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Urological Research

Abstracts

IX Congress of the European Society for Urological Oncology and Endocrinology (E.S.U.O.E.), October 29–31, 1992, Trento, Italy

Congress chairman: Prof. L. Luciani

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Editorial

The present issue of *Urological Research* includes the papers presented at the meeting of the European Society for Urological Oncology and Endocrinology (E.S.U.O.E.), Trento, Italy, October 29–31, 1992.

The influx of contributions to the subjects proposed by the scientific board (growth factors and oncogenes, molecular oncology and endocrinology, markers for clinical behaviour, image analysis) reflects the fervent current research in many urological centers and provides deep insight into highly specific experimental and clinical uro-oncological topics, together with the stimulus to conduct further investigations.

The results of this investigative activity can be seen in the large number of excellent abstracts submitted which constitute a body of scientific knowledge of great relevance that is both promising and a challenge. Once again, this makes this Society's congress a productive meeting point linking scientists and clinicians.

All this work demonstrates the concern, effort, and dedication of urologists involved in research and in clinical medicine who are willing to collaborate to face the present and future urological dilemmas and thus assure constant progress in urology.

Lucio Luciani, Trento

State-of-the-art lectures

1

INACTIVATION OF TUMOR SUPPRESSOR GENES IN HUMAN BLADDER CANCER. Peter A. Jones, Seth P. Lerner, Yvonne C. Tsai, Aria F. Olumi, Peter W. Nichols, and Donald G. Skinner. Kenneth Norris Jr. Comprehensive Cancer Center, 1441 Eastlake Avenue, Los Angeles, CA 90033, USA.

Recent studies on the molecular genetics of human cancers have suggested that the sequential inactivation of tumor suppressor genes is an essential part of the carcinogenic process. We have investigated the molecular events associated with the development of human bladder cancer, focussing particularly on the role of putative tumor suppressor genes in this process. Allelic loss of chromosome 9 was found in a high percentage (± 60%) of all human bladder cancers examined regardless of grade or stage. Inactivation of a tumor suppressor gene on this chromosome is therefore likely to be an early event in the development of neoplasia. We are currently mapping the region of chromosome 9q which is most commonly deleted. We have additionally shown that chromosome 17p is often reduced to homozygosity in high grade and high stage transitional cell carcinomas and have shown that mutations in the p53 gene are commonly associated with these tumors. Mutations in p53 therefore seem to be a late event in the development of bladder cancer and might have a role in tumor progression. Analysis of the spectra of mutations found within the p53 gene shows that the patterns in different tumors are markedly different suggesting that examination of mutational spectra may be useful in defining the etiology of these cancers.

2

HUMAN PROSTATE CANCER MODEL: NEW STRATEGIES PROBING BONE-PROSTATE INTERACTION. Leland W. K. Chung. The University of Texas M. D. Anderson Cancer Center, Houston, Texas USA.

A human prostate cancer model was established by inoculating a prostate-specific antigen (PSA)-producing LNCaP cell line with either prostate or bone fibroblasts or specific growth factor(s) (GFs) and extracellular matrix (ECM) immobilized on Gelfoam®. The resulting LNCaP tumors were used to evaluate PSA production and excretion in athymic hosts. This model was also employed to examine the biochemical nature of mesenchymal cell-derived growth-promoting protein(s) and to assess the efficacy of potential chemotherapeutic agents against human prostate cancer. Because of the propensity of human prostate cancer to metastasize to the bone, this study defined a 1.0 M NaCl-eluted fraction, MS1, from the conditioned medium of a bone stromal cell line, MS, by heparin-affinity column chromatography. The growth-promoting activity was assayed both *in vivo* (*e.g.*, tumor formation) and *in vitro* (*e.g.*, soft agar colony formation). We found that the growth-promoting activity was trypsin- and heat-sensitive, partially degraded by acid and dithiothreitol. Immunochemical studies indicate that the polyclonal antibody raised against MS1 blocked the growth-promoting effect elicited by the partially purified bone conditioned media. We have fractionated further the MS-1 proteins by high-performance liquid chromatography and have identified a single immunoreactive band that has the ability to stimulate anchorage-independent growth of the prostatic epithelial cells. This immunoreactive band was also found to be present in human bone marrow aspirates obtained from prostate cancer patients. This growth-promoting factor was found to be immunochemically dissimilar to KGF, HGF, and bFGF. However, addition of bFGF, HGF, and NGF but not aFGF, TGFβ$_1$, IGF1, IGF2, PDGF, EGF, TGFα, and KGF stimulated anchorage-independent growth of prostate cells, a condition closely mimicked the tumor formation *in vivo*. We found that the MS1 fraction also contains fibronectin and tenascin but not laminin or collagen IV. None of the ECM proteins induced soft agar colony formation by normal prostate epithelial cells. Therefore, it is possible that the ECM protein(s) may potentiate the tumor-inducing activity of locally produced GFs by the competent fibroblasts.

3

EPITHELIAL CELL PLASTICITY IN BLADDER CARCINOMA.

