p53 lesions with genomic instability and tumor cell proliferation. 135 formalin fixed bladder cancer specimens were examined. The p53 gene expression was studied by immunohistochemistry (CM1). The presence of 17p deletions was examined by dual labeling fluorescence in situ hybridization (FISH), using centromeric probes for chromosome 17 (p17HU8) and a gene specific probe for p53 (17p13.1). DNA ploidy (measured by flow cytometry) as well as gene amplifiation of the oncogenes erbB-2 (17q21) and bcl-1 (determined by FISH) were examined as parameters for genomic integrity. Tumor cell proliferation was measured by assessing the percentage of Ki-67 positive tumor cells using the antibody MIB-1.

Comparison of different methods for analysis of p53 gene aberrations revealed only a weak association of p53 protein expression with 17p deletion. Interestingly, the combination of FISH and immunohistochemistry allowed distinction of 4 groups with phenotypic differences. Tumors with neither 17p deletion nor p53 expression (n=38) showed low prevalence of aneuploidy (32%) and low proliferation rate (Ki67 Labeling Index (Ki67 LI) = 12%). Tumors with p53 expression but no 17p deletion (n=45) showed a similar Ki67 LI (13%) but an increased prevalence of aneuploidy (65%, p=0.002). Tumors with 17p deletion but no p53 expression (n=14) exhibited an increased Ki67 LI (20%) but no increased tendency for aneuploidy (36%). Tumors with both p53 expression and 17p deletion (n=38) showed highest prevalence of aneuploidy (90%, p = 0.002) and also a high Ki67 LI (20%). No association was seen between presence of p53 alterations and gene amplification.

Our results are consistant with a key role of the p53 gene for preservation of genomic integrity and for regulation of tumor cell proliferation. Tumors with both 17p deletion and p53 protein expression may constitute a highly aggressive subgroup of bladder tumors.

P54

p53 MUTATIONS HAVE NO ADDITIONAL VALUE OVER STAGE IN BLADDER CANCER.

Vet J.A.M.[1], Bringuier PP.[1], Poddighe P.J.[2], Karthaus H.F.M.[3], Debruyne F.M.J.[1], Schalken J.A.[1].
Dept's of Urology[1] and Pathology[2], University Hospital, Nijmegen and Dept. of Urology[3], Canisius Wilhelmina Hospital, Nijmegen, the Netherlands.

Evidence is accumulating that the tumor suppressor gene p53 is involved in the development of bladder cancer. We studied p53 mutations in 47 bladder tumors using polymerase chain reaction/ single-strand conformation polymorphism (PCR-SSCP) analysis. Eight out of 24 invasive tumors appeared to have a p53 mutation while no p53 mutations were found in the superficial tumors. All the p53 mutations were found in grade 3 tumors. The tumors with altered p53 showed a higher frequency of allelic loss (FAL) compared to the tumors without a mutation (55.8% versus 21.1%, $p<0.05$, by Chi-square test). This increase in FAL suggests a correlation between p53 mutations and genetic instability. A significant correlation between mutated p53 and poor survival in the whole group studied was found ($p<0.001$, by log rank test). However, within the group of muscle-invasive tumors the occurrence of p53 mutations had no additional prognostic value. Therefore, even though p53 mutations were found in aggressive tumors, the clinical usefulness of its detection seems limited. Nevertheless, these results implicate that p53 is involved in the clinical behaviour of bladder cancer, and especially its role in the progression of superficial cancer to invasive disease needs further attention.

P55

SQUAMOUS CARCINOMA OF THE URINARY BLADDER - A MOLECULAR EPIDEMIOLOGY STUDY

P-U Malmström (1), P Ohneseit (3), A Shibata (3), C Spruck (3), M Shamaa (4), M Elbaz (4), C Busch (2), P Jones (3).
Dept. of Urology (1) and Pathology (2), Uppsala University, Sweden, Kenneth Norris Jr. Comprehensive Cancer Center (3), Los Angeles, USA and Urology and Nephrology Center (4), Mansoura , Egypt .

Squamous carcinoma of the urinary bladder is rare in Europe while it constitues the most common histological type in Egypt. Epidemiological data support the etiological role of Schistosomiasis for the Egyptian type. Specific carcinogens can give rise to specific mutations of p53. The aim of the present study was study the occurrence and pattern of p53 gene mutations in these two different populations.

Formalin fixed specimens from 15 Egyptian and 5 Swedish cases were examined with SSCP analysis and immuno-histochemistry (DAKO, D0-7). SSCP detected p53 mutations in 8/15 (53%) Egyptian specimens while 8/15 (53%) also stained positive for p53. The corresponding Swedish figures were 4 of 5 (80%) and 4 of 5 (80%). There was, however, no exact correlation between the SSCP and the immunohistochemistry data as four cases with mutations stained negative. No distinct difference in codon locations of the mutations were observed between the two populations. However, three of the mutations were CGC (Arg) to CAC (His). Base changes at codon 175, a mutational type not previously detected in carcinomas of the bladder, has been associated with chronic inflammation of the urothelium.

From the present data, mutations of p53 seems to occur more often in Swedish than in Egyptian samples. Further studies are ongoing to analyze other genetic events in this material.

P56

COMPUTER-ASSISTED CHROMATIN TEXTURE CHARACTERIZATION OF FEULGEN-STAINED NUCLEI IN 331 TRANSITIONAL BLADDER CELL CARCINOMAS.

Roland van Velthoven[1], Michel Petein[2], Alexandre Zlotta[3], Willem J. Oosterlinck[4], Adrian van der Meijden[5], Robert Kiss[6] and Claude Schulman[3].
[1]Dept. Surgery, Div. Urology, and [2]Dept. Pathology, Institute J. Bordet; [3]Dept. Urology, Erasmus Hospital, [6]Lab. Histology, Fac. Medicine, Free University of Brussels, Brussels; [4]Dept. Urology, University Hospital, University of Gent; Belgium; [5]Div. Urology, Bosch Medisch Centrum, 's Hertogenbosch, The Netherlands.

The chromatin patterns of Feulgen-stained nuclei in a series of six normal mucosa and 331 transitional bladder carcinomas (including 293 superficial (Ta and T1) and 38 invasive (T2-T4) cases) were quantitatively described by means of eight parameters relating to densitometric, run-length distribution and co-occurrence matrix features. The results show that the chromatin texture of the superficial bladder tumours was markedly different from that of the invasive ones. Indeed, the latter exhibited a distinctly more dense and heterogeous chromatin pattern than the former. Furthermore, the data also showed that the increasing level of malignancy revealed by the increasing clinical stage was accompanied by an increase in the overall chromatin condensation level. In fact, there were only some areas of the nucleus which increased in density in view of the fact that pale areas appeared concomitantly with these increasingly denser chromatin areas. This chromatin density increase (occurring as a function of increasing clinical stage) corresponded to a marked increase in the frequency of small and dense chromatin clumps joining together into very large dense chromatin clumps which were distributed more and more heterogeneously in the nucleus as the clinical stage of the tumour increased.

P57

MUTANT p53-EXPRESSION IN HUMAN PROSTATIC TUMORS MAY PREDICT NON-APOPTOTIC RESPONSE TO CASTRATION

Patrick Westin*, Per Stattin#, Jan-Erik Damber#, Anders Bergh*
Departments of Pathology (*) and Urology & Andrology (#)
University of Umeå, Umeå, Sweden

Prostatic tumors are considered to be either androgen-dependent or androgen-independent. According to current opinion androgen withdrawal induces programmed cell death and apoptosis in the normal prostate and in androgen-dependent tumors, but not in androgen independent ones. Our recent observation that castration does not induce apoptosis in the highly differentiated androgen sensitive Dunning R 3327 PAP prostatic tumor in rats indicate that this opinion may be an oversimplification. In this study we examine the morphological response to castration therapy in human prostatic carcinoma patients. Ultrasound-guided needle biopsies were taken from 9 patients with high or moderately differentiated prostatic cancers, before and 7 days after castration. The biopsies were examined by image-analysis to determine tumor cell nuclear area, by immunohistochemistry using an antibody against mutant human p53 protein (Ab-6, Oncogene), and by *in situ* end labelling of fragmented DNA in order to visualise apoptotic cells. Before castration, apoptotic tumor cells were extremely rare. Four of the nine examined tumors showed positive immunohistochemical staining for mutant p53. In these patients the number of apoptotic tumor cells was unaffected by castration. In contrast, about 3 % of the tumor cells were apoptotic 7 days after castration in the p53 negative tumors. Interestingly, all the nine examined tumors were androgen-sensitive since they all showed a castration-induced decrease (from 8 to 40%) in nuclear cell area, and in serum PSA. The magnitudes of these responses were not related to changes in apoptotic index. Our results demonstrate that there are at least two different responses to hormone withdrawal therapy in human prostatic tumors; cellular atrophy and/or DNA-fragmentation and cell death. It appears that castration only induces apoptosis in a subgroup of presumably androgen dependent tumors that can possibly be identified prior to therapy (p53 negative).The hypothesis that mutant p53 could prevent castration-induced apoptosis should be tested in a larger group of patients.

P58

EXPRESSION OF THE RETINOBLASTOMA TUMOUR SUPPRESSOR GENE IN PROSTATIC CANCER SPECIMENS

Johannes M Wolff, Rothewelle J Tate, Geoffrey D Chisholm, Fouad K Habib
Department of Urology, Klinikum RWTH Aachen, Aachen, Germany Department of Surgery/Urology, University of Edinburgh (WGH), Edinburgh, UK

The retinoblastoma tumour suppressor gene (RB gene) has been reported to be altered in a number of human cancers indicating that its dysfunction is a critical event in the development in many tumours. Point mutation at exon-intron boundaries of the RB gene have been reported in several cell lines. These mutations cause loss of splice-donor sites and prevent normal splicing of mRNA transcripts leading to a truncated protein product. We have analyzed 14 primary prostate specimens (BPH n=5, CaP n=9) for the presence of a short-sized mRNA transcript within exon 13 - 18 and exon 18 - 23 by reverse transcriptase polymerase chain reaction (RT-PCR). None of the prostatic tissues showed any evidence of an aberrant short-sized mRNA. In addition a potential hot spot for recombination has been predicted, as a number of deletions in various tumours have been found to contain clustered the region between exon 13 and exon 17. Employing DNA-PCR 18 primary adenocarcinomas of the prostate were investigated for mutational changes within exon 13 and 17. We failed to demonstrate any indication for deletions of the RB gene within exon 13 and 17 at DNA level in these cancer specimens. We therefore conclude that alterations of the RB gene are not involved as a causative factor in the development of adenocarcinoma of the prostate.

P59

PROGNOSTIC MARKERS FOR PROSTATIC CARCINOMA.

Maureen.E.Harper, Lindy Goddard, Eve Glynne-Jones & Keith Griffiths. Tenovus Cancer Research Centre, University of Wales College of Medicine, Cardiff, Wales, UK.

Prognostic markers for prostatic cancer should include those factors intimately involved in malignant progression- components of proliferation, growth responses, particularly those suggestive of autocrine stimulation, and modulators of invasion locally and distantly. In our studies the estimation of the growth fraction in prostatic tumours by analysis of the Ki-67 expression was found to be significantly correlated with grade and metastatic status and by life table analysis to relate to survival. Proliferating cell nuclear antigen (PCNA) expression was also shown to be of prognostic value by life table analysis. Multivariate analysis of the data revealed that Ki-67 was superior to PCNA and was an independent variable from that of histological grade and metastatic status. The MIB antibody which can be used with formal saline fixed tissue has been found to correlate with the Ki-67 assay and can be substituted for it in proliferation assays on these tumours. Expression of growth factors and their receptors by both immunocytochemistry (ICC) and northern blot analysis of the mRNA's on these tumours has shown epidermal growth factor (EGF) is rarely expressed and that the message is absent in all the primary tumours but present in the prostatic cell lines used as controls. TGFα was found in the majority of prostatic tumours by both ICC and mRNA analysis and a correlation with tumour grade was observed. EGF receptor which can bind both EGF and TGFα was detected in some prostatic carcinomas by ICC (Amersham antibody) but did not appear to be prognostic by life table analysis. All samples however appeared to express the message for EGF receptor which requires further clarification using *in situ* studies with an EGF receptor probe. Nuclear androgen receptor (AR) measurement by ligand binding assays was not found to be prognostic and an ICC assay for AR has recently shown a relationship with histological grade. AR as determined immunocytochemically has not yet been analysed by life table analysis. The cadherin expression (ICC using HECD-1), metalloprotease expression (ICC) and angiogenic potential, as assessed by estimation of capillary distribution and density using endothelial cell markers, are more recent tumour characteristics being assayed on the prostate specimens.

P60

A CRITICAL EVALUATION OF SERUM TPS MEASUREMENT IN THE DIAGNOSIS AND MONITORING PROSTATE CANCER PROTOCOLS

Marko TARLE and Ksenija KOVAČIĆ, Nuclear Medicine and Oncology Clinic, University Hospital Sestre Milosrdnice, ZAGREB 41000, CROATIA

Serum TPS concentration is an inappropriate tool for distinguishing benign prostatic tumor (BPH) from local carcinoma (P> 0.05) but is highly effective in discriminating primary tumor from metastatic adenocarcinoma (P< 0.05).
In all first presented cancer patients serum TPS concentration serves as a reference for possible further respective measurements during monitoring. In addition, the reliability of a single bone scan may be rather low. Hence, an ambiguous finding may be tested and/or confirmed by serum TPS value.
The raise in normal TPS level in subjects with local disease is indicative of the development of metastases. The assessment of serum TPS level is more cost-effective than bone scintigraphy and may be used in discriminating candidates for a skeletal scan from those with Stage Do disease.
Numerical TPS serotest values exert a linear correlation with both tumor spread (Tarle et al., Prostate, in press) and differentiation grade in hormonally treated Stage D2 cancer patients. This does not hold true for the assessment of hormone dependent PSA and PAP serotests. Accordingly, the addition of serum TPS values to PSA and PAP measurement in monitoring metastatic patients seems to be of utmost clinical importance.
Prostate cancer is characterized with an extreme clonal heterogeneity especially in advanced subjects. According to the UICC standards an obvious healing in bone and the improvement in a lymphatic system may lead to the classification of a disease into a "no change" ("stabilization") or even "partial remission" category despite the fact that in parallel some minor osseous lesion(s) may be suspective of a progress. Such aggressive and relatively small lesions that will soon become predominant and a widespread cell subpopulation(s) are detected easily by measuring serum TPS level. This fact may give a new dimension to the estimation of tumor status. Accordingly, some fundamental standards of tumor follow-up protocols may be subjected to changes by using cell proliferation serotest measurements.
In up to 10% of advanced prostate adenocarcinomas areas of small cell carcinoma are detected by both pathohistological and immunohistochemical means. A significant contribution of the neuroendocrine component we have early recognized during tumor progression by a decline in serum PSA, PAP and TPS values and a simultaneous elevation in blood neurone-specific enolase (NSE) concentration (Tarle et al., Prostate, in press). The applied procedure is not only noninvasive but is also readily accomplished.

P61

INCREASED LEVEL OF ANEUPLOIDY IN PERIPHERAL BLOOD LYMPHOCYTES DERIVED FROM PATIENTS WITH PROSTATE CANCER. Irit Bar-Am, Eliahu Mukamel, Hava Schaham and Lydia Avivi, Tel Aviv, Israel. (Presentation by Dr. Bar-Am)

The frequency of aneuploidy in human peripheral blood lymphocytes was examined in patients with metastatic prostate cancer before and after hormonal therapy. The mean percentage of aneuploid cells in a group of 12 patients was twice that observed in a group of 7 age-matched males with benign prostate hyperplasia (20.3 ± 1.7 vs. 10.3 ± 2.3, respectively; $p < 0.01$), in another 7 patients who underwent orchiectomy the mean level of aneuploidy was 10.7 ± 1.9, similar to that noted in patients with benign prostatic hyperplasia. The same effect on aneuploidy was noted in patients with prostate cancer who were treated with GNRH analogues.

Our data suggest that a high percentage of chromosomal aberrations is present in peripheral blood lymphocytes in patients with metastatic prostate cancer. Hormonal manipulation reduces the level of these aberrations to almost normal levels.

P62

THE LNCaP MOUSE MODEL OF HUMAN PROSTATE CANCER:ANDROGEN - INDEPENDENT CANCER PROGRESSION AND OSSEOUS METASTASIS

[1]George N. Thalmann, [1] Ploutarchos Anezinis, [1]Shi-Ming Chang, [1]Haiyen Zhau, [1]M.Craig Hall, [2]Sen Pathak, [1]Andrew C. von Eschenbach, [1]Leland W.K. Chung, [1]Departments of Urology an [2]Cell Biology, Universitiy of Texas, M.D. Anderson Cancer Center, Houston, Tx, USA.

Malignant growth progression of prostate cancer is marked by development of androgen-independence and non-random dissemination to the axial skeleton. Our laboratory has developed the first human prostate cancer model in which metastasis of the primary prostate cancer to lymph nodes and the bone can be observed. Based on this model, a series of LNCaP cell sublines were derived from LNCaP tumors maintained in castrated and intact athymic male mice.We have characterized androgen-dependence, anchorage-independent growth and prostate-specific antigen (PSA) expression in these sublines. The C4-2 subline, derived by inoculating castrated male hosts twice with LNCaP cells, acquired tumorigenic and metastatic potential. We suggest that the host microenvironment may play a key role in determining the behavior of these LNCaP sublines. The C4-2 subline expressed decreased androgen receptor levels and proved to be less androgen-regulated. This subline expressed 5- to 10-fold higher basal PSA mRNA in the absence of androgen stimulation. The parental LNCaP cell line remained nontumorigenic over more than 50 passages in culture.

We recently determined the tumorigenic and metastatic potential of the C4-2 subline, which mimics androgen independent prostate cancer in patients. C4-2 derived tumors were transplantable for 4 consecutive generations with a yield of 33 - 100 % tumorigenicity for an observation period between 4 to 14 weeks.

When the C4-2 subline was injected either orthotopically or subcutanously it metastasized to lymph node and bone in 10 % to 50 % of the animals with a higher incidence in castrated hosts. Cytogenetic analysis indicated that all of the tumor-derived sublines were human and share common marker chromosomes with the parental LNCaP cells, as well as chromosomal deletions such as 8p, which is often found in human prostate cancer. Immunohistochemistry demonstrated staining for PSA and cytokeratin in all sublines. In anchorage-independent growth assays the subline derived from osseous metastasis showed a decreased intrinsic activity in comparison to sublines derived from primary tumor or lymph nodes when assayed under serum-free conditions.

In conclusion, this metastatic LNCaP human prostate cancer model may permit for the first time to study the molecular mechanism of prostate cancer progression and metastasis.

P63

REGULATION OF TISSUE-TYPE TRANSGLUTAMINASE IN HUMAN PROSTATIC CANCER CELL LINES AND NON-MALIGNANT PROSTATIC CELLS

U. Rausch, H.-W. Goebel, A. Wunsch, P.J. Schiemann, and G. Aumüller
Department of Anatomy and Cell Biology, Philipps-University, D-35033 Marburg

Tissue type transglutaminase (TGase) is a cytosolic enzyme the primary function of which is to catalyze the covalent cross-linking of proteins. It has been suggested to be involved in a number of (intra)-cellular processes[1] in normal and malignant cells, such as changes in cellular morphology, adhesiveness and programmed cell death[2].

We have studied the regulation of tissue-type TGase expression by steroid hormones, anti-androgens, and sodium butyrate in the LNCaP and the PC-3 prostate cancer cell lines and in BPH (benign prostate hyperplasia) derived cells, both at protein and RNA levels. Tissue-type TGase could be detected in all cells excluding in LNCaP. While steroids and anti-androgens had no influence on cell growth, enzyme activity, and TGase-mRNA content, sodium butyrate and retinoic acid (DU-145) significantly increased TGase activity and TGase-mRNA content as shown by Northern and Slot Blot analysis. At the same time, sodium butyrate and retinoic acid decreased cell growth. In LNCaP, sodium butyrate depressed PSA-mRNA content to the baseline level. In all other cells PSA was nearly undetectable under all conditions tested.

These results are not in favour with a specific hormone dependence of tissue-type TGase in human prostatic cancer but point to a role of the enzyme in apoptotic processes.

[1] Greenberg, C.S. et al. (1991), FASEB J. 5, 3071-3077
[2] Fésüs, L. et al. (1991), Eur. J. Cell Biol. 56, 170-177

This study was supported by the Deutsche Krebshilfe (Grant W6/90, Au-1).

P64

PREDICTIVE INDICATORS FOR SUBSEQUENT BLADDER TUMORS FOLLOWING NEPHROURETERECTOMY IN PATIENTS WITH UPPER URINARY TRACT TRANSITIONAL CELL CARCINOMA.

Eliahu Mukamel, Dan Simon, Ofer Nativ, Ciro Servadio, Petah Tiqva, Israel (Presentation by Dr. Mukamel)

The predictive indicators for developing subsequent bladder tumors in patients with upper tract transitional cell carcinoma (TCC) were determined in 55 patients who were followed for 2 to 15 years after nephroureterectomy. Of the 55 patients, 30 developed subsequent bladder tumors, 28 (93%) of these tumors appeared within two years following the nephroureterectomy. Nineteen of 39 (48.7%) patients with low grade (I, II) tumors and 11 of 16 (68.7%) patients with high grade (III, IV) tumors developed subsequent bladder tumors. None of the patients with a single calyceal tumor developed bladder tumors, whereas 21 of 34 (60.1%) patients with a single pelvic or ureteral tumors developed bladder tumors. Seven of 8 patients (87.5%) patients with multifocal calyceal and renal pelvic tumors developed subsequent bladder tumors. DNA ploidy analysis showed that subsequent bladder tumors were detected in 42% of patients with DNA diploid neoplasms compared to 70% of those with abnormal DNA content ($p < 0.001$). Patient survival curves showed a significantly higher survival rate for those patients who had no tumor appearance in the bladder compared to those who developed subsequent bladder tumors. Our data suggest that patients with high grade DNA non-diploid and multifocal upper tract TCC are at high risk for developing subsequent bladder tumors within 2 years following nephroureterectomy.

P65

HPV INFECTION AND CELL PROLIFERATION IN URINARY BLADDER CARCINOMA.

P. Ferrari,° E. Righi, G. Ferrari,° S. Bettelli, G. Castagnetti,° C. De Gaetani, G.P. Trentini.° Department of Urology and Institute of Pathologic Anatomy, University of Modena, Italy.

The unpredictability of evolution of urinary bladder carcinoma emphasizes the need for valid predictors of clinical course. In recent years, the attention has been focused on biologic markers like cell proliferation rate. Analogously, the possible role and significance of HPV infection has been sought with controversial results. Aim of this study was to investigate the presence of HPV-DNA and the changes in cellular proliferation indices and in the expression of p53 protein in a series of 101 histologic samples of 43 patients with bladder carcinoma. HPV-DNA was searched by a non-isotopic in situ hybridization technique using both two wide-spectrum and three virus-type specific biotinylated probes for HPV 6/11, 16/18,31/33/35. The cell proliferation activity was studied by immunohistochemical demonstration of cellular proliferation-associated nuclear antigens like Ki-67 and PCNA or by study of nucleolar organizer region proteins (AgNORs). The expression of p53 was evaluated immunohistochemically by an anti-p53 moAb (Do7) . The results obtained with the wide spectrum probes showed HPV-DNA in 17 cases (40 %). 62.5 % of these cases appeared to harbour HPV types 31/33/35, either alone or in association with other virus types. No significant correlations were observed between virus infection and tumor grade. But, a higher number of T1 tumors and of disease-free survivals were found in the group of virus-negative cases. Concerning proliferative activity and p53 expression, all tested parameters did not appear to change in relation with HPV infection. Analogously, no significant differences were found between the cell proliferation rate and the clinical course of the disease in terms either of grade and stage or of survival. Instead, the expression of p53 was significantly increased in T3 tumors. About AgNOR expression, only AgNOR number was significantly increased in HPV positive cases, but no differences were found in relationship with grade and stage or follow-up. In conclusion, HPV infection seems to affect the progression and the course of bladder carcinoma, a phenomenon that does not appear to be related with cell proliferative activity.

P66

PLOIDY LEVEL DETERMINATION BY MEANS OF IMAGE CYTOMETRY IN 314 TRANSITIONAL BLADDER CELL CARCINOMAS.

Roland van Velthoven[1], Michel Petein[2], Willem J. Oosterlinck[3], Alexandre Zlotta[4], Adrian P.M. Van der Meijden[5], Robert Kiss[6] and Claude Schulman[4].
[1]Dept. Surgery, Div. Urology, and [2]Dept. Pathology, Institute J. Bordet; [4]Dept. Urology, Erasmus Hospital, [6]Lab. Histology, Fac. Medicine, Free University of Brussels, Brussels; [3]Dept. Urology, University Hospital, University of Gent; Belgium; [5]Div. Urology, Bosch Medisch Centrum, 's Hertogenbosch, The Netherlands.

Image cytometry was carried out on four normal bladder mucosae, 281 superficial (Ta and T1) and 33 invasive (T2-T4) bladder cancers. The parameters used to characterize these bladder tumors were 1) histopathological grading, 2) clinical staging, 3) tumor size, 4) the DNA index (DI), 5) DNA histogram type (DHT), 6) the percentage of euploid (diploid + tetraploid) cells, 7) the percentage of polyploid cells (>5C DNA content), 8) proliferative activity (SPF value), and 9) nuclear area (NA). The proliferative activities of the tumors were not related either to histopathological grade or to clinical stage, but it was to the DHT parameter which made it possible to identify diploid, hyperdiploid, triploid, hypertriploid, tetraploid and polymorphic tumors. The hypertriploid tumors exhibited a significantly lower proliferative activity than the non-hypertriploid ones. While both the DI and the NA values correlated significantly with the histopathological grading, only the NA values correlated significantly with clinical staging. We further observed that some grade III bladder tumors were definitely diploid, while some grade I tumors were highly aneuploid. We thus hypothesise that the ploidy level of a given tumor reflects its age directly and its aggressiveness only very indirectly. In our opinion, aneuploidy is only an indirect marker of aggressiveness because it reflects the fact that a malignant tumor is old, i.e. has been present in a patient over a long period and has had ample time to express its malignancy at the clinical level. A significant relationship was accordingly obtained between tumor size and ploidy level, with the highest proportion of aneuploid tumors and the highest percentage of polyploid cell nuclei being observed among the largest bladder tumors.

P67

BIOLOGICAL MARKERS IN TRANSURETHRAL BIOPSIES FROM BLADDER CANCER: PRELIMINARY RESULTS

Torelli T.[1,2], Benini E.[2], Costa A.[2], Pizzocaro G.[1,2], Campo B.[1], Milani A.[2], Veneroni G.[2], Ordesi G.[1], Leidi G.L.[1], Pilotti S.[2] and Silvestrini R.[2]
[1]Ospedale Predabissi, Melegnano (Milano)
[2]Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano

A biologic profile including proliferative activity, evaluated as ^{3}H-thymidine labeling index (^{3}H-dT LI), DNA ploidy, p53 tumor-suppressor gene and P-glycoprotein (P-170), as an expression of the multidrug resistance gene, was defined for 50 primary transitional cell carcinomas of the bladder. ^{3}H-dT LI was evaluated by autoradiography on histologic sections after incubation of fresh tumor biopsies with ^{3}H-thymidine. Ploidy was defined by flow cytometric analysis of DNA content on nuclei suspensions obtained from frozen material. Expression of p53 protein and P-170 glycoprotein was detected by immunohistochemistry using the PAb1801 and C219 monoclonal antibody respectively, on sections from paraffin-embedded tumor biopsies. Invasive tumors showed a higher median ^{3}H-dT LI (12.7% vs 4.2%) and a higher frequency of aneuploidy (73% vs 43%) and more frequently expressed p53 (82% vs 36%) than superficial tumors. Further analysis showed that proliferative activity was higher in invasive than in superficial cancers only in p53-positive or aneuploid tumors and not in p53-negative or diploid tumors. Moreover, proliferative activity and p53 overexpression, but not ploidy, were directly related to histologic grading and tumor stage. Generally, P-170 was not significantly related to any biologic or clinico-pathologic factor. Kinetic and phenotypic biologic markers are differently related to clinico-pathologic factors.

P68

INVERSE RELATION OF E-CADHERIN (E-CAD) AND AUTOCRINE MOTILITY FACTOR RECEPTOR (AMFR) EXPRESSION AS PROGNOSTIC FACTORS IN PATIENTS WITH BLADDER CARCINOMA

Thomas Otto [1], Avraham Raz [2], Walter Birchmeier [3], Friedhelm Noll [1], Herbert Rübben [1]

1 Department of Urology, University of Essen, Medical School, Hufelandstr. 55, 54122 Essen, FRG
2 Metastasis Research Program, Michigan Cancer Foundation, 110 East Warren Avenue, Detroit, Mi, USA
3 Institute of Cell Biology (Tumor Research), University of Essen, Medical School, Virchowstr. 173, 45122 Essen, FRG

The level of expression of two cell surface antigens, the cell adhesion molecule E-cadherin and the autocrine motility factor receptor gp 78, was analyzed in various histological types of bladder cancer.

Monoclonal antibodies against E-cadherin (6F9) and gp 78 (3F3A) were used to stain serial frozen sections of normal bladder and bladder carcinomas from 123 patients.

Positive expression of E-cadherin and negative expression of gp 78 were associated with the low risk patient group; only one of sixteen patients underwent tumor progression and none of these died of cancer. However, reduction of E-cadherin expression accompanied by increased gp 78 expression was associated with poor prognosis; 71 % of the patients (n = 30) with E-cadherin-negative or -reduced and gp 78-positive tumors underwent rapid cancer progression, and 32 % of the patients died of cancer-related disease at a median of twenty months after initial diagnosis.