THIERY, J.P., BELLUSCI, S., BOYER, B., DELOUVEE, A., JOUANNEAU, J., MOENS, G., RADVANYI, F., SAVAGNER, P., TUCKER, G.C. and VALLES, A.-M. Laboratoire de Physiopathologie du Développement, CNRS URA 1337 and Ecole Normale Supérieure 46, rue d'Ulm, 75230 Paris Cedex 05 France.

A rat bladder carcinoma has been used as a model system to study the conversion of an epithelial to a migratory fibroblast-like state. This morphological transformation is triggered by collagens but not by fibronectin or laminin. A similar conversion is induced by acidic Fibroblast Growth Factor (aFGF) in subconfluent cultures while this multifunctional growth factor acts as a mitogen on high density cultures. In low density cultures, aFGF and several other growth factors acting through tyrosine kinase receptors induce a rapid internalization of desmosomes, a major adhesive structure of epithelia. The newly formed fibroblasts progressively lose their cytokeratins which are replaced by vimentin intermediate filaments. The transformation is fully reversible upon removal of the growth factor. Acidic FGF also triggers cell motility and production of gelatinases. On collagen substrates, the speed of locomotion is enhanced in the presence of aFGF and under these conditions the bladder carcinoma cells readily invade 3D collagen gels. The bladder carcinoma line can also become fibroblastic after transfection with an expression vector coding for aFGF, most likely through an autocrine mechanism. Clones producing the growth factor can efficiently invade rat bladders maintained in organotypic cultures. These clones grow much more rapidly in nude mice than the original cell line and micrometastases are detected in less than two weeks following subcutaneous injection. Thus, this model system may offer a unique opportunity to evaluate the role of growth factors in the triggering of carcinoma invasion and metastasis.

Workshop: Growth factors and oncogenes

4

ANALYSIS OF THE DNA SEQUENCE OF THE LIGAND- AND THE DNA-BINDING DOMAINS OF THE ANDROGEN RECEPTOR IN PROSTATIC TUMOR CELL LINES AND TISSUE SPECIMENS

Culig Z., Klocker H., Hobisch A., Eberle J., Kaspar F., Cronauer M., Bartsch G. Department of Urology, University of Innsbruck, Austria

Almost all initially anti-androgen responsive prostatic carcinomas relapse to an androgen-insensitive stage. Changes in the androgen signalling chain are possibly responsible for development of androgen insensitivity. The purpose of this study was to investigate whether androgen receptor (AR) mutations occur in prostatic tumor tissue in vivo.

The experiments were designed to isolate, by means of the polymerase chain reaction (PCR) technique, AR complementary DNA fragments containing both the ligand- and the DNA-binding domains, from prostatic tumor cell lines LNCaP, PC-3, DU-145, 10 tissue specimens obtained by radical prostatectomy and 6 fine-needle aspirates obtained from patients receiving androgen withdrawal therapy. AR fragments were detected in all samples except in androgen-independent DU-145 cells. The fragments were isolated and subsequently analyzed by DNA sequencing. No alterations were detected in the tissue specimens and in the fine-needle aspirates. In the three tumor cell lines which represent late stages of prostatic tumor, different findings were obtained. The androgen-independent DU-145 cells did not express ARs, whereas the PC-3 cells, which are also androgen-independent, expressed very low levels of normal AR. In contrast to this, the androgen-dependent LNCaP cells expressed high levels of structurally abnormal ($G^{3157}\rightarrow A$, thr→ala) AR.

Our results suggest that AR mutations are probably uncommon molecular events in the early and intermediate stages of prostatic carcinoma (represented by tissue specimens from radical prostatectomy and fine-needle biopsies). Qualitative and quantitative changes, however, seem to occur in late stages of prostatic cancer e.g. metastases (represented by tumor cell lines).

5

TESTOSTERONE PROMOTES ONCOGENE-INITIATED PROSTATE CANCER USING THE MOUSE PROSTATE RECONSTITUTION MODEL SYSTEM Alain Mottaz, Dov Kadmon, Sang H. Park, Peter T. Scardino and Timothy C. Thompson. (Presentation to be made by Dr. Mottaz). Scott Department of Urology, Baylor College of Medicine, Houston, Texas, USA

The concept that testosterone promotes prostate cancer by complementation of specific oncogene activities was tested using the mouse prostate reconstitution (MPR) model system. MPRs were produced following the introduction of the *ras* oncogene alone or the *ras* and *myc* oncogenes together into Balb/c urogenital sinus cells (epithelium alone or epithelium and mesenchyme) using the helper virus-free recombinant retroviruses Zip/ras/ßgal and Zipras/myc 9, respectively. Adult intact male host animals received either empty silastic tubing implants or implants containing 25 mg testosterone propionate at the time of grafting. Because the Balb/c mouse is normally resistant to *ras+myc*-induced carcinogenesis, the background level of malignant transformation was minimized. More severe hyperplastic and dysplastic changes were seen in *ras*-transformed epithelial cells under conditions where the mesenchyme was also induced with this single oncogene. In the presence of pharmacological levels of testosterone under conditions of *ras*-induced epithelium and mesenchyme, a focal carcinoma was produced in one case indicating that testosterone and *ras* alone can cooperate during carcinogenesis. Restricted introduction of *ras* and *myc* to the epithelium did not produce any malignancies (n=11). Low frequency progression to malignancy was seen under conditions where the two oncogenes were introduced into both the epithelium and the mesenchyme (1/27). Interestingly as seen with the *ras* oncogene alone, pharmacological levels of testosterone significantly increased the frequency of malignancy when the two oncogenes were present in both the epithelium and mesenchyme (8/17). All *ras+myc*-induced carcinomas demonstrated elevated mRNA levels for transforming growth factor-beta 1 (TGF-ß1) as well as increased extracellular TGF-ß1 demonstrated by immunohistochemical analysis. In contrast the single *ras* + testosterone-induced focal carcinoma was negative for TGF-ß1 immunoreactivity. These studies demonstrate a tumor promoting activity for testosterone *in vivo* under conditions that closely mimic the initiation of human prostate cancer.