Reduction of E-cadherin expression and increased levels of gp 78 in bladder tumors define a high risk group of cancer patients. The use of these parameters can markedly improve early diagnosis of high risk bladder cancer patients and may influence decision for treatment.

P69

ENHANCED URINARY sICAM-1 LEVEL IN BLADDER TUMOR PATIENTS REFLECT INVASION AND MALIGNANCY

D. Rohde, D. Küsters, W. Stieghorst, R. Sikora and G. Jakse; Dept. of Urology, RWTH Aachen, Germany

The intercellular adhesion molecule ICAM-1 participates in tumordefense mechanism. A soluble form (sICAM-1) has been identified in sera, cerebrospinal fluid and bile sofar. Due to the expression of ICAM-1 at urothelial carcinoma cells in-vitro and on tumor specimen, we investigated the appearance of sICAM-1 in human urine and serum from patients with bladder carcinoma and volunteers. Analysis contained urine analysis, blood chemistry, microbiology, CEA, CA-19-9, tumor stage, grade and volume in comparision to sICAM-1 or bFGF level obtained by an ELISA. In addition the response of bladder tumor cell lines to cytokines (IFN-α, IFN-τ) were observed.
Results: Enhanced sICAM-1 level in urine reflected an increase in tumor invasion and malignancy more sensitive than in serum or compared to bFGF. Significant differences were found between superficial (pTa) and invasive (pT1-4) carcinoma. Moreover sICAM-1 was constitutively shed by several bladder tumor cell lines and could be stimulated with cytokines.
Conclusions: sICAM-1 could be detected by an simple and reproducible assay. It is suggested as a new tumor marker for invasion and biology of bladder carcinoma. Being involved either in immunosurveillance or tumor defense of tumor cells the analysis of sICAM-1 in urine and serum may recommend itself as a prognostic factor for patients with a bladder carcinoma or an indicator for the response and efficacy of intravesical immunotherapy.

P70

MECHANISMS OF DEFECTIVE E CADHERIN FUNCTION IN UROLOGICAL TUMORS. ASSESSMENT OF THE ROLE OF CATENINS GIROLDI L.; BRINGUIER P.P.; QUAX P.; SCHALKEN J. Urological Research Laboratory. University Hospital Nijmegen, Nijmegen, The Netherlands.

Attempts to understand the molecular mechanisms enabling the metastasizing cells to detach from the primary tumor site led researchers to focus on an epithelial cell-cell adhesion molecule: E-cadherin. Decreased immunoreactivity is correlated with aggressiveness in several cancers including prostate and bladder tumors and it is believed that the cells loosening due to impaired E-cadherin function plays a major role in invasion. Elucidating the mechanisms of defective E-cadherin function should then yield useful insights on the invasion process.

Decreased immunoreactivity does not always correlate with decreased mRNA expression. SSCP analysis of the relevant prostate and bladder tumors did not provide evidence for mutations suggesting that post transcriptional mechanisms may be involved.

Catenins α, β and γ are co-immunoprecipitated with an E-cadherin antibody. These 3 proteins anchor E-cadherin to the cytoskeleton, conditioning its adhesive function. Immunoprecipitation analyses of a panel of 6 prostate and 10 bladder tumor lines shows that 3 prostate and 4 bladder lines have no or considerably reduced amounts of E-cadherin. One prostate and 2 bladder lines yield no or almost no γ catenin. One prostate line, PC3, lacks α catenin; consequently E-cadherin staining in this line is cytoplasmic. Transfection of αN catenin restores a membranous E-cadherin staining and is associated with a morphological change to an epithelial phenotype. Preliminary Elisa experiments show a 10 time uPA decrease in the epithelial transfectants.

Northern analyses of 16 bladder tumors revealed the presence of the 2 α catenin transcripts in all of them. 29 out of 30 tumors display 2 β catenin transcripts, 1 tumor has only 1 transcript.

Thus, in urological tumors, impaired E-cadherin function is most of the time due to unstable E-cadherin expression, however lack of catenins can also lead to defective E cadherin function. Complete assessment of the importance of this event in tumors awaits immunohistochemical analysis.

P71

LEVELS OF CIRCULATING INTERCELLUAR ADHESION MOLECULE-1 IN PATIENTS WITH METASTATIC CANCER OF THE PROSTATE AND BENIGN PROSTATIC HYPERPLASIA

Johannes M Wolff, Richard N Stepenson, Geoffrey D Chisholm, Fouad K Habib
Department of Surgery/Urology, University of Edinburgh (WGH), Edinburgh, UK

Current reports put forward a role for intercellular adhesion molecule 1 (ICAM-1) in the progression of human malignancy. The availability of a new antibody made it possible to detect circulating ICAM-1 (cICAM-1) in human body fluids including serum. It has been suggested that serum ICAM-1 levels may be a useful marker in cancer patients to monitor tumour burden and provide additional prognostic information.

Methods: Serum levels of cICAM-1 were measured in patients with benign prostatic hyperplasia (BPH, n=20). The values obtained were compared to those in patients with metastatic cancer of the prostate (CaP, n=25) before and after androgen deprivation treatment. Healthy men under the age of 40 served as a control (n=8).

A commercial available ELISA-Kit to detect soluble ICAM-1 was employed. The assay utilises an immunoenzymatic technique for quantification of cICAM-1 in human body fluid including human serum.

Results: The mean cICAM-1 levels observed in BPH patients was 339.52+/-15.3 ng/ml. CaP-patients demonstrated a lower mean serum concentration of 263.6+/18.5 ng/ml and 266.8+/-22.1 ng/ml before and after therapy respectively. The mean cICAM-1 levels of cICAM were found to be significantly elevated in the BPH and CaP group compared to healthy controls ($p < 0.01$). Although the difference between BPH and untreated as well as treated CaP was significant ($P < 0.05$) if failed to reflect the malignant state of the prostatic disease on an individual basis.

Conclusion: On the evidence of this study we suggest that cICAM-1 levels do not provide additional information in patients with metastatic CaP.

P72

DIFFERENCES IN EXPRESSION OF VOLTAGE GATED ION CHANNELS BETWEEN LOW AND HIGHLY METASTATIC DUNNING PROSTATE CANCER CELL LINES IN VITRO. Julia A. Grimes, Mustafa B.A. Djamgoz, James E.G. Downing, Marc E. Laniado, Christopher S. Foster and Paul D. Abel, London, U.K. (Presentation by Julia Grimes).

It is currently not possible to predict the clinical course of human prostate cancer in individual patients. Ion channel expression is as yet unexploited in the study of cancer cell behaviour. This report describes the types of voltage gated ion channels present in 2 Dunning cell lines of markedly differing metastatic ability (the AT-2 which displays a low metastatic rate to non-specific sites and the MAT LyLu which displays a high metastatic rate specifically to lymph node and lung) using the whole cell patch clamp technique in-vitro. In all AT-2 cells, only outward currents were recorded. These activated at -40mV, had a current density of 13.8 pA/pF and were partially blocked by tetraethylammonium (TEA) with an IC50 of about 12mM, all of which are characteristics of potassium channels. Outward currents were also present in all MAT LyLu cells, activated at -40mV and with a current density of 6.75 +/- 1.06 pA/pF. These were also partially blocked by TEA with an IC50 of about 12mM, consistent with potassium channels but with lower current density compared to the AT-2 cells. Inward currents were also identified but these were present in only 41% of MATLyLu cells. These were activated at -20mV, were transient taking 2.1 +/- 0.17 milliseconds to peak, had a density of 4.08 +/- 1.13 pA/pF and were blocked by tetrodotoxin at concentrations of 1 microM, characteristic of sodium channels. These studies have shown for the first time differential expression of ion channels in epithelial cells of low and high metastatic ability derived from the same parent cancer. Current research is directed to determining the relevance of differential ion channel expression to metastasis in-vivo.

P73

HUMAN SEMINOMA SYNTHESIZE AND SECRETE FREE α- AND ß-SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN (hCG)

S. Dirnhofer°, S. Madersbacher*, W. Kranewitter°, R. Gerth°, A. Hittmair#, A. Hobisch+ and P. Berger°
°Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria; *Dept. of Urology, University of Vienna, Austria; #Dept. of Pathology, University of Innsbruck, Austria; +Dept. of Urology, University of Innsbruck, Austria

Human chorionic gonadotropin (hCG) is a well established tumour marker for certain malignancies, the most prominent of which is testicular cancer. Among these the diagnostic value of hCG-like molecules for seminomatous cancer is, however, still a matter of debate. Thus, we investigated the production of hCG and its free, non assembled α- and ß-subunits by human seminoma using reverse-transcriptase polymerase chain reaction (RT-PCR) and determined the influence of peripheral metabolism on levels of these molecules in body fluids (hydrocele fluid, cubital veinous blood, urine) by a set of highly sensitive (2 pg/ml) and specific (cross-reactivity <0,01%) time-resolved 2-site fluoro-immunoassays using our own panel of monoclonal antibodies. All seminoma patients (n=6) were negative in serum and urine for hCGß (<5 pg/ml) but, surprisingly, revealed detectable free ß-subunit levels in the hydrocele fluid (range: 2,6-14,5 ng/ml). Moreover, five out of six patients had hCGα in hydrocele fluid. Final proof that hCGα and hCGß is indeed produced by previously termed "marker negative" seminomas has been achieved by RT-PCR using primers specific for these gene products. Three testes obtained from seminoma patients undergoing inguinal semicastration (2 of them hCGß negative in serum) were examined for hCG-subunit gene expression. We were able to detect hCGß in all three cases investigated and hCGα was expressed in two of them. These findings indicate that the distinction between hCG positive and negative testicular cancer should be reconsidered. Furthermore, they point at significant influences of peripheral hormone clearence on hCG levels in various body compartments.

Oral presentations: Tumor progression, metastasis

O74

HUMAN PROSTATE CANCER PROGRESSION CORRELATES WITH GLUCOSE TRANSPORTER NO.4 EXPRESSION

Beniers, A.J.M.C.[1], Tamimi, Y.[2], Effert, P.[1], Handt, S.[3], Jakse, G.[1].
Dept's of Urology[1] and Pathology[3], University Hospital Aachen, Germany and Dept. of Urology[2], University Hospital Nijmegen, The Netherlands.

Cell motility and metastatic potential has been found to correlate with aerobic glycolysis in the Dunning rat prostatic adenocarcinoma model. This prostate cancer model comprises several biologically diverse cell lines that represent a wide range of differentiation states and metastatic ability.

Using the human (Hep G2) glucose transporter (GT) cDNA as probe in Northern analysis we studied the expression of the glucose transporter no. 4 in the Dunning rat prostatic adenocarcinoma model. A close correlation was found between expression level and differentiation, however, no relation with metastatic capacity could be found. Recloning the Hep G2 cDNA into the plasmid pGEM-3Zf(+) allowed us to perform sense and antisense RNA in situ hybridization (RISH) on human prostate tumor samples. This enabled the evaluation of the mRNA levels and hence the GT expression in these tumors. Radioactive (^{35}S) and non-radioactive (digoxyginine, DIG) RISH was performed on frozen sections of tumor samples of 45 prostate cancer patients. Using sense- and anti-sense ^{35}S labelled 28s rRNA probes (non specific signal) in control hybridizations, the quality of the RNA preserved in the tumor samples could be checked. A strong correlation was found between the ^{35}S and DIG labelled hybridizations. The slides with ^{35}S labelled GT antisense RNA were used to quantify the GT expression using an image analysis system. A high expression of the glucose transporter no. 4 was measured in regions with high Gleason grade (4 and 5), whereas in lesions of Gleason grade 3, a weak expression was observed. In areas of grade 1 and 2, only low or no expression could be measured. These results suggest that GT expression as determined by RISH correlates well with tumor differentiation in prostate cancer indicating that GT expression should be investigated for further study of prostate cancer tumor biology.

O75

DECREASED E-CADHERIN EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS PROSTATE CANCER PATIENTS.

Rainy Umbas, William B. Isaacs, Pierre P. Bringuier, H. Ewout Schaafsma, Herbert F.M. Karthaus, Gosse O.N. Oosterhof, Frans M.J. Debruyne, Jack A. Schalken. Urology Research Laboratory, University Hospital Nijmegen.

Decreased levels of the cell-cell adhesion molecule, E-cadherin are associated with loss of differentiation in a number of human carcinomas. However, the value of E-cadherin as a prognostic marker in these cancers is largely undetermined. A previous study of E-cadherin levels in prostate cancer revealed that almost 50% of tumors examined had reduced or absent levels of this protein (Umbas et al. Cancer Res., 52: 5104). To determine potential prognostic significance of this finding, prostate cancers specimens from 89 patients were evaluated immunohistochemically for E-cadherin expression, and the results related to histopathological grade, tumor stage, presence of metastases, and survival.

As previously observed, a significant inverse correlation was found between E-cadherin expression and tumor grade. Importantly, we also found significant correlations between E-cadherin expression and tumor stage and overall survival. Sixty-three per cent of the tumors that extended beyond the prostate capsule (T3-4) *versus* 33% of the tumors confined to the prostate (T1-2) had aberrant expression (chi-square=8.1, $p<0.005$). Seventy-six per cent of the primary tumors from patients that presented with metastases showed aberrant staining compared to 32% from patients without metastases (chi-square=14.9, $p<0.001$). Log linear multiple regression analysis revealed that E-cadherin expression has additional value to clinical stage in predicting metastases. The life table analysis showed a significantly higher survival rate from patients with normal staining compared to patients with aberrant expression (log rank test: chi-square= 20.4, $p<0.001$). *These results suggest that E-cadherin expression can serve as prognostic indicator for the biological potential of prostate cancer.*

O76

POLYAMINES AND PROSTATE CANCER CELL MIGRATION: POSSIBLE IMPLICATIONS FOR TUMOR METASTASIS

Johannes C. Romijn, Sigrun Erkens-Schulze and Fritz H. Schröder;
Dept. of Urology, Erasmus University Rotterdam, The Netherlands.

Polyamines (putrescine, spermidine and spermine) are ubiquitous cellular components of which the physiological functions are not entirely clear yet. Circumstantial evidence suggests that polyamines are required to support cell proliferation. Other studies have led to the assumption that polyamines also play an important role in tumor metastasis. Since in vivo models are unable to discriminate between proliferative effects and influences on the metastatic process itself, we performed in vitro studies on the polyamine dependence of tumor cell migration. We previously demonstrated a correlation between induceability of cell migration and metastatic potential of a series of Dunning rat prostate cancer cell lines.

For the present study we used the metastatic Dunning MAT-LyLu cell line. Manipulation of intracellular polyamine concentrations was achieved by the exposure to polyamine synthesis inhibitors and exogenous polyamine addition. Cell migration, induced by either fibronectin or motility factors from serum free conditioned media, was assessed in a modified Boyden chamber assay.