6

The Molecular Basis of Androgen-Regulated Prostate Cell Apoptosis: Evidence That Castration Initiates a Defective Cell Cycle.

Marc Colombel, Carl A. Olsson and Ralph Buttyan, Columbia University College of Physicians and Surgeons, New York, NY, U.S.A.

The rat ventral prostate provides an excellent model system to study the molecular basis of apoptosis. Within weeks after castration of an adult male rat, 85% of the cells of this gland, consisting mainly of secretory epithelial cells, die by apoptosis. In the past, this model has been utilized to identify unique gene products induced during apoptosis. These include *sulfated glycoprotein-2*, *cathepsin-D* and *tissue transglutaminase*. While there was original speculation that some of these gene products might be lethal, and therefore the cause of apoptosis, it seems more likely based on recent functional studies, that these products are required for the removal of the apoptotic bodies and the recovery of the surrounding tissues.

More remarkable, however, is the molecular evidence implicating cell cycle events in prostate cell apoptosis. Whereas the adult rat prostate gland has very few actively proliferating epithelial cells, castration initiates massive reentry onto the cell cycle as identified by the early synthesis of G_1 proteins, c-*fos* and c-*myc*, extensive incorporation of bromodeoxyUridine into high molecular weight nuclear DNA of epithelial cells and induced expression of the proliferating cell nuclear antigen, PCNA. These prostate epithelial cells never reach mitosis, and we propose that they exit from this defective cell cycle (onto apoptosis) somewhere in S-phase or in the G_2-phase.

While we are currently investigating the growth factor environment of the regressing prostate in an attempt to identify the stimulus that initiates cell cycle reentry after castration, we have also identified a potential molecular switch from the productive cell cycle to apoptosis. Based on an earlier report showing that reintroduction of wild type p53 into p53-deficient rapidly cycling cells was sufficient to initate apoptosis, we examined for the expression of p53 in the regressing prostate gland. Both mRNA and protein studies show dramatically enhanced expression of p53 in the prostate epithelial cells after castration. Our studies indicate that androgen-regulated apoptosis is a cell-cycle dependent event that might occur when the suppressor gene, p53, is inappropriately expressed during the cell cycle.

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7

EPIDERMAL GROWTH FACTOR RECEPTOR IN EXPERIMENTAL PROSTATIC CANCERS. EFFECTS OF ENDOCRINE TREATMENT.

Jan-Erik Damber, Anders Bergh, Bodil Assarsson and Mats Gåfvels
Departments of Urology & Andrology, Pathology and Physiology, University of Umeå, S-901 87 Umeå, Sweden

The amount of epidermal growth factor receptor (EGF-R) has been shown to be increased with increasing grade of malignancy in several tumors such as breast and bladder cancers. In prostatic tumors EGF-receptor binding has been demonstrated both in vivo and in vitro and there are several lines of evidence suggesting a relationship between EGF-R and androgens. However, the relationship between the grade of malignancy and EGF-R in prostatic tumors is more unclear since both increased and decreased level of this receptor has been found in low differentiated prostatic tumors. It has also been shown that the EGF-receptor expression increases in the ventral prostate of rats after castration, but if this also is true for prostatic tumors is unknown. It was the purpose of the present work to elucidate the possible role of EGF-receptors in the Dunning prostatic tumor system by examine the effect of different endocrine treatment such as castration and estrogen treatment and furthermore to evaluate if the EGF-receptor expression varies in accordance with malignancy grade.

Method: Rats bearing the high differentiated, hormone dependent R3327 Dunning tumor and the undifferentiated, hormone independent Dunning AT-1 was used. Castration was performed via the scrotal route. Estrogen was given as oil suspension in a dose of 50 µg/day. Treatment period lasted for 6 weeks and thereafter the animals were killed by decapitation and tumor tissue was excised and quickly frozen in liquid N_2. EGF-receptor binding assay was performed on membrane fractions using the method of Scatchard. The amount of EGF-receptor mRNA was quantified by using solution hybridization. Furthermore localization of the EGF-receptor was performed by the use of immunohistochemistry and autoradiography.

Results: EGF-receptor binding capacity and mRNA was demonstrated in R3327 prostatic tumors from both control and castrated animals while no such activity was found in the AT-1 tumors. Castration did not induce any quantitative changes of the EGF-R. Estrogen treatment induced a significant reduction of the binding capacity of EGF-R and its mRNA. By immunohistochemistry and autoradiography EGF-receptors were demonstrated in the epithelial cells in the R3327 tumors of both control animals and animals treated with castration or estrogens. The AT-1 tumor was lacking EGF-receptor immunoreactivity.

Conclusions: The present study demonstrates the presence of high affinity EGF-receptors in the high differentiated, hormone dependent R3327 Dunning tumors. Castration did not promote any effect on EGF-R. The reduction of EGF-receptor binding and its mRNA after estrogen treatment is probably related to the reduced number of epithelial cells seen in such tumors after estrogen treatment. In Dunning tumors with undifferentiated morphology such as the AT-1 tumors no signs of EGF-R activity could be found. It is suggested that the Dunning prostatic tumor model looses its EGF-receptor when developing into a more undifferentiated state.

8

DIFFERENTIAL EXPRESSION OF FERRITIN HEAVY CHAIN IN A RAT TCC PROGRESSION MODEL

Jacqueline A.M. Vet, Reindert J.A.van Moorselaar, Marion J.G.Bussemakers, Frans M.J. Debruyne and Jack A. Schalken. Urological Research Laboratory, department of Urology, University Hospital Nijmegen, The Netherlands.

A major problem in the management of transitional cell carcinoma (TCC) of the bladder (comprising >95% of all bladder tumors) is the fact that 10-25% of the superficial tumors clinically progress. To find molecular parameters with predictive value for progression of superficial bladder cancer the technique of differential hybridization analysis was used on a rat transitional cell carcinoma progression model. This progression model was derived from two independent spontaneously arisen rat TCCs. In one line (RBT 323) the tumor progressed rapidly to a highly metastatic state, in another line (RBT 157) slow progression to a moderate metastatic phenotype was observed. A cDNA library of RBT 323-passage 10 was constructed and differentially screened with probes derived from mRNA of RBT 323-pass.10 (highly metastatic) and RBT 157-pass.1 (low metastatic capacity).
Out of 22 differential clones overexpressed in RBT 157-pass.1 we thus far characterized pVet13. pVet13 had an interesting expression pattern: expression of a 1 kb transcript detected by clone pVet13 was higher (>10-20 fold) in RBT 157-pass.1 than in RBT 323-pass.1 and RBT323-pass.10. Sequence analysis revealed that pVet13 was highly homologous to the rat ferritin heavy chain, a iron-binding protein. Ferritins have been proposed to play a role as regulators of cellular differentiation, whereas cell differentiation appears to favor accumulation of ferritin H-subunit mRNA.
We conclude that the differential clone ferritin might be a good candidate as molecular marker for predicting bladder tumor progression. On-going investigations study ferritin expression in all passages of the rat TCC progression model and test its value in predicting progression in human bladder cancer.

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E-CADHERIN AND AUTOCRINE MOTILITY FACTOR RECEPTOR: PROGNOSTIC PARAMETER IN BLADDER CANCER

[1] T. Otto, [2] W. Birchmeier, [3] U. Schmidt, [4] A. Raz, [1] H. Rübben
[1] Department of Urology, [2] Institute of Cell Biology, [3] Institute of Pathology, University of Essen, Medical School, 4300 Essen 1, Hufelandstraße 55, FRG
[4] Michigan Cancer Foundation Center, Detroit, Michigan, USA

INTRODUCTION: Loss of cell-cell adhesion and vigorous cell motility are essential requirements of tumor invasion and metastasis [1,2].
It is the major problem to select those patients who are at risk for tumor progression and who may profit from aggressive therapy, i.e. radical cystectomy.
RESULTS: We determined the expression of AMF-receptor in bladder cancer and found a strong correlation not only to tumor grade and stage but also to E-cadherin expression.

		n	E-Cadherin* normal ++	E-Cadherin* decreased [+]/-	AMF-receptor* normal [+]	AMF-receptor* increased ++
normal bladder tissue		31	31	0	31	0
bladder carcinoma						
Grading	G1	5	4	1	4	1
	G2	25	8	17	10	15
	G3/4	20	5	15	1	19
Staging	Ta/1	17	11	6	8	9
	T2-4	31	6	25	7	24
	M1	2	0	2	0	2

*=Expression was measured by indirect immunofluorescence microscopy [1,2]

[1] Frixen, U.H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Lachner, D., Birchmeier, W.: E-Cadherin mediated cell-cell adhesion prevents invasivness of human carcinoma cells. J. Cell. Biol. 113:173 (1991)
[2] Nabi, i.r., Watanabe, h., Siletti, S., Raz, A.: Tumor cell autocrine motility factor receptor. In: Goldberg, I.D. (ed). Cell motility factors, Basel, Birkhäuser Verlag, p. 184 (1991)

Oral communications: Molecular oncology and endocrinology I

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ANDROGEN RECEPTOR EXPRESSION IN HUMAN PROSTATIC CARCINOMA CELL LINES.

Ewan Grant, Kenneth Batchelor*, Fouad K Habib
University Department of Surgery (WGH), Western General Hospital, Edinburgh, UK. *Cellular Biochemistry Department, Glaxo Research Institute, Research Triangle Park, North Carolina, USA.