Polyamine depletion, induced by α-difluoromethylornithine (DFMO), an ornithine decarboxylase inhibitor, or by methyl bis(guanylhydrazone) (MGBG), an inhibitor of S-adenosylmethionine decarboxylase, resulted in a substantial reduction (over 70%) of induced cell migration, regardless the stimulating factor used. This effect was polyamine depletion-specific, as shown by the complete reversal of inhibition when also exogenous polyamines were added. The time course of migration inhibition caused by DFMO-treatment (maximal after 4 days) coincided with the disappearance of intracellular spermidine. Inhibition by MGBG-treatment (resulting in lowered spermidine but increased putrescine levels) was abolished by spermidine addition. These results suggest that spermidine is of critical importance for maintaining migration activity.

Spermidine has been described to promote polymerization of monomeric G-actin into the filamentous F-actin form in cell-free systems. We observed that polyamine-depleted MAT-LyLu cells indeed had a significantly reduced intra-cellular F-actin content, which was restored to normal values after spermidine addition. Dynamic changes of the cytoskeleton are crucial for cell migration and, therefore, in tumor metastasis. Our results may indicate that polyamines, and spermidine in particular, may have a regulatory function in cytoskeletal organization. Pharmacological interference with intracellular polyamines may thus provide possibilities to control the metastatic process.

O77

ROLE OF BONE MATRIX PROTEINS VITRONECTIN, OSTEOPONTIN AND α2HS-GLYCOPROTEIN ON PROSTATE CANCER PROGRESSION AND OSSEOUS METASTASIS

[1]George N. Thalmann, [1]M. Craig Hall, [1]Robert A. Sikes, [2]Robert E. Devoll, [2]Mary C. Farach-Carson, [1]Andrew C. von Eschenbach, [1]Leland W.K. Chung,
Departements of [1]Urology an [2]Biochemistry, U.T ,M.D.Anderson Cancer Center, Houston TX, USA

Prostate cancer metastasizes in a non-random manner to osseous tissue forming osteoblastic-/lytic lesions. These findings suggest a bidirectional paracrine interaction between bone stroma and tumor cells.Previous experiments from our laboratory have demonstrated the importance of stromal-epithelial interaction on prostate development and prostate cancer progression. The role of cell adhesion molecules in the process and regulation of invasion, progression and metastasis is still poorly understood. To elucidate the role of bone-derived matrix proteins on prostate cancer growth and progression, we analysed the role of the biologic activity of bone matrix proteins in a soft agar colony formation (SACF) assay known to correlate with in vivo tumorigenicity.

Non-collagenous bone matrix protein fractions were prepared by molecular sieve and ion-exchange chromatography as described *(Bone and Mineral 16:1,1992).* Of the three fractions analysed FS1, a high molecular weight fraction containing a mixture of phospho- glycoproteins including osteopontin enhanced SACF in a biphasic manner with a 4-fold increase at 10 ug/ml ($p<0.5$). ES2, as low molecular weight fraction containing primarily osteocalcin did not significantly increase of SCAF ($p>0.05$). D2, an ES1 subfraction of α2HS-glycoprotein subfraction stimulated SCAF in a biphasic manner similar to FS1 with a 2- to 3-fold increase at 10 ug/ml ($p<0.01$). Conditioned medium from bone fibroblasts (positive control) yielded a 2- to 3-fold increase of SCAF.

These bone matrix protein fractions were further purified and separated; The fraction D3b, containing vitronectin, increased SCAF 6- to 7-fold. The D4a fraction, containing osteopontin (also called OPN, SPP1), stimulated SCAF growth 3- to 7-fold.

Anchorage-independent growth is stimulated by bone matrix protein fractions containing vitronectin, osteopontin a α2HS-glycoprotein. These findings suggest that specific bone matrix proteins may play an important role in prostate cancer progression and non-random osseous metastasis. Altered adhesion, cell-matrix interaction and growth promoting properties provided by bone matrix proteins in the host environment may help to explain the mechanisms of metastasis of prostate cancer.

Oral presentations: Immunology, diagnosis, treatment

O78

THE ROLE OF MONOCYTES/MACROPHAGES, T HELPER CELLS AND PENTOXIFYLLINE DURING THE INDUCTION OF BACILLUS CALMETTE-GUÉRIN-ACTIVATED KILLER CELLS AGAINST BLADDER CARCINOMA CELLS *IN VITRO*.

A. Böhle*, A. Thanhäuser, D. Jocham*, B. Schneider*, N. Reiling, H.-D. Flad, and A.J. Ulmer; Forschungsinstitut Borstel, D-23845 Borstel, Germany and *Department of Urology, Medical University of Lübeck, D-23562 Lübeck, Germany.

Intravesical Bacillus Calmette-Guérin (BCG) is a successful therapy against superficial bladder carcinoma recurrences. To understand the immunotherapeutic mechanisms of BCG we investigated cytotoxic mechanisms induced by BCG *in vitro* using human peripheral blood mononuclear cells (PBMC). Previously we demonstrated that BCG is able to induce MHC-unrestricted killer cells (BCG-activated killer, BAK cells) against various bladder tumor cell lines. The effector cells are characterized by the coexpression of the surface markers CD8 and CD56. We could now demonstrate the participation of monocytes/macrophages (Mφ) and T helper (CD4+) cells during the activation period of BAK cells. The cytotoxicity of PBMC depleted of Mφ by counterflow elutriation and subsequent nylon wool column could no longer be induced by BCG. Mφ could easily restore the induction of the cytotoxicity after re-addition of at least 1% Mφ in the starting population. PBMC depleted of CD4+ lymphocytes also failed to be rendered cytotoxic after stimulation with BCG. This is probably due to a dramatic decrease of interferon-γ (IFN-γ) release, a cytokine of high relevance for the BAK cell phenomenon. In addition, we could elucidate that pentoxifylline (POF), a phosphodiesterase inhibitor, is able to inhibit the production of IFN-γ and interleukin-2 at the protein and mRNA level in PBMC stimulated with BCG. This inhibitory capacity of POF, consequently, resulted in the inhibition of BAK cell induction. We conclude from our data that the activation of cytotoxic effector cells by BCG *in vitro* requires the co-operation of different cell populations and cytokines. In patients treated with intravesical BCG this is reflected by the infiltration of the bladder wall with mononuclear cells, especially Mφ and CD4+ cells, and by the detection of cytokines in the urine. *Supported in part by a grant of Institut Mérieux, Leimen, Germany.*

O79

PROGNOSTIC SIGNIFICANCE AND STABILITY OF URINARY CYTOKINES AND ALBUMIN FOLLOWING BCG THERAPY OF SUPERFICIAL BLADDER CARCINOMA E.C. De Boer, D.H.J. Schamhart, T.M. De Reijke, K.H. Kurth, Dept. Urology, Univ. Amsterdam, Amsterdam, The Netherlands.

Introduction. An accurate prognostic indicator for response on BCG therapy is urgently needed. Goal of this study was to assses the prognostic value of BCG-induced urinary IL1, IL2, IL6, TNF, IFNγ, and albumin. In addition, to assure a standard for further investigation, the stability of cytokines and albumin in urine, and the effect of leukocyte presence was examined.

Patients and methods. The study included 32 cases of BCG treatment courses (24 pts.) with primary (solitary TaG1 excluded) or recurrent superficial bladder carcinoma (4 TaG1, 6 TaG2, 3 TaG3 (1 associated with TisG3), 1 T1G2, 6 T1G3 (2 ass. TisG3), 4 TisG3 only). A BCG course consisted of 6 weekly intravesical instillations. Pts. were followed for recurrence of disease by 3-monthly cystoscopy. Cytokines were determined in serial urine samples with commercially available, highly specific and reproducible ELISAs (oligoclonal system). For albumin a nephelometric microalbumin assay was performed. Data were standardized to urine creatinine. To investigate stability, positive urine samples from 3 patients were incubated for 0.5, 2, 6, 12, and 24 hours at 4, 20, and 37°C, and subsequently measured.

Results. Pre-instillation values of 3.7±6.2 pg/μmol creat IL1, 0.0±0.1 U/μmol creat IL2, 5.9±12.8 pg/μmol creat IL6, and 0.2±0.8 pg/μmol creat TNF (n=134) were established. For IL2, using a threshold level of 0.40 U/μmol creat, a significant prognostic value was found for prediction of patients with (A) "EARLY" tumor recurrence (≤month 6), or (B) "LATE" tumor recurrence (>month 6): $IL2^+$, 7/19 EARLY and 12/19 LATE; $IL2^-$, 11/13 EARLY and 2/13 LATE (P=0.018, Fisher's exact test). For the other cytokines and albumin P values were lower: IL6, P=0.023 (n=32); TNF, P=0.026 (n=29); IL1, P=0.034 (n=15); albumin, P=0.099 (n=24). No progn. value of IL2 was observed using a 12-month cut-off for EARLY/LATE recurrence (P=0.208, n=31).

Albumin was stable in urine at all temperatures. IL1β, IL2, and IL6 were found to be stable at 4 and 20°C., but for TNFα 81±25% and 98±0% decrease of the initial concentration was observed after 24h at 4 and 20°C., respectively. After 2h at 37°C. for TNF, IL2, and IL6 100±1%, 33±36%, and 13±21% decrease were observed already, respectively. After 24h at 37°C. 75±28%, 85±8%, 90±7% and 99±1% decrease were observed for IL1β, IL6, IL2, and TNFα, respectively. BCG-induced IFNγ was extremely unstable and could only be detected in immediately dialysed fresh urine samples. The amount of IFNγ was significantly correlated with IL2 (P<0.001, n=16). Immediate removal of leukocytes from fresh urine samples did not significantly influence quantification of IL2 and IL6 (98±21% and 102±25% of initial conc.), whereas for IL1 and TNF this resulted in determination of lower and higher levels, respectively.

Conclusions. Urinary IL2 induction was found to have the highest prognostic significance following 6-week BCG treatment of superficial bladder carcinoma. Absence of IL2 induction was predicting for early recurrence (≤month 6). This may be of value for individual adjustment of the treatment to improve therapy. Considering the instability of cytokines in urine, monitoring of IL2 may be of value provided that samples are collected serially (max.2-hourly) and immediately cooled.

O80

EXPRESSION OF MAGE GENES BY BLADDER CANCER. AN RT-PCR STUDY IN 49 TUMORS.

Jean-Jacques Patard[1], Francis Brasseur[1], Sixtina Gil-Diez[3], François Radvanyi[3], Dominique K. Chopin[2], and Thierry Boon[1].
(1) Institut Ludwig pour la Recherche sur le Cancer, Bruxelles, Belgique
(2) Service d'Urologie, Hôpital Henri Mondor, Créteil, France
(3) Laboratoire de Physiopathologie du Développement, Paris, France

Bladder cancer is one of the rare location where the natural history of the tumor has been favorably altered by local immunotherapy using bacillus Calmette Guérin (BCG). In previous work, we have shown that intravesical BCG is associated with activation of T cell-MHC restricted pathways suggesting the search for tumor rejection antigens. In this study, we investigated the expression of human gene MAGE family in bladder cancer.

Human gene MAGE-1 directs the expression of a tumor-rejection antigen, named MZ2-E, that is recognized by Cytotoxic T Lymphocytes (CTL) (Van der Bruggen, 1991). MAGE-2,-3, and -4 are genes closely related to MAGE-1. All four genes are expressed in many tumors (notably melanomas, non-small cell lung carcinomas, head and neck carcinomas and breast carcinomas), while no expression has been found in normal adult tissues except testis (Brasseur, 1992; Chambost, 1993; De Smet, 1993; Rimoldi, 1993; Weynants, 1993).

We report here the expression of MAGE-1, -2, -3, 4 analyzed by RT-PCR in 49 bladder carcinoma samples. 43% of these tumors were positive for at least one of the four genes. MAGE-1,-2,-3,-4 were expressed in 18.4%, 28.6%, 32.7% and 32.7% of the tumors respectively. A positive correlation was also noted between tumor agressiveness (stage and grade) and MAGE expression: 70% of the invasive tumors (stage T2) were positive for at least one of the four genes, while only 25% of the superficial tumors (stage T0TA) expressed these genes. Similarly the proportion of MAGE positive was 50% for the high grade tumors (G3), and only 25% for the low grade tumors (G1). The expression of MAGE genes by a significant fraction of bladder tumors has a great therapeutic interest: antigens encoded by MAGE genes could constitute useful targets for specific immunotherapy.

O81

IMAGING EFFICACY OF ^{131}IODINE MONOCLONAL ANTIBODY (mAb) G250 IN PATIENTS WITH RENAL CANCER (RC). Neil H. Bander, Chaitanya R. Divgi, Egbert Oosterwijk, Jeannette C. Wakka, Elizabeth C. Richards, Ronald B. Finn, Steven M. Larson, Lloyd J. Old. New York Hospital-Cornell University Medical Center, Ludwig Institute for Cancer Research and Memorial Sloan-Kettering Cancer Center, New York, N.Y. U.S.A.

To date, 34 patients with RC have been studied with ^{131}I-mAb G250. The first group of 16 patients received doses of G250 ranging from 0.2-50.0 mg with a fixed dose of 10 mCi ^{131}I one week before surgery. Tissue was obtained at surgery to confirm pathology and quantitate G250 biodistribution. A second group of 18 patients with metastatic disease has been entered in an ongoing phase I therapy trial and their mAb imaging studies have been reviewed. The G250 dose has been fixed at 10 mg (determined as the optimal dose in group 1) with escalating doses of ^{131}I (to date, doses range from 30 - 75 mCi/m^2). In these patients, no post-mAb biopsies were available; mAb images were compared to conventional staging modalities (CT, MRI, bone scan).

In group 1, 12 of 16 patients had G250$^+$ RC and all 12 patients were successfully imaged in primary as well as bone and soft tissue sites. In 3 patients (25%), sites of disease unsuspected from conventional images were imaged and pathologically proven.

In group 2, where no biopsy data is available, 16 of 18 patients had all known sites of disease successfully imaged. In 3 patients (19%), additional sites were found which could subsequently be confirmed as metastatic disease by conventional imaging studies.

In summary, G250 imaging demonstrated a specificity of 100%. Furthermore, the sensitivity of G250 scans exceeded that of conventional staging studies and could be used in lieu of CT, MRI or bone scans. Given the high level of sensitivity and specificity demonstrated, G250 appears promising as a clinically useful staging modality.

O82

MOLECULAR STAGING OF PROSTATE CANCER WITH THE USE OF AN ENHANCED REVERSE TRANSCRIPTASE-PCR ASSAY Aaron E. Katz, Carl A. Olsson, Anthony Raffo. Cristoforo Cama, Harris Perlman, Mitchell C. Benson, Ralph Buttyan. Columbia University, New York, NY. U.S.A.

Up to 40% of surgically-treated prostate cancer patients are subsequently found to be clinically understaged. The need for a more sensitive staging modality to identify the presence of extraprostatic disease prior to surgery is required. We developed an enhanced reverse transcriptase (RT)-PCR assay utilizing oligonucleotide primers specific for the human prostate specific antigen (PSA) mRNA that identifies PSA-synthesizing cells from reverse transcribed mRNA. This assay was applied to RNAs extracted from the peripheral blood lymphocyte fraction of 48 patients with clinically localized prostate cancer. In addition, bloods from females (n=20), young males (n=20), age-matched control males under treatment for benign prostatic hypertrophy (BPH) (n=25), and men with established, untreated metastatic prostate cancer were tested (n=10).

This enhanced RT-PCR assay can recognize 1 PSA-expressing cell diluted into100,00 lymphocytes. Although none of the specimens from female or non-cancer male patients were positive, 8 of 10 metastatic prostate cancer patients were positive in this assay. Additionally, 11 of 48 (22.9%) patients with clinically localized disease (T1-T2b) were positive from blood specimens obtained prior to surgery. Retrospective pathology from this group of patients identified a correlation between positivity on this assay and the presence of capsular tumor penetration (sensitivity 73%, specificity 89.2%) as well as a strong correlation with the finding of carcinoma at the surgical margin (sensitivity 90%, specificity 94%). Logarithmic regression analysis of the results of the RT-PCR assay indicates its remarkable superiority to DRE, CT scan, endorectal coil MRI, PSA, PSAD, or Gleason score for predicting the true pathological stage of prostate cancer in these surgically-treated patients. This molecular assay provides a highly sensitive and specific means to correctly stage apparent localized prostate cancer prior to surgical committment.

O83

GENETHERAPY TO INTENSIFY CHEMOTHERAPY OF UROLOGIC CANCER

Gerald H. Mickisch and Fritz H. Schroeder
Dept. of Urology, Erasmus University Rotterdam, The Netherlands

Many human tumors such as bladder carcinoma that are initially responsive to chemotherapy eventually fail to respond to treatment. For most drugs, dose escalation that may be required for a cure cannot be achieved because sensitive tissues such as bone marrow limit cytotoxic therapy. Approaches to prevent or circumvent myelosuppression are therefore a high priority of research on dose intensification protocols. One such strategy is to protect bone marrow cells by virtue of MDR1 gene expression encoding for P-glycoprotein (Pgp). In our first set of experiments, we have transplanted bone marrow cells derived from transgenic mice that constitutively express MDR1 to lethally irradiated recipients (n = 36). From 6 weeks to 10 months after the transplant, all animals contained MDR1 DNA in spleen and bone marrow specimens as indicated by Southern blot analysis, and expressed MDR1 RNA in bone marrow samples as detected by slot blot analysis. In addition, these animals were resistant to the myelosuppressive effect of doxorubicin, daunomycin, taxol, vinblastine, vincristine, etoposide and actinomycin D, whereas control animals that were reconstituted with normal bone marrow reacted with a significant decrease in their white blood counts.

In a second set of experiments, we retrovirally transfected a construct consisting of a murine LTR promoter and the human MDR1 gene into CD34-positive bone marow stem cells from rhesus monkeys using the same technique as in the in our institution ongoing clinical ADA gene therapy protocol. Upon transplantation, high and long lasting expression of the human MDR1 gene was observed in recipient monkeys. Based on this preclinical analysis, we have, in close cooperation with the Dutch Gene Therapy Group, submitted clinical protocols to test myeloprotection afforded by MDR1 gene transfer to intensify chemotherapy in otherwise incurable human cancer for institutional and state approval. The Dept. of Urology will investigate metastatic, refractory bladder carcinoma using the MDR-related drugs vinblastine and doxorubicin for myeloablative support of bone marrow transplantation, selection of MDR-positive clones, and dose escalation of chemotherapy in patients. Objectives of these phase I/II studies are feasibility and toxicity of transplanting retrovirally transfected MDR-bone marrow stem cells, and eventually efficacy of this innovative approach.

Poster session: Immunology treatment

P84

CONSTITUTIVE EXPRESSION OF CYTOKINES IN RENAL CELL CARCINOMA

S. Pomer, I. Drehmer, D. Brkovic, R. Waldherr
Department of Urology and Pathology, University of Heidelberg, Germany.

Cytokines play an important role in the induction and maintainance of specific and nonspecific defense mechanisms by immunocompetent mononuclear cells in RCC. However, little information is available on expression of cytokines in RCC in situ. Therefore in a preliminary study we have analyzed the expression of selected cytokines and growth factors in a series of RCC-patients.
Tumor tissues from 20 patients (14 men, 6 women, mean age 63 years, range 39-83) with primary RCC were prepared for polymerase chain reaction (PCR), immunocytochemistry and in situ hybridization. PCR analysis demonstrated transcripts for TNF-Alpha in 90% and for IL-1-Beta in 85% of the tumors examined. Tumor cell cultures available from the primary tumors and analyzed by reverse-transcription/PCR expressed IL-1-Beta and TNF-Alpha as well. TNF-Alpha was negative in papillary tumors. IL-2, IL-2R and IFN-Gamma were consistently negative in tumor cells. A few tumor cells expressed PDGF-BB/AB, PDGF-Beta-R. IL-2R, IFN-Gamma and TNF-Alpha positive mononuclear infiltrating cells were found almost consistently in tumor stroma and occasionally interdispersed between tumor cells. TGF-Beta was expressed by stromal fibroblasts and in addition by some mononuclear infiltrating cells. In 18/20 cases a diffuse or focal expression of mRNA for IL-1-Beta and TNF-Alpha was found in tumor cells by in situ hybridization. Further studies are necessary to elucidate whether local production of IL-1-Beta and TNF-Alpha is related to the metastatic capacity and behaviour of renal cell carcinoma.

P85

EXPRESSION OF HLA-A, B ANTIGENS IN RENAL CELL CARCINOMA ANALYZED BY ISOELECTRIC FOCUSING

Hans-Joachim Luboldt [1], Britta Kubens [2], Herbert Rübben [1], Hans Grosse-Wilde [2]

1 Department of Urology, University of Essen, Medical School, Hufelandstr. 55, 45122 Essen, FRG
2 Institute of Immunolgy, University of Essen, Medical School, Hufelandstr. 55, 45122 Essen, FRG

HLA class I expression is frequently altered in tumors such as colon carcinoma or squamous cell carcinoma compared to the tissue from which they originate. Most research has been done by immunohistochemical methods using a variety of different monoclonal antibodies. In order to identify discrete changes of HLA antigens we analyzed in 30 patients with the definite diagnosis of renal cell carcinoma the HLA-A, B antigen expression on tumor tissue specimens, normal organ tissue and blood lymphocytes by one dimensional isoelectric focusing (1D-IEF) followed by Western-blot. The immunoprecipitates were prepared from tissue extracts by anti-HLA classe I mAb w6/32 bound to immunomagnetic beads (Dynal ®). In all cases the HLA phenotypes of the patients typed by the NIH-Test were confirmed by 1D-IEF. In 3 tumors (10 %) a loss of HLA-A1 expression was observed in comparison to the healthy tissue. A definite decreased expression of an HLA allele was detected in another 2 tumors (HLA-A1; HLA-A28). These findings reflect selective variations of HLA class I expression in renal cell carcinomas which may result in an escape mechanism of tumor cells from immune surveillance by HLA class I restricted CD8 T-lymphocytes.

P86

MHC-DEPENDENT INHIBITION OF T-CELL PROLIFERATION BY RENAL CELL CARCINOMA CELLS (RCC)

G.A. Banat*, S. Pomer, K. Pechold*, D. Kabelitz*

Departements of Immunology * and Urology, University of Heidelberg, Germany.

Although T-lymphocytes are thought to be critical in the control of tumor growth in vivo, it appears that the presence of tumor infiltrating lyphocytes (TIL) suggests an uneffective defense mechanism against tumor cells. Therefore we analyzed the MHC-dependent antigen specific stimulation of T-lymphocytes under the influence of renal cell carcinoma cells (RCC) in vitro. We could show, that T cells activated by an antigen specific stimulus (EBV transformed B cells) were inhibited in their proliferation (75%) by additional cell-cell contact with RCC-cells. Also, these tumor cells appear to have an influence on neighbouring RCC cells, so that they also send out a soluble substance or a signal, leading to an even stronger inhibition of the T-cell proliferation. These powerful effects seem to be dependent on the development of surface receptors on the tumor cells; we could show that the booster phenomenon exists only in the presence of RCC cells, which express the IL-1β receptor on their surface. The inhibition could only be demonstrated if the stimulation of the T-cells was in a MHC-dependent manner, neither with lectin activated T-cells nor with streptococcus enterotoxin A (SEA) activated T cells. These results could give an explanation for the poor stimulation rate of the ex vivo isolated TILs, and sometimes disappointing results of TIL-based immunotherapies.

P87

INDUCTION OF ANTI-TUMOR CYTOTOXICITY IN VITRO IS DEPENDENT ON THE PRESENCE OF TUMOR CELLS AND ANTIGEN-PRESENTING CELLS. Michael Eisenmenger, Georg Steiner, Andrea Schöllhammer, Gero Kramer and Michael Marberger. Departments of Urology and Rheumatology, University of Vienna, Austria.

Although IL-2-driven tumor-infiltrating T-lymphocytes (TITL) are reported to have anti-tumor activities, the clinical usefulness of TITL is limited. Current immunotherapeutic approaches primarily rely on propagation in vitro of effector cells generated in vivo. Antigen presentation and generation of primary immune responses in vitro might increase both the amount and specificity of effector cells. The information available on tissue T-lymphocytes (TTL) derived from normal kidney is limited, as a test system enabling direct comparison of all three T-cell types without in vitro propagation is not available. Therefore a method was established to enrich TTL, TITL and peripheral blood T-lymphocytes (PTL) of the same patients immediately after tumor nephrectomy. T cells treated with antibody plus complement were cultured alone or together with tumor cells (TC) for 8 days in combination with IL-2, syngeneic B cells (as semiprofessional antigen-presenting cell) or OKT3. All T-cell populations contained substantial numbers of $CD3^+$/ $CD8^+$/$CD28^+$ (pre)cytotoxic T cells. Both $CD4^+$ and $CD8^+$ T-cell types expressed significant amounts of activation markers such as HLA-DR, VLA-1, CD69, IL-2Rα and IL-2Rβ, indicating that TTL and TITL are extremely activated. Functional characterization revealed no cytotoxicity against TC of any of the three T-cell types in any combination without stimulation by TC. Addition of TC resulted in 6% lysis by TTL, which was increased by adding IL-2 (10%) or syngeneic B cells (13.5%). Combination of TTL, syngeneic B cells and IL-2 was most effective, resulting in up to 38% TC lysis. Interestingly, TITL and PTL showed only 10% TC lysis regardless of stimuli. In summary, these data suggest that the frequency of T cells capable of killing TC is increased in nonmalignant surrounding renal tissue, probably reflecting a nonspecific influx due to the tumor as demonstrated by higher numbers of $CD3^+$/ $CD45RA^+$/Leu-8^+ T cells in TITL than in TTL suspensions.

P88

DISTRIBUTION OF TUMOR-INFILTRATING LYMPHOCYTES (TIL) IN PROSTATE CANCER (PCa). Gero Kramer, Georg Steiner, Christian Reinwald, Susanne Binder, Michael Marberger. Depts. of Urology and Pathology, University of Vienna, Austria

A method was established to obtain exact information on the location and activation marker expression of individual lymphocyte subsets in situ to arrive at inferences about their function in vivo. Serial sections of tumor areas defined by a pathologist of 20 previously untreated, surgery-derived PCa tissues were analyzed for intraepithelial (i.e.), periepithelial (p.e.) and perivascular (p.v.) $CD3^+$ tumor-infiltrating lymphocytes (TIL). In general, $CD3^+$ TIL ranged from 12 cells/ mm^2, as seen in normal prostate, to 217/mm^2 independently of diagnosis. The distribution pattern showed an increased affinity close to PCa cells, indicated by 22.7% of $CD3^+$ TIL located p.e. within 0.05 mm of the epithelium, whereas the majority (65%) were more distant yet still tended to accumulate near PCa cells. In contrast, i.e. $CD3^+$ TIL only account for 8.3% of all $CD3^+$ TIL, reflecting the inability of T cells to invade closed glandular PCa structures. The small percentage of 3.9% p.v. $CD3^+$ TIL suggests that T-lymphocytic traffic is rather limited. This immunological vacuum, possibly indicating massive immunosuppression by PCa, is confirmed by the finding that the CD4:CD8 ratio, which was 2.3:1 overall, showed a tremendous shift toward $CD8^+$ cells of 1.08:1 in p.e. areas, continued to 0.34:1 in i.e. areas. To further investigate TIL regarding lymphokine receptor expression and to evaluate which cell type would respond to future local lymphokine therapy, we analyzed TIL for IL-2R and IL-7R expression, both receptors for ILs capable of inducing vigorous TIL proliferation in our in vitro cultures. Overall, TIL showed significant reactivity with anti-IL-7R (29.4/mm^2) and anti-IL-2R (15.9/mm^2) antibodies, consistent with a strong ongoing immune response. In contrast, the pattern of p.e. $IL\text{-}7R^+$ (3.68/mm^2) and $IL\text{-}2R^+$ (2.86/mm^2) cells reconfirms the drastic decrease seen in i.e. areas with $IL\text{-}7R^+$ (0.03/ mm^2) and $IL\text{-}2R^+$ (0.13/ mm^2) cells. Interestingly, the ratio of IL-7R:IL-2R-bearing cells showed a drastic shift toward IL-2R expression from 4.2:1 in p.v. to 0.22:1 in i.e. areas.

P89

FILTER-BASED IMMUNOCYTOLOGY IN TRANSITIONAL CELL CARCINOMA.

Klaus Decken, Wolfgang Strich, Renate Enners, Roger Gerke, Thomas Ebert, Bernd J. Schmitz-Dräger

Urologische Klinik, Heinrich-Heine-Universität Düsseldorf, Germany

Because of its high sensitivity the method of immunocytology is highly interesting for the diagnosis of transitional cell carcinoma (TCC), especially in the follow-up of TCC patients. Disadvantages of immunocytology are the technically demanding and time consuming procedure. Additionally, a considerable number of specimens is not evaluabe due to pyuria, hematuria degradation of cells or too low numbers of urothelial cells. To gain a higher percentage of evaluable specimens some investigators chose barbotage specimens in stead of spontaneous urine, thus, loosing the advantage of non-invasiveness.

In our laboratory a filter-based immunocytology method was developed. A prefixation of spontaneous urine samples at the place of collection rid us of the necessity of immediate processing and allowed up to 14 days of storage. In contrast to fibrous filters isoporous membranes allow to perform staining and evaluation on the filter. A pore width of 10 µm was selected to allow a passage of the erythrocytes and granulocytes through the pores while epithelial cells are retained. Thus, the percentage of non-evaluable specimens because of pyuria or hematuria was drastically reduced. An additional decrease of the fraction of specimens with too low cell numbers was achieved as the staining and evaluation on the membranes reduced the cell losses. One-step immunostaining employed a preformed complex of monoclonal antibody Due ABC 3 and phosphatase labelled anti-mouse Ig. By these means, total assay time could be reduced from more than 5 hours using the cytocentrifugation method to less than 30 minutes in our filter-based assay. The number of assay steps was reduced from 39 to 12 steps.