The progression of prostate cancer from androgen-dependent to androgen-independent growth is thought to be associated with the outgrowth of androgen insensitive cells (1). Androgen insensitivity in the prostate cancer cell lines DU145 and PC3 may be due to the absence of androgen receptor (AR). cDNA was prepared from total RNA derived from DU145, PC3 and the hormone sensitive prostate cancer cell line LNCaP. PCR amplification of a 228bp stretch of the AR cDNA demonstrates the presence of AR mRNA in all three cell lines, though with higher levels of expression in LNCaP cells. PCR amplification of genomic DNA from LNCaP, DU145, PC3 and female placenta reveal differences in the length of polymorphic (CAG)-repeat (encoding a polyglutamine sequence) in exon 1, but no differences were observed in exons 2-8. However, variations within the polymorphic region in exon 1 did not appear to correlate with mRNA levels. Further studies on the transcriptional control of AR gene expression in DU145 and PC3 are ongoing.

1. Isaacs, J.T. et al. (1978) Cancer Res. 38, 4353-4359.

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REGULATION OF ANDROGEN RECEPTOR EXPRESSION IN THE HUMAN HETEROTRANSPLANTABLE PROSTATE CARCINOMA PC-82

Jacobus A. Ruizeveld de Winter, Wytske M. van Weerden, Peter W. Faber, Gert J. van Steenbrugge, Jan Trapman, Albert O. Brinkmann, Theodorus H. van der Kwast

Depts of Pathology, Endocrinology & Reproduction and Urology, Erasmus University Rotterdam, The Netherlands

In vivo effects of androgen manipulation on hAR expression were examined both at the mRNA and at the protein level in the androgen dependent human prostatic carcinoma tumor model PC-82. HAR protein expression in PC-82 tumor tissue was studied by immunohistochemistry and immunoblotting using several antibodies reactive with different epitopes of the hAR molecule. HAR mRNA levels in PC-82 samples were determined with a S_1- nuclease protection assay.

The majority of PC-82 tumor cells from testosterone supplemented mice showed hAR expression immunohistochemically. The almost complete reduction of nuclear hAR immunoreactivity after castration was restored after androgen supplementation after one day. The immunohistochemical data and Western blot results were in line. In contrast, no changes were observed in the hAR mRNA content of PC-82 tumor cells after androgen withdrawal.

The proliferative activity of PC-82 tumor tissue decreased after castration. Providing castrated mice with androgens restored proliferation. However, this increase of proliferative activity lagged at least 24 h behind the normalization of the hAR protein expression.

Our results support the concept of hAR up-regulation by androgen in contrast to the in other models observed down-regulation. The expression of the hAR in PC-82 is thought to be modulated by translational and/or post-translational mechanisms because hAR mRNA levels remained unchanged.

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SELECTION OF GENES PLAYING A ROLE IN CYTOKINE ACTION BY MEANS OF DIFFERENTIAL HYBRIDIZATION.

A.J.M.C. Beniers, F.M.J. Debruyne, J.A. Schalken.
Dept. of Urology, University Hospital Nijmegen, The Netherlands.

Cytokines are a broad class of protein cell regulators with a variety of regulatory effects on different cell types. Many cytokines are involved in the immune response. Despite the vast quantity of papers about cytokine action, still very little is known about the pathways by which they transmit signals to the nucleus which results in de novo or enhanced transcription or inhibition of genes. Sofar, these genes playing a role in the ultimate action of the cytokines and which may determine sensitiveness/resistance of particular cells to cytokines, are unidentified.

A practical approach to compare gene expression in different cell types is the comparison of cDNA library's by means of differential hybridization. We compared gene expression in the NU-12 and NU-20 renal cell carcinoma (RCC) xenografts which have a relatively high- and low sensitivity towards cytokine (IFN-alpha/gamma and TNF) treatment, respectively. cDNA library's of poly A+ selected mRNA were made by cloning into the Lambda ZAP II cloning vector which allows directed cloning of the cDNA's. Differential screening of both library's with probes derived from poly A+ selected mRNA of NU-12 and NU-20 revealed 15 clones overexpressed in the NU-12 tumor.

Screening of a panel of sensitive to insensitive tumors with probes of the selected clones with Northern blot analyses revealed three clones which were highly expressed in sensitive lines, but to a much lower level in insensitive or intermediate sensitive lines (difference: 10-20 fold). The clones recognized transcripts of 1.9, 2.7 and 3.3 Kb respectively. Initial sequence analyses has not revealed any homology with sequences of known genes and therefore no conclusions can be drawn concerning genes playing a role in cytokine sensitivity.

We conclude that the selected (sofar unknown) genes may play an important role in conferring sensitivity to RCC cells for IFN-alpha -gamma and TNF. Ongoing research in characterizing these genes ultimately will increase our knowledge of the genetic action of these cytokines

13

GROWTH FACTORS INVOLVED IN PROSTATIC SKELETAL METASTASES.

Shona Lang, *William R Miller, Fouad K Habib, University Dept. of Surgery (WGH), Western General Hospital, Edinburgh, UK, *ICRF/Medical Oncology Unit, Western General Hospital, Edinburgh, UK.

Prostate cancer is a major cause of male cancer mortality, which seems to have a selective metastatic spread to bone tissue.