The portion of evaluable specimens in filter-cytology was 89 %, compared to 70 % in conventional immunocytology. The specificity of the method was 86 % (31/36 correct negative) compared to 82 % (30/37) while the sensitivity reached 91 % (50/55 correct positive specimens) compared to 85 % (53/60). Thus, the results appear comparable to conventional immunocytology. Further studies to define the clinical impact of this assay are currently ongoing.

P90

REVERSAL OF P-GLYCOPROTEIN MEDIATED MULTIDRUG RESISTANCE BY KETOCONAZOLE

Michael Siegsmund[1], Ivan Aksentijevich[2], Ira Pastan[3], Michael M. Gottesman[2]
[1]Department of Urology, Klinikum Mannheim, University of Heidelberg, Germany
Laboratories of [2]Cell Biology and [3]Molecular Biology, National Cancer Institute, NIH, Bethesda, MD USA.

The antifungal drug ketoconazole has been shown to potentiate the cytotoxic effectiveness of anthracyclines and etoposide on prostate carcinoma cells, therefore making it potentially useful in the treatment of prostate cancer.

To further investigate this effect, we evaluated the ability of ketoconazole to overcome multidrug resistance mediated by P-glycoprotein and additionally examined a panel of 11 prostate carcinomas for the expression of the *MDR*1 gene using a RNA-PCR assay.

In our *in vitro* system, using well defined KB cell lines, we found, that ketoconazole potentiated the cytotoxicity of vinblastine and doxorubicin on the multidrug resistant KB-V1 cell line, which is several hundred-fold more resistant than the parental cell line KB-3-1. Ketoconazole had little or no effect on the parental KB-3-1 cells. By means of a functional assay with the fluorescent dye rhodamine, which is a substrate of P-glycoprotein, we demonstrated that ketoconazole inhibited the efflux pump function of the protein.

Using a RNA-PCR assay, *MDR*1 expression was detectable in 8 of 11 examined prostate carcinoma samples suggesting a possible role of this gene in the drug resistance of prostate carcinoma.

Our data suggest that ketoconazole might be a promising chemosensitizer and merits further investigation.

P91

Proliferation of the rat Dunning R3327(H) prostate adenocarcinoma can be inhibited by active immunization to GnRH

J.FÜRST, U.PÖTSCHER, E. FIEBIGER, J.FRICK[2], G.Hacker[3] and E.ROVAN
Institute of Zoology, University of Salzburg, Austria, [2]Department of Urology,General Hospital of Salzburg, Austria and [3]Institute of Pathology, General Hospital of Salzburg, Austria.

Observation of an massive immune-response within the prostate of rats immunized to GnRH leads us to conclude, that the rat prostate in vivo contains considerable amounts of GnRH. The physiological role of prostatic GnRH is uncertain, nontheless, there might be a new circuit in the regulation of the prostate which could be utilized for the management of prostate cancer, even when testosterone-independent. In order to examine the effect of active immunization to GnRH on prostate cancer, male Copenhagen/Fischer F1 rat were inocculated subcutaneousely with the Dunning R3327(H) prostate adenocarcinoma and subjected to vaccination. Test animals received 5 intramuscular injections of 5µg/100g bodyweight GnRH-diphteria toxoid conjugate. The anterior pituitary becomes subsequently understimulated and therefore testicular testosterone synthesis blocked. Assesment of tumour-proliferation by means of PCNA analysis revealed, that vaccination to GnRH has a more pronounced inhibitory effect on proliferation than orchiectomy (control 39%, orchiectomy 29%, vaccine 18% proliferating cells). Removal of local GnRH seems therefore to contribute to the observed effects. Histopathological evaluation showed necrosis as well as a marked mastocytopoiesis in tumours of vaccinated rat, indicating at least partially non reversible effects.

P92

NEGATIVE GROWTH CONTROL BY ESTRADIOL ON HORMONE NON RESPONSIVE PC3 HUMAN PROSTATE CANCER CELLS

G. Carruba, R. Farruggio, E. Fecarotta, G. Oliveri, M. Pavone-Macaluso* and L. Castagnetta
Hormone Biochemistry Laboratories and *Institute of Urology, University of Palermo, Italy

We have investigated the effects of estradiol (E_2) on growth of androgen receptor negative, hormone non responsive PC3 human prostate cancer cell line. After 6 days exposure to 0.01 up to 100 nM E_2, a significant inhibition of proliferative activity of PC3 cells was observed. In order to ascertain whether or not E_2 acts via its own receptors, we have assessed presence of estrogen receptors (ER) by multiple experimental approach. Firstly, high affinity ER were detected in the nuclear fraction of PC3 cells using radioligand binding assay. In second place, positive staining for HSP27, which is thought to represent a marker of estrogen sensitivity in target cells, was revealed using D5 monoclonal antibody. Finally, RT-PCR approach showed concurrent expression of normal and variant (lacking exon 4) ER mRNAs. The likelihood that E_2 effects are mediated by TGFß has also been explored. Use of neutralising antibody against $TGF\beta_1$ produced a three fold increase of growth of PC3 cells, which was reversed by concomitant addition of 100 nM E_2. However, we failed to demonstrate any increase of $TGF\beta_1$ mRNA after E_2 administration. Further studies are needed to better define if E_2 effect on PC3 cells implicates a rise in $TGF\beta_1$ at protein level or other TGFß species may be involved.

Studies partly supported by Italian AIRC and CNR (Special Project *Aging*, c. no. 93.435.PF40).

P93

REACTIVATION OF TWO MUTANT ANDROGEN RECEPTORS BY HIGH ANDROGEN CONCENTRATIONS - A MOLECULAR BASIS FOR A TREATMENT OF A MALE PSEUDOHERMAPHRODITISM PATIENT WITH A QUALITATIVE ANDROGEN RECEPTOR DEFECT

Christian Radmayr, Felizia Kaspar, Alfred Hobisch, Georg Bartsch and Helmut Klocker
Dept. of Urology - Innsbruck University - Austria

Male sex differentiation and the development of a normal male phenotype depend on the secretion and action of fetal androgens. Disturbances of normal androgen physiology in genetic and gonadal males result in defective virilization. Affected patients cover the whole spectrum from phenotypic females to patients with partial disorders and phenotypic males with infertility. Androgen receptor mutations have been identified in many patients with androgen insensitivity syndromes but in many cases there is no clear cut relationship between clinical symptoms and receptor abnormality.

We report here the functional characterization of two mutant androgen receptors detected in a newborn boy with a perineoscrotal hypospadia and a 46XY phenotypic girl with complete androgen insensitivity. Both receptors showed a qualitative androgen binding defect caused by single base substitutions in exon E and exon H, respectively, of the receptor gene. Cloning and functional characterization of these two receptors revealed that their function was restored at high androgen concentrations. In the transactivation assay mutant androgen receptor $ARgly^{703}$ which was found in the patient with perineoscrotal hypospadia showed wild-type function when the androgen concentration was raised to about three times the normal concentration. Restoration of the function of the receptor $ARleu^{916}$which was found in the patient with testicular feminization was achieved at androgen concentrations ten to twenty times above normal levels.

These findings offered a possible treatment for the newborn one. Increasing serum androgen levels should activate the mutant receptor of this patient and improve virilization according to the results of the transactivation assay. Therefore, prior to surgical correction of his perineoscrotal hypospadia, three cycles of pregnyl treatment were applied in 6 months intervals. This treatment resulted in good growth and development of his external genitalia.

Our investigations demonstrate 1) that the functional difference between a mutant AR that causes partial and one that causes complete androgen insensitivity may be small, and 2) that the functional characterization of a mutant AR of a patient with incomplete androgen insensitivity offers a treatment modality.

P94

INFLUENCE OF DIFFERENT NATURAL AND SEMI SYNTHETIC SULFATED POLYSACCHARIDES ON THE IN VITRO MOTILITY OF THE HUMAN PROSTATE CANCER CELL LINE TSU-PR1

Klaus Rembrink*, Johannes C. Romijn, Lucheng Cao, Fritz H. Schröder
Dept. of Urology, Erasmus University Rotterdam, The Netherlands

Sulphated polysaccharides, such as pentosan polysulphate (PPS), were previously shown to inhibit human tumor growth in athymic nude mice (Zugmeier et al 1992) and tumor cell motility in vitro (Pienta et al 1992). We have studied the effect of four different sulphated polysaccharides, the semi synthetic compounds G872, G911 (kindly provided by Prof. H. Guan, University of Qingdao, China) and PPS as well as the natural glycosaminoglycan hyaluronic acid (HA), on the motility of the hormone insensitive human prostate cancer cell line TSU-PR1.

Tumor cell motility was determined by use of the Boyden Chamber (BC) assay. The lower BC compartment was either filled with control medium, conditioned medium (CM) derived from the Dunning R3327 subline AT 2.1, or rat fibronectin (FN) in a concentration of 10μg/ml. The filter between both compartments had pores of 8μm in diameter. Cells were seeded in a concentration of 5 x 10^5/ml. The polysaccharides (HA, PPS, G872, G911) were added (200μg/ml) in either the lower, the upper or both compartments. Motility was quantitated by using an image analysing system (IBAS/Zeiss).

The results showed only minor inhibiting effects on TSU-PR1 motility by any of the polysaccharides tested. Identical results were obtained with either FN (see table) or CM as migration stimulating factor. Inhibition was not increased by exposure of the cells to 100 or 200 μg/ml PPS for a period up to 144 hours prior to performance of the assay.

	NO DRUG	UPPER C	LOWER C	BOTH C
PPS	100 %	85 %	84 %	87 %
G872	100 %	98 %	81 %	98 %
G911	100 %	85 %	81 %	76 %
HA	100 %	100 %	96 %	90 %

It is concluded that the inhibitory effect of PPS as reported by others cannot be confirmed in our model. It is possible that the described effects of polysaccharides on tumor cell motility are indirect and caused by interaction with migration-stimulating agents that are not present in our test system.

* Recipient of a research grant of the Deutsche Krebshilfe

P95

DIFFERENTIAL RESPONSIVENESS OF SUBLINES OF THE HUMAN PROSTATIC CARCINOMA CELL LINE LNCAP UPON ANDROGENS AND 1,25-DIHYDROXYVITAMIN D_3

Gert Jan van Steenbrugge[1], Jos Veldscholte[2], Monique H.A. Oomen[1], Gert Jan C.M. van den Bemd[3], Johannes P.T.M. van Leeuwen[3], Eppo Mulder[2] and Fritz H. Schröder[1], Depts. of Urology[1], Endocrinology & Reproduction[2] and Internal Medicine III[3], Erasmus University Rotterdam, The Netherlands.

1,25-dihydroxyvitamin D_3 (VD_3) is the biological most active form of vitamin D_3, and has been shown to induce differentiation and to inhibit proliferation of many types of cancer cells. In the present study, the effect of combined treatment with VD_3 and the non-metabolizable androgen, R1881, was determined on growth and differentiation (production of prostate-specific antigen (PSA)) of three sublines of the human prostatic carcinoma cell line LNCaP, the hormone-sensitive LNCaP-FGC (FGC) and the hormone-independent LNCaP-r (R) and LNO. Cell proliferation was assessed by measurement of the content of total DNA and the concentration of PSA in the culture media was measured with an automated enzyme immuno-assay (IMx-MEIA).

Ligand binding experiments demonstrated specific VD_3 receptors (VDRs) in all three LNCaP sublines with binding capacities of 14, <10 and 34 fmol/mg protein in the FGC, R and LNO lines, respectively. In addition, one major 4.6 kb VDR messenger RNA transcript was identified in all three lines. VD_3, which did not bind to the androgen receptor (AR), inhibited androgen-induced proliferation of the FGC line at concentrations between 1 and 100 nM, but did not change the rate of proliferation when given alone. Exposure of FGC cells to R1881 in combination with 100 nM VD_3 resulted in a (R1881) dose-dependent stimulation of PSA secretion. In contrast to the FGC line, the R and LNO sublines, when cultured in androgen-depleted medium showed a relatively high basal growth, which was further stimulated by low androgen concentrations (10^{-11} and 10^{-12} M). In spite of the proliferative status of the R cells under these conditions, VD_3 at high concentration of 100nM slightly stimulated, rather than inhibited the growth, whereas at higher concentrations of R1881 (10^{-7} - 10^{-10} M) VD_3 dose-dependently inhibited growth of the R cells. In the presence of all concentrations of R1881 tested (10^{-7} - 10^{-12} M) growth of the LNO cells was almost completely suppressed when treated with 100 nM VD_3, a concentration which did not affect the relatively high basal growth rate of these cells. In conclusion, growth of the FGC line is inhibited by VD_3 when the cells are in a proliferative status, whereas in the R and LNO sublines a strong antiproliferative effect was only seen in the presence of androgens. The underlying mechanism is not yet clear, but does not involve androgen receptor binding. Study supported by the Dutch Cancer Society (grant IKR 90-13).

P96

ANTI-TUMORAL EFFECTS OF LIAROZOLE ON THE GROWTH OF TRANSPLANTABLE HUMAN DU-145 PROSTATIC CARCINOMA IN ATHYMIC MICE.

G. Smets, R. Van Ginckel, B. Xhonneux, J. Van heusden, B. Janssens, M. Callens, M. Borgers, R. De Coster.
Janssen Research Foundation, Beerse, Belgium.

The anti-tumoral activity of liarozole has been studied in the androgen-independent human DU-145 PCa cell line, subcutaneously implanted in athymic mice. Liarozole is an imidazole-containing compound that inhibits the cytochrome P-450 dependent catabolism of all-trans-retinoic acid (RA). Oral administration b.i.d. of liarozole at dose levels of 20, 10, 5 mg/kg reduced median tumor volume by 26%, 51% and 23% respectively. Lower doses were inactive. RA administration b.i.d. at dose levels of 2.5, 1.25, 0.63, 0.31 mg/kg reduced median tumor volume by 27%, 35%, 25% and 6% as compared with tumor growth in controls. The tumors from castrated animals were statistically significantly larger than those from the control group. Histologically, the solid DU-145 tumors were typical carcinoma as defined immunocytochemically by the presence of cytokeratins. To investigate the influence of liarozole and RA on cell-cell adhesion, the expression of E-cadherin was monitored by immunocytochemistry and Western blotting. In control tumors, the E-cadherin expression was heterogeneous with large cell areas negative for E-cadherin alternating with fields positive for E-cadherin. Treatment of the tumors by liarozole or RA resulted in the same picture of E-cadherin expression with cell fields positively or negatively stained for E-cadherin but with the positive cells much more intensely stained. In both treated and untreated tumors, E-cadherin was always expressed at the cell membrane. Western blotting of detergent (Triton X-100, SDS, deoxycholic acid salt) extracted proteins, revealed that treatment induced a shift of proteins with molecular weights ranging between 40 and 120 kDa. In view of the prognostic importance of E-cadherin, densitometry of E-cadherin immunoblots was performed. A decrease of expression was noted after treatment with liarozole at 10 mg/kg b.i.d. The intensified immunostaining together with the apparent contradiction of a decrease of tumor volume and decrease of E-cadherin expression might reflect treatment-induced functional shifts in the molecule. In an attempt to understand the molecular histology of this tumor model, further investigation by e.g. immunoprecipitation and localisation at the ultrastructural level, is necessary.