To investigate the reasons for this selective spread, the project has looked for growth factors produced by the bone environment that stimulate prostate cancer growth. This was achieved by producing primary cultures of human bone cells (BC). Serum free conditioned medium (SFCM) was collected from confluent cell cultures and used to stimulate the growth of two human prostatic carcinoma cell lines DU145 and PC3. In addition various human recombinant haemopoietic growth factors (HGF) were also used to test for growth stimulation. Following the plating of the cell lines, the cells were exposed to HGF and BCCM in a serum free environment and growth was subsequently measured every 24h, using thymidine uptake and cell counts. Results have indicated that the BCCM produces a 50-100% growth stimulation of PC3 and DU145 cells. SFCM taken from other cell lines representing different metastatic sites were also tested but these had no effect on growth. Of the HGF's tested both erythropoietin (100mU/ml EPO) and granulocyte macrophage colony stimulating factor (100U/ml GM-CSF) produced a 50-100% stimulation of PC3 and DU145 growth. Interleukin-3 and granulocyte colony stimulating factor both had little effect. Initial experiments have shown the presence of GM-CSF receptors on DU145 cells and additional experiments are being carried out to further characterise these. Our results indicate that both known and unknown growth factors from bone tissue stimulate the growth of prostate cancer cell lines.

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THE ROLE OF *neu* ONCOGENE IN PROSTATIC EPITHELIAL CARCINOGENESIS.

Haiyen E. Zhau, Robert A. Sikes, and Jianxin Zhou. The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, U.S.A.

Amplification and overexpression of c-*erb* B-2/*neu* oncogene have been associated with the progression and possible prognosis of human cancers. In this study, we demonstrated that *neu* oncogene may also play an important role in human prostate cancer. Our conclusion is based on the following experimental results: (1) a monoclonal antibody, c-*neu* Ab-3, reacted positively with 69% (11/16), and 80% (12/15) of the human prostatic cancer tissues studied using immunohistochemical and western blot analysis, respectively; (2) the c-*erb* B-2/*neu* oncoprotein ($p185^{neu}$) was detected in both androgen-responsive LNCaP cells, and androgen-unresponsive PC-3, and DU-145 cells with distinct subcellular localizations; $p185^{neu}$ was found to be associated with cytoplasmic and perinuclear regions of the LNCaP cells, but was associated with the cell membranes of the PC-3 cells and the perinuclear region of the DU-145 cells. The differential subcellular localization of $p185^{neu}$ may indicate the status of differentiation of the prostatic cancer cells; (3) the mRNA expression of c-*erb* B-2/*neu* oncogene was positively regulated by androgen both in cultured LNCaP cells and in the LNCaP prostatic tumors maintained in athymic adult male hosts (Zhau et al., Mol. Carcinogenesis, in press, 1992).

To assess the transforming potential of the activated rat *neu* oncogene in prostatic epithelial carcinogenesis, we transfected a cloned rat ventral prostatic epithelial cell line, NbE-1.4 with an activated, point-mutated *neu* oncogene. This transfection procedure resulted in the recovery of rat prostatic epithelial cell clones that have altered cell morphology, increased in efficiency of soft agar colony-formation, and a conversion from nontumorigenic to a tumorigenic phenotype. We demonstrated by a polymerase chain reaction followed by restriction enzyme digestion that the expression of point-mutated *neu* oncogene depressed the expression of the wild-type *neu* oncogene by the transformed rat prostatic epithelial cells (Sikes and Chung, Cancer Res., in press, 1992).

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LOSS OF HETEROZYGOSITY WITHIN 22 LOCALLY ADVANCED PROSTATE CANCERS.

Stewart Phillips, Susan Lee, Dion Morton, Paul Cairns, Michael Wallace. Depts of Surgery and Urology, Queen Elizabeth Hospital, Birmingham, UK.

Prostate cancer is the commonest malignancy in men. There is a wide spectrum of this tumour, ranging from a high incidence of latent disease, to locally advanced or metastatic disease accounting for 3% of male deaths in the UK. In common with other solid tumours, the activation of oncogenes and inactivation of tumour suppressor genes is thought to be important in tumourigenesis. The latter may be indicated by loss of heterozygosity within the tumour DNA.

Little work to date has been carried out on primary locally advanced tumours. We have identified allelic loss within a series of 22 locally advanced tumours (T2-T4), with or without bone metastases. Tumour samples were obtained when these patients underwent TURP for obstructive symptoms, and stored frozen until required. A blood sample was also taken as a source of genomic DNA. Tumour and blood DNA were extracted by standard techniques, digested by restriction enzymes and run on agarose gels. Southern blots were set up and the resulting filters hybridised with DNA probes. Autoradiographs were used to reveal the allelic status of the tumours, and detect loss of heterozygosity in sites of known or suspected tumour suppressor genes.

Within the 22 patients studied, loss was detected in probes to the FAP and Rb genes, and also to chromosomes 10p and 10q. 3 out of 15 informative probes (20%) to 10p showed deletions. Between 3 out of 14 (21%) and 5 out of 16 (31%) deletions were noted in informative probes to 10q. With respect to Rb gene, deletions were noted in 3 of 8 (37.5%) of informative cases. Deletions were also noted in a third of informative FAP gene probes.

This work suggests that losses of Rb and FAP genes, and the losses of the suspected tumour suppressor genes on chromsome 10, are a common event in the pathogenesis of the locally advanced and metastatic tumours responsible for the morbidity and mortality of prostate cancer.

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MOTILITY INDUCTION OF METASTATIC DUNNING RAT PROSTATE CANCER CELLS. Johan C. Romijn, Sigrun Erkens, Diederick M. Keizer and Fritz H. Schroeder; Dept. of Urology, Erasmus University, Rotterdam, The Netherlands.

Increased cell motility is believed to be one of the early events in the metastatic cascade. The stimulation of tumor cell motility can be brought about by factors that are produced and secreted by the tumor cells themselves. We have used the Dunning rat prostate cancer model to evaluate the production of such factors, often referred to as autocrine motility factors, and to examine the response to these by a number of tumor cell variants with different metastatic abilities. The results showed that the motility response, measured as cell migration in a modified Boyden chamber assay, was clearly different in the various cell lines: highly metastatic cells (MAT-LyLu) were much more motile than the poorly metastatic lines (AT-2, G). Motility-inducing activity was found to be secreted in the (serum-free) conditioned medium of both MAT-LyLu and AT-2 cells, but not of G-cells. Inhibition of protein synthesis by cycloheximide (10 μM) blocked motility factor production and/or secretion. The activity was also abolished by precoating the Boyden chamber filter with bovine serum albumin. This and other observations strongly suggested that tumor cell migration was driven by haptotaxis (directed migration in response to a solid factor), which is in contrast to the general idea that migration is mostly induced by chemotactic or chemokinetic signals. We also observed a strong responsiveness of (metastatic) cells to rat-fibronectin and rat-laminin. To examine the possibility that the secreted motility factor was identical to either laminin or fibronectin, we quantitated the amount of these components in the conditioned medium by means of dot-blot experiments. Although laminin and fibronectin were detectable in the medium, their levels (< 0.1 μg/ml) were too low to account for the observed migratory response, implicating that another, sofar unidentified factor is involved in migration induction. Biochemical characterization of the motility-inducing activity indicated that the factor is a protein (all activity was lost following trypsin treatment) that is sensitive to exposure to dithiothreitol (10 mM), to oxidation and to temperatures above 60°C. The activity was stable in the pH range from 2 to 12 and in the presence of urea (6 M). The factor was concentrated by ammonium sulfate precipitation and by lyophylization. For further characterization and purification the concentrated preparation was subjected to gel filtration using a Sephacryl S-300 column. All migration-inducing activity was eluted in fractions corresponding to a molecular size of approximately 250 kDa and was not associated with any of the major protein peaks. Attempts to identify this factor are currently underway.

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THE CONCEPT OF MOLECULAR TARGETING FOR REVERSAL OF MULTIDRUG RESISTANCE.

Gerald H. Mickisch, Ira Pastan, Fritz H. Schröder, Dept. of Urology, Erasmus University Rotterdam, The Netherlands and Lab. of Molecular Biology, National Cancer Institute, NIH, Bethesda, Md., USA.

Using renal carcinoma and prostate carcinoma cell lines, we investigated the concept of targeting and killing multidrug resistant cells in urogenital cancers. Renal carcinoma lines HTB44, 45, 46, and 47 expressed a relatively low, but easily detectable level of *MDR*1 mRNA as indicated by Northern blot analysis, whereas prostate lines LNCaP and DU145 were found to be *MDR*1-negative. Anti-P-glycoprotein monoclonal antibody MRK16 was conjugated to Pseudomonas exotoxin (PE) by a stable thioether bond and purified by HPLC procedures. Treatment with MRK16-PE resulted in a dose-dependent killing of multidrug resistant renal carcinoma cells, while non-MDR expressing prostate carcinoma cells were not affected. Addition of excess MRK16 blocked the effect of MRK16-PE. Furthermore, MOPC-PE, a non-MDR associated monoclonal antibody control conjugate, did not target and kill multidrug resistant renal carcinoma cells. Having established that MRK16-PE was active against and specific for multidrug resistant cells in culture, we also tested bioactivity in MDR-transgenic mice, whose bone marrow cells express the human *MDR*1 gene at a level approximately equal to that found in many human cancers. Again, MRK16-PE killed multidrug resistant bone marrow cells with high efficiency in an intact animal, and killing was blocked by excess amount of unconjugated MRK16. Thus, these studies provide the basis for the further investigation of MDR-directed immunotoxins in the treatment of drug-refractory urological malignancies such as renal cell carcinomas.

Oral communications: Markers for clinical behaviour

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ENHANCED ERYTHROCYTE POLYAMINES IN RENAL CELL CARCINOMA (RCC)

B. CIPOLLA, F. GUILLE, Y. BLANCHARD*, L. CORBEL, F. STAERMAN, J.Ph. MOULINOUX*, B. LOBEL
Departments of Urology and *Therapeutique anti-cancereuse
C.H.U. RENNES - FRANCE

Polyamines spermidine (Spd) and spermine (spu) are ubiquitous amines involved in normal and tumoral cell proliferation. They are mainly transported in the blood by erythrocytes. Their erythrocyte levels are increased in malignant gliobastomas, bronchopulmonary cancers and leukemias. We have already shown an increase of erythrocyte polyamines in prostatic cancer and they could be of prognostic importance. As few tumor and prognostic markers are available in renal cell carcinoma, the aim of this study is to assess the value of erythrocyte polyamines in this kind of tumor.