P97

ENHANCED INHIBITION OF HUMAN RENAL CELL CARCINOMA IN NUDE MICE BY GEMCITABINE WITH INTERFERON-α.

D. Rohde, M. Goertz, R. Sikora, G. Jakse

Dept. of Urology, Aachen, Germany

2',2'-Difluorodeoxycytidine (dFdC; LY188011; Gemcitabine) has been shown to inhibit tumor cells in various experimental models by blocking DNA-synthesis. Following previous in-vitro data, where we observed a nearly equal cytotoxicity of gemcitabine (GEM) and vinblastin (VBL) against renal cell carcinoma (rcc) cells at peak-plasma level, an in-vivo study was employed to elaborate the potency of GEM against xenografts of human rcc alone or in combination with interferon-α (IFN-α).
Methods: Cells of human rcc (SN12C, ACHN) have been inoculated subcutanously (s.c.) in 3-4 week old nude mice, and afterwards by tail vein injection at first tumor appearance. Therapy was initiated at a s.c. tumorvolume of 100 mm^3 and lasted 16 weeks either with GEM and rhu-IFN-α2a (Roferon A), VBL and IFN-α, or GEM alone.
Results: S.c. tumors derived from ACHN cells were inhibited by GEM+/-IFN-α in a more sufficient way than by VBL+IFN-α. S.c. xenografts of SN12C cells didn't respond at all while GEM+IFN-α was effective to reduce the incidence of pulmonary metastasis. All animals that underwent a remission recieved GEM+/-IFN-α exclusively. GEM+IFN-α enhanced survival and health status most.
In conclusion GEM has overall been shown to be superior to VBL+IFN-α against metastatic rcc. In combination with IFN-α, GEM recommends itself as an most interesting chemotherapeutic drug for patients with advanced renal cell carcinoma, thus clinical studies should be initiated.

P98

Treatment with tumor necrosis factor α and interferon α of a human kidney cancer xenograft in nude mice: Evidence for an anticachectic effect of interferon

In the present study the in vivo effect of tumor necrosis factor α (TNFα) or/and interferon α (IFNα) on the macroscopic tumor growth (external caliper measurements of tumor size) and on the cell proliferation (in vivo ^{3}H-thymidine labelling index, LI, and mitotic index, MI) of a human RCC xenograft line in nude mice has been investigated. Neither of these substances alone nor their combination was effective in changing statistically significantly the time course of the tumor sizes and the growth patterns of the treated tumors as compared to the untreated controls. Also the cell kinetic parameters were only marginally affected by these treatments, whereby TNFα alone proved to be more effective than IFN α alone. However, compared to the effect of TNFα alone the combination with IFNα leads to some amelioration of the cell kinetic perturbations and also to an appreciable shift in the growth patterns of the tumors from distinct Gompertzian (under TNFα alone) to near exponential (under the combination treatment; $p<0.05$). As a consequence the tumors grow more slowly under the combined treatment during the observation time, and on the other hand, their growth does not decelerate as much as under TNFα alone. Actually, if tumor growth should continue in the same way, the extrapolation of the present data predicts smaller and greater tumors than the control tumors in the TNFα and in the combination treatment groups respectively. Notably in the combination the effect of the IFN α seems to predominate. This is also seen in the effect of this combination on the cachexia of these tumor-bearing animals: Either alone or in combination with TNF α, IFN α partially protects the animals from tumor-growth associated weight loss. Although the direct antineoplastic in vivo effect of the present cytokine combination against this human RCC xenograft line is rather limited, the potential antagonizing effect of IFN α on the development of cachexia should be further explored.

P99

SPECIFIC HUMORAL RESPONSES DIRECTED AGAINST HUMAN RENAL CELL CARCINOMA INDUCED BY VACCINATION WITH INTERNAL IMAGE MONOCLONAL ANTI-IDIOTYPE ANTIBODIES.

Hirotsugu Uemura[1,2], Eigoro Okajima[2], Wilhelmus P. Peelen[1], Frans M.J.Debruyne[1] and Egbert Oosterwijk, [1]Dept. of Urology, University Hospital Nijmegen, Nijmegen, the Netherlands and [2]Dept. of Urology, Nara Medical University, Nara, Japan

Anti-idiotype antibodies (Ab2) are defined as antibodies directed against determinants located in immunoglobulin molecules. Ab2s directed against the idiotopes of anti-tumor antibodies antigenically mimicking the nominal antigen (internal image Ab2) may function as tumor vaccine. We have isolated six murine monoclonal Ab2s directed against mAbG250, a murine monoclonal antibody (Ab1) which specifically reacts with a large number of human renal cell carcinoma. The six Ab2 (NUH31, 51, 71, 82, 91;IgG1, NUH44;IgG2a) demonstrate specificity for the Ab1 combining-site idiotope, and recognize partly overlapping epitopes, as revealed by cross-blocking RIA. In the current study, we characterized their ability to induce tumor antigen specific humoral responses in mice and rabbits.

Serum from mice and rabbits immunized with Ab2s cross-linked to keyhole limpets hemocyanin showed reactivity with antigen-positive ($G250^+$) cell lysates but not with antigen-negative ($G250^-$) cell lysates, indicating the induction of specific anti-tumor-associated antigen (anti-TAA) antibodies. To investigate whether the anti-TAA specific antibodies mimicked mAbG250 (so-called Ab1'), competitive RIA were performed. These showed that all NUH induced sera inhibited ^{125}I-mAbG250 binding to $G250^+$ cells, indicating a specific humoral anti-TAA response inductions by the immunization with Ab2.

To investigate whether subtle differences between the Ab1's existed, fine specificity studies of these immune sera were carried out. Competitive RIA of Ab3 sera and ^{125}I-Ab1 with individual Ab2 showed that the six Ab2 could be divided into four mutually exclusive groups. Competition of NUH71 and NUH91 induced Ab3 sera with Ab1 was only effective when the corresponding Ab2 was presented as binding moiety, suggesting that they bear unique epitopes. The remaining NUH-Ab3 sera could be divided into two groups: NUH31- and 51-Ab3 sera, and NUH44- and 82-Ab3 sera. NUH31-Ab3 sera bound equally well to NUH51 and NUH44-Ab3 sera to NUH82, and vice versa. Taken together, these results indicate that all Ab2s induce RCC-specific Ab1', but that subtle differences between the six Ab2s exist. Most likely these six Ab2 recognize four slightly different idiotopes in the mAbG250 binding pocket. Our findings demonstrate that all six Ab2s can induce specific humoral responses against human RCC and suggest that such Ab2s might be useful as tumor vaccines in patients with RCC, since they seem to function as TAA surrogate.

P100

ANTIGEN SPECIFIC CELLULAR RESPONSE INDUCED BY VACCINATION WITH ANTI-IDIOTYPE ANTIBODIES BEARING THE INTERNAL IMAGE OF THE HUMAN RENAL CELL CARCINOMA-ASSOCIATED ANTIGEN G250.

Hirotsugu Uemura[1,2], Eigoro Okajima[2], Wilhelmus P. Peelen[1], Frans M. J. Debruyne[1], and Egbert Oosterwijk[1]. [1]Dept. of Urology, University Hospital Nijmegen, Nijmegen, The Netherlands and [2]Dept. of Urology, Nara Medical University, Nara, Japan.

We have previously isolated and characterized six different murine monoclonal anti-idiotype antibodies (Ab2s) directed against monoclonal antibody G250 (mAbG250) which reacts specifically with a large proportion of human renal cell carcinoma (RCC). These six Ab2s (NUH31, 51, 71, 82, 91;IgG1, NUH44;IgG2a) inhibit binding of mAbG250 to RCC; i.e., compete with the nominal G250 antigen, indicating that they bear the internal image of the RCC-associated antigen. Cross-blocking RIA showed that the epitopes recognized by the Ab2s are partly overlapping. In the current study, we investigated whether immunization with Ab2 could induce antigen specific cell mediated immunity.

Eight to 10-week-old female BALB/c mice were immunized with Ab2 cross-linked to keyhole-limpets-hemocyanin and subsequently boosted twice at 2 week intervals. Eight days after the last booster, mice were challenged with 2.5×10^5 antigen-positive ($G250^+$) and/or -negative ($G250^-$) cells in the ear pinna. Ear thickness were measured before, 2, 6, 24 and 48 hrs after tumor challenge to assess delayed type hypersensitivity reactions (DTH). For all six Ab2, challenge with $G250^+$ cells resulted in a profound DTH reaction whereas no DTH reaction was observed with $G250^-$ cells. The G250-specific DTH reaction demonstrated a biphasic pattern with an early (2 hr) and late (classical) reaction (24 and 48 hr). Both early and late reactions were anti-idiotype mAb specific as immunization with irrelevant IgG did not result in any DTH response ($p<0.05$). The differences between challenge with $G250^+$ and $G250^-$ cells was highly significant ($p<0.01$). Six hours after tumor challenge no reaction was observed in either group.

To confirm the cellular nature of the DTH responses, lymphocytes or serum harvested from mice sensitized with Ab2 were transferred to naive mice which were subsequently challenged with $G250^+$ and $G250^-$ cells. Tumor challenge of cellular transferred mice resulted in similar DTH responses as compared to sensitized mice, whereas serum transfer only showed a reaction 2 hours after tumor challenge, confirming that the DTH reactions were cell mediated. The 2 hour response of the serum transferred mice could be explained by the presence of tumor-specific T cell products. Our findings demonstrate that all six Ab2s can induce specific cellular responses directed against human RCC and suggest that they might be useful as RCC-TAA surrogates in patients with RCC.

P101

ANTI-TUMOR EFFECTS OF VACCINATION WITH ANTI-IDIOTYPE ANTIBODIES IN MICE WITH HUMAN RENAL CELL CARCINOMA.

Hirotsugu Uemura[1,2], Eigoro Okajima[2], Antonius JMC Beniers[3], Frans MJ Debruyne[1] and Egbert Oosterwijk[1]. [1]Dept. of Urology, University Hospital Nijmegen, Nijmegen, The Netherlands, [2]Dept. of Urology, Nara Medical University, Nara, Japan and [3]Dept. of Urology, University Hospital Aachen, Aachen, Germany.

In previous studies we have shown that six different internal image anti-idiotype antibodies (Ab2) raised against the binding site of MAbG250 (Ab1) which specifically reacts with high percentage of human renal cell carcinoma (RCC), induce antigen specific humoral and cellular responses in mice and rabbits. Based on the induction of Ab3 in mice and rabbits, the Ab2s can be divided into four mutually exclusive groups: I) NUH-31 and -51, II) NUH3-44 and 82, III) NUH-71, IV) NUH-91. To investigate the anti-tumor effects of Ab2 treatment, mice were challenged with antigen-positive ($G250^+$) tumor cells and treated with Ab2-induced Ab3 sera.

BALB/c mice were immunized and subsequently boosted 3 times with Ab2s (NUH31, 71, 82, 91) or MOPC21 (control) cross-linked to keyhole limpets hemocyanin. Ab3 sera were harvested one week after the last booster and used for treatments. Nu/nu BALB/c mice carrying small established NU12 human RCC xenografts ($G250^+$, 20 mm^3) or receiving a s.c. injection of 2×10^5 SK-RC-52 ($G250^+$) RCC cells were treated by i.p. injection of 0.2 ml Ab3 sera. Mice were treated 3 times a week for 4-6 weeks. Control mice were treated with sera from MOPC21 immunized mice (Ab3-MOPC) or normal mouse sera. The tumor-take rates and tumor growth were determined every week.

Treatment of SK-RC-52 injected mice with any of the Ab3 sera from Ab2 immunized mice resulted in significant tumor growth inhibition ($p<0.01$) and lower tumor take as compared to control groups. These results indicate that the mice were protected against tumor cell challenge. In mice with established RCC xenografts, significant reduction in tumor growth was achieved. In some NUH-Ab3 sera treated mice, complete tumor regression was observed 6-8 weeks after initiation of treatment. In contrast, treatment with control sera resulted in 100 % tumor-take and ineffective tumor cell doubling time. The therapeutic efficacy of Ab3 sera was higher than MAbG250 treatment. Histological examination revealed massive infiltration of NUH-Ab3 treated tumors, whereas no infiltrates were observed in control tumors.

These findings demonstrate that immunization with Ab2s elicits powerful anti-tumor effects in immune competent animals. A clinical trial is necessary to investigate whether immunization of RCC patients with Ab2 will result in similar specific anti-RCC responses.

P102

SIGNIFICANCE OF TOPOISOMERASE-II ACTIVITY FOR THE MULTI DRUG RESISTANCE PHENOTYPE OF RENAL CELL CARCINOMA.

Scheltema, J.M.W., Steenbrugge G.J. van, Romijn, J.C., Schröder, F.H. and Mickisch, G.H., Erasmus University Rotterdam, The Netherlands.

The simultaneous resistance to the cytotoxic effects of several classes of antineoplastic drugs, multidrug resistance (MDR), is a common feature of Renal Cell Carcinomas (RCCs). Knowledge of the mechanisms involved will facilitate the design of rational approaches for overcoming MDR in RCC. The expression in RCC of the human MDR1 gene and its product, P-glycoprotein (Pgp), has already been identified as one of these mechanisms. The possibility to inhibit Pgp, which eliminates intracellular cytotoxic drugs, has recently led to clinical investigations. A second mechanism of drug resistance in RCC could be reduced activity of DNA-topoisomerase-II (Topo-II), which is a target of several antineoplastic drugs. Reduction of Topo-II activity results in decreased drug-induced DNA cleaving. In our laboratory, we have established 3 non-chemoselected human RCC cell lines (RC2,RC21,RC43) in a murine xenograft model, and propagated them in long-term tissue culture. We analyzed chemoresistance patterns of the lines against vinblastine (VBL), doxorubicin (DOX), and etoposide (ETO) with a microculture tetrazolium test (MTT). Resistance to VBL is known to be associated with the MDR1 phenotype, whereas DOX and ETO are considered to be Topo-II-active drugs. MDR1 expression was analyzed by Northern blotting using the MDR5A probe, and by immunohistochemistry using antibodies JSB1 (anti MDR1). IC50 values for VBL were 0.01 μg/ml (RC21), 3.3 μg/ml (RC2), and not demonstrable for RC43 within a reasonable dose range ($>10\mu$g/ml). These results correlated with MDR1 expression in that high expression corresponded to strong resistance, and vice versa. Sensitivity testing to ETO revealed high resistance for RC2, but not for RC43 (IC50 3μg/ml) and for RC21 (IC50 1μg/ml), thus confirming that resistance to ETO involves a mechanism different from MDR1. RC2 revealed a decreased topo-II content using antibody anti-top2 as compared to RC43 which is thought to be typical for the topo-II type of MDR. These results appear to indicate that altered topo-II activity is functional, at least in part, for the MDR-phenotype in cultured RCC.

P103

CYCLOSPORIN A AGAINST BLADDER CARCINOMA: RISK OR BENEFIT?

D. Rohde[1], S. Biesterfeld[2], B. Totten[1], R. Sikora[1] and G. Jakse[1]
[1] Dept. of Urology, RWTH Aachen, Germany
[2] Dept. of Pathology, RWTH Aachen, Germany

The aim of this study was to evaluate the cytotoxicity of cyclosporin A (CyA) against urothelial carcinoma in-vitro and after intravesical (i.ves.) instillation in-vivo.
Using a colorimetric cytotoxicity assay we found a growth inhibition of all human cell lines (HT1376, J82, 647V, RT112, RT112DDP, RT112MMC) by CyA. In addition a preincubation of the cells with CyA before exposition to platinum-derivatives enhanced the cytotoxic potency, leading to a reduction of EC_{50}-values by halve (8 μM CyA). In contrast tumor burden increased in BBN-fed animals after nine i.ves. instillations (12,8 μg CyA/w) and tumors could be primarily induced even in normal rats (2/6).
In conclusion CyA provides strong cytotoxicity against bladder cancer cells in-vitro but not after topical instillations in-vivo. This diminished efficacy may be due to the immuno-suppresive component of the drug. Nevertheless, the synergism found with platinum-derivatives and its general ability to sensitize cells against drugs related to P170 (e.g. doxorubicin or etoposide) may recommend CyA as an interesting substance to support conventional polychemotherapeutic protocols (M-VAC, M-VEC). Altough current clinical trials do not report a propagation of tumors using CyA in combination with chemotherapy, the use of non-immunosuppressive and less nephrotoxic cyclosporin-analogues like PSC 833 might be a better choice.

P104

BCG SORPTION TO THE BLADDER WALL: A (MATHEMATICAL) APPROACH CONSIDERING THE PHYSICOCHEMICAL PROPERTIES OF THE BLADDER WALL AND BACTERIAL SURFACE AND EFFECTS OF EXOGENOUS POLYELECTROLYTES. Denis H. Schamhart, Liesbeth C. de Boer and Karl-Heinz Kurth, Dept. Urology, Univ. Amsterdam, Amsterdam, The Netherlands.

BCG sorption to the lumenal surface of the bladder wall is probably a crucial factor in the efficacy of BCG treatment of superficial bladder cancer. According to the theory of lyophobic colloid stability, bacterial sorption can be described as the net interaction force between the surfaces of both the bladder wall and the bacterium, being a balance of attraction and repulsion forces. Assuming a general observed bacterial surface potential of ≈ -15 mV, computer modeling suggests that under conditions existing in the uninjured bladder, the probability of irreversible sorption of BCG to the bladder wall is very low, due to the high repulsion barrier. These theoretical considerations are in agreement with experimental, electronmicroscopic observations [Teppema et al, Urol Res 20:219, 1992].
A study was initiated to improve the attachment of BCG to the bladder wall by adding polyelectrolytes (PEs). Physicochemical considerations predict a profound effect of PEs on the sorption of bacteria to surfaces, but the effect should depend severely on the intrinsic properties of the surfaces involved, and the concentration of PE. A narrow range of concentrations determines whether repulsion (by steric interaction) or bridging between the bacterium and bladder wall occurs. Binding of PPS, a highly sulfated PE to the bladder wall of human, guinea pig and rat was found to be 4 ± 2 ng, 76 ± 1 µg, and 40 ± 3 µg per bladder, respectively. Furthermore, a significant (p=0.002) binding of PPS to several BCG substrains was observed: 3.4 ± 0.3 µg/mg dry weight. Vigorous washing indicated a strong binding of PPS to the bladder and BCG. The binding of PPS to both the bladder wall and BCG bacilli suggests a PPS associated enhancement of BCG sorption to the bladder at low PPS concentrations ("bridging"), in contrast to high concentrations ("steric interaction"). Improvement by PPS of BCG-induced immune reaction (inflammatory reaction in the bladder wall [IR]; weight (g) [Wi] and number of lymphocytes [Li x 10^7] of iliac lymph nodes) was studied in the guinea pig. After 6 intravesical PPS (10, 0.1 mg/ml for 0.5 h) instillations prior to BCG (10^7 CFU) the various parameters after PPS instillation prior to BCG were compared with BCG alone and analyzed by the Wilcoxon two-sample test for the unpaired case. The means of IR, Wi and Li were 2.1 (p=0.24), 0.21 (p=0.13) and 4.3 (p=0.15) at PPS (10 mg/ml) + BCG, and 2.4 (p=0.05*), 0.28 (p=0.04*) and 6.6 (p=0.04*) at PPS (0.1 mg/ml) + BCG compared to 1.5, 0.06, 1.7 at BCG alone. PPS alone had no effect.
In conclusion, the results suggest that low PPS concentrations (0.1 mg/ml) enhance BCG-induced immune reactions in the guinea pig probably as a result of an increased BCG sorption to the bladder wall. It can be speculated that PPS may increase the efficacy of BCG provided conditions of bridging are met: low PPS concentration or coverage of only one of the surfaces (bladder wall or BCG).

P105

INTERNALIZATION OF BCG BY BLADDER TUMOR CELL LINES IS CORRELATED WITH EXPRESSION OF FIBRONECTIN - A FLOW CYTOMETRIC METHOD

E.C. De Boer, D.H.J. Schamhart, T.M. De Reijke, K.H. Kurth.
Dept. Urology, Univ. Amsterdam, Amsterdam, The Netherlands.

Introduction. The glycoprotein fibronectin (FN), present in basement membranes and variably expressed on epithelial cell surfaces, has been suggested to play a role in retention of BCG within the bladder and subsequent immune activation and antitumor activity in intravesical BCG immunotherapy of superficial bladder cancer. Furthermore attachment and internalization of BCG by the bladder cancer cell line T-24 have been reported to be dependent on FN.
Methods. In this study the cell lines TCC-SUP, J-82, BT-B, SBC-7, SBC-2, RT-4, and T-24, derived from human bladder carcinomas with different grade of differentiation, were investigated on FN expression and ingestion of BCG (Connaught) by FACS analysis. Ingestion was quantified by in vitro incubation of cell monolayers with (chemically) FITC-labelled BCG and subsequent labelling with rabbit anti-BCG antibodies (Ab) and goat anti-rabbit PE-conjugated second Ab. The percentage of BCG-ingesting cells was expressed as the % of cells with intra+extracellular BCG (FITC-stained) minus the % of cells with extracellular BCG only (PE-stained).
Results. The amount of FN expression of SBC-2, SBC-7, BT-B, RT-4, T-24, J-82 and TCC-SUP were 1, 1, 3, 5, 29, 33, and 58 (median fluorescence intensity), respectively. The percentages of BCG-ingesting cells were 0, 2, 0, 0, 68, 51, and 34 %, respectively, which was significantly correlated ($R=0.95$, $P<0.01$) with FN expression. Internalization did not occur at 4°C. and was inhibited by Cytochalasin-B. Ab directed to BCG did inhibit ingestion but Ab to FN did not.
Conclusions. FN was variably expressed on bladder tumor cell lines and was significantly correlated with internalization of BCG. However, a causal relation could not be assessed by inhibition with Ab to FN. The phenomenon may play a role in the antitumor effect of BCG against bladder cancer.

P106

INTERLEUKIN-8 (IL-8), INTERLEUKIN-6 (IL-6), TUMOR NECROSIS FACTOR-α (TNF-α), SOLUBLE TNF RECEPTORS (sTNFR) 75 kDa AND 55 kDa AFTER BCG THERAPY OF UROTHELIAL CANCER (TCC): CYTOKINE KINETICS OVER 6 INSTILLATIONS

[1]George N. Thalmann, [2]Beatrice Dewald, [3]Harald Gallati, [2]Marco Baggiolini, [1]Urs E. Studer, [1]Department of Urology Inselspital, [2]Theodor Kocher Institute Berne, [3]Hoffmann-La Roche Basle, Switzerland.

The role of different cytokine mediators in BCG therapy of TCC is not well understood. The majority of cells in the urine after BCG are polymorphnuclear leukocytes (PMN). IL-8 is a potent chemoattractant for PMN. IL-8 is biologically stable in the urine. IL-6 is an acute phase protein. TNF-α plays a central role in the cytokine cascade. Soluble receptors to TNF-α have been reported, however, their function is not clear. After instillation of 120 mg or after perfusion of the upper urinary tract with 360 mg of BCG Immun Pasteur F we determined the expression and time course of IL-8, IL-6, TNF-α and its soluble receptors TNFR 75 kDa and 55 kDa during BCG therapy of TCC.

Urine from 8 patients was collected in hourly intervals during the first 6 h after instillation/perfusion of BCG, thereafter in 6 h intervals up to 24 h. Serum samples were obtained in 4 patients during the first 6 h. Cytokine levels were determined by a solid phase double-ligand ELISA/ELIBA method.

IL-8 titers increased to a maximum of 1100 ng ± 1250 (S.D.) after the first instillation to 2965 ng ± 1560 after the 5th. Over 24 h titers were highest during the first 6 h period (10184 ng ± 4682). IL-8 was measurable in the urine up to 90 h after BCG. IL-6 attained a maximum 4 h after instillations 1 - 5 (62 ng to 125 ng ± 85) and 2 h after instillation 6 (140 ng ± 57). Thereafter it decreased to a steady level for up to 42 hours. TNF-α reaches a maximum value 1 h after instillation with a maximum after the 3rd. sTNFR 75 kDa and 55 kDa increase 1 - 4 h after instillation/perfusion. sTNFR 75 kDa values vary from 567 ng ± 315 to 1567 ng ± 864, sTNFR 55 kDa from 490 ng ± 398 to 1222 ng ± 1159. In the serum IL-6 titers rise 12-fold and TNF-α titers 2-fold.

In conclusion, cytokines play a mediating and modulating role in BCG antitumor activity in a para- and autocrine manner. The kinetics and levels of cytokines such as IL-8 may prove to be prognostic factors of responsiveness to BCG therapy.

P107

LOCAL IMMUNOLOGICAL RESPONSE FOLLOWING INTRA-VESICAL BACILLUS CALMETTE GUERIN (BCG) PROPHYLAXIS FOR BLADDER CANCER.

Jean-Jacques Patard*, Zivko Popov*, Béatrice Muscatelli-Groux* Sixtina Gil-Diez*, Yves Fradet, Claude Abbou* and Dominique K. Chopin***, *Hôpital H. Mondor, Créteil, France, **Université Laval, Québec, Canada.

BCG has proven effective against recurrence and progression in the prophylactic treatment of superficial transitional cell carcinoma (TCC) of the bladder, however the optimal protocol has not been defined and mechanisms of action remains unclear. This study was focused on cytokine production and antigen presentation to effector cells after BCG instillations. 64 urines were tested for IFN γ production after BCG treatment in 11 patients, using a modified commercial ELISA assay (Holland Biotechnology). Urines were collected before and 2h, 4h, 6h, 8h, 10h, 12h and 24h after BCG instillations. An immunohistological study was also conducted using a two step phosphatase alcaline technique to explore the expression of tumor associated antigens (TAA) (E7, 19A211, T138), molecules of the major histocompatibility complex (MHC) and lymphocyte-subset infiltrates (CD3, CD4, CD8, CD45) on bladder biopsies before and 3 weeks after completion of treatment in nine patients. IFN γ could be found only 4h, 6h and 8h after instillation with a maximum of detection at 6 h. Urinary levels ranged from 2,9 to 35 UI/ml in 10 patients. During a six week course of BCG IFN γ could be detected at minimum levels during the two first instillations and progressively increased from the third instillation. TAA and MHC CL II antigens who were not or faintly expressed on normal urothelial cells before treatment showed a strong expression in five patients after treatment. A local recruitment of immunocompetent cells was also established. These data suggest that local immune response after endovesical BCG could be quantified by simple ELISA tests. These tests could be useful to define objectives criteria for treatment rationalisation (dose and duration) and to correlate immune response to antitumoral activity. There are some evidences that mechanisms involved in antigen presentation are increased after BCG instillations suggesting that a T cell-MHC restricted pathway might be involved in the antitumor response. This study supports the search for tumor rejection antigens in bladder cancer.

P108

A NEW MODALITY IN THE TREATMENT OF CANCER USING A HIGH TEMPERATURE RADIOFREQUENCY (RF) HEATING DEVICE

A.R. Zlotta[1], R. Kiss[2], C.C. Schulman[1], Depts. Urology[1] and Histology[2], University Clinics of Brussels, Erasme Hospital, Brussels, Belgium

Neoplastic cells are more sensitive to heat than normal cells. Most clinical hyperthermia protocols deal with 43-45°C temperatures delivered for 30-60 minutes. RF has been recently used in urology to selectively ablate prostatic tissue through a transurethral catheter (TUNA) outfitted with deploying needles delivering up to 15 watts for 5 minutes and obtaining over 100°C. This concept has been applied in the present work with respect to the experimental MXT mouse mammary adenocarcinoma model, which is subcutaneously transplanted onto the flank of B6D2F1 mice. These MXT-bearing tumor mice were treated with a special RF catheter with single deploying needle. Animals were sacrificed at days 1, 5 and 15 post-treatment, i.e. at days 18, 22, and 32 post-transplantation. Central temperatures reached up to 110°C. Histological examination revealed no apparent changes at 1 days post-treatment, while extensive necrosis affected the tumors at days 5 and 15 post-RF treatment. Sharp delineation with adjacent normal structures was noted. A second study compared RF heating with chemotherapy and combination of both modalities. Four groups of 15 mice with same age tumors were analyzed. The four groups included one control and three treatments, i.e. 1) 9 intraperitoneal chemotherapeutic injections of adriamycin (1 mg/kg), etoposide (10 mg/kg) and cyclophosphamide (40 mg/kg), 2) RF heating, and 3) combination of RF + chemotherapy, conditions. Mean tumor area values (15 days post-treatment) were 497 +/- 55 mm^2 for control group, 340 +/- 115 mm^2 for FR group ($P < 0.05$), 333 +/- 83 mm^2 for chemotherapy group ($P < 0.05$) and 157 +/- 63 mm^2 for combination of chemotherapy + RF ($P < 0.001$). This study demonstrates the efficacy of tumor destruction by RF heating, safety to adjacent organs and increase in the effectiveness of chemotherapeutic effects by RF heating. A new field of future investigations in cancer treatment is open.

List of contributors*

* Numbers after the author's names are **abstract numbers**