Patients and Methods. 34 patients admitted for non malignant renal pathologies (Renal Lithiasis, pyelonephritis, renal cysts or pyelo ureteral junction anomalies) served as controls. 51 patients with pathologicaLly proved renal cell carcinoma were stratified into 4 groups according to stage : pT 1-2 No Mo, pT 3-4 NoMo, pTx N+ Mo, pTx Nx M+. All patients had pretherapeutic spd and spm erythrocyte determination by H.P.L.C from 5ml blood collected in a peripheral vein.

Statistics were performed with non parametrie Wilcoxon test and Spearman regression test.

Results : 34 controls (mean age = 48 +/- 3) had mean spd = 10 +/- 3,6 and spm = 4,9 +/- 3 erythrocyte levels. 51 Rcc patients (mean age = 64 +/-11) as a whole had mean spd = 17 +/- 13 and spm = 9,5 +/- 12 erythrocyte levels. Spd and spm erythrocyte levels are significantly increised in RCC patients compared to controls ($p < 0,01$). When compared to controls spd and spm levels of stage pT1-2 No Mo patients are not increesed whereas spd levels are increased in stage pT3-4 No Mo, N+ and M+ patients ($p < 0,01$). Compared to stage pT1-2 No Mo patients, spd of pT3-4 NoMo, N+ and M+ patients are increased ($p < 0,05$). No differences are observed between pT3-4 No Mo and N + or M+ patients. No correlation is found between spd or spm and blood count erythrocytes or hemoglobine levels.

Conclusions. Erythrocytes polyamines are increased in RCC patients compared to controls. Furthermore they are significantly increased in locally extensive (pT3-4) and metastatic disease compared to local disease (pT1-2 No-Mo). Further evaluation is necessary to confirm these results. Follow-up studies are under way to assess their prognostic value.

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BIPARAMETRIC ANALYSIS OF p53 PROTEIN EXPRESSION AND DNA CONTENT OF RENAL CELL CARCINOMA BY FLOW CYTOMETRY.

R.Verardi*, A.R.Magalini^, L.Garza^, G.Ruggeri* A.Cozzoli°, M.Scanzi°, A.Albertini*, D.Primi^, S.Cosciani Cunico°, P.G.Grigolato §, A.Benetti §, A.Berenzi §, G.L.Mantero^. * Institute of Chemistry, School of Medicine, University of Brescia, ^ Consorzio per le Biotecnologie, Consiglio Nazionale delle Ricerche, Brescia, ° Department of Urology, University of Brescia, § Department of Pathology, University of Brescia (Italy).

The p53 protein was first detected as complex with simian virus 40 T antigen. Subsequent studies have shown that the wild type protein is able to suppress cell proliferation and transformation. On the other hand, the mutated gene facilitates cell transformation and cooperation has been demonstrated between mutant p53 and mutant *ras* genes in cellular transformation. The normal p53 undergoes to self-oligomerization and it is been suggested that a single allelic mutation may cause a significant increase of half life, oligomerization and inactivation of wild type. Overexpression of p53 has recently been reported in several human tumors including breast, colon, lung, prostate and bladder. In most cases, overexpression of p53 has been found to be associated with mutations in the p53 gene.

Our study was aimed at the evaluation of p53 expression in renal cell carcinoma.

Surgical fragments of macroscopically determined tumoral and normal tissue were collected and immediately stored at - 80°C, from 16 patients undergone to radical nephrectomy.

Dual parameter flow cytometry was performed by simultaneous staining of DNA with propidium iodide (PI) and p53 protein with the monoclonal antibody PAb1801. The expression of p53 was evaluated as percentage of positive cells in relation to the DNA content. Adjacent tissue sections were also evaluated by immunohistochemical staining with the same monoclonal antibody. Ten of 16 cases (62,5%) showed an enhanced level of p53 compared to the corresponding normal tissue. Cell positive percentage ranged from 5 to 52%. No significant correlation was observed between p53 overexpression and DNA index. However, in some of positive cases (30%) the protein overexpression was found in the aneuploid cell populations. Interestingly, in 2 cases different level of p53 were observed in different tumor tissue fragments, suggesting heterogeneity of p53 status in some tumors. The same pattern was confirmed by immunohistochemistry demonstrating a good correlation between the two methods. In order to assess the presence of p53 gene mutation in the positive samples, we are now analyzing DNA from portions of the samples studied for protein expression by Single Strand Conformation Polymorphism (SSCP) of exons 4 to 9, after enzymatic amplification with polimerase Chain Reaction (PCR).

Taken together these results suggest the reliability of flow cytometric analysis for screening of p53 alterations in human tumors.

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PHASE I RADIOIMMUNOSCINTIGRAPHY TRIAL OF ^{131}I-LABELED MONOCLONAL ANTIBODY G250 IN RENAL CELL CARCINOMA.

Egbert Oosterwijk, Jeannette C Wakka, Neil H Bander, Sydney Welt, Chaitanya R Divgi, Steven M Larson, Lloyd J Old. New York Unit Ludwig Institute for Cancer Research, and Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, NY10021, USA.

G250 is a cell surface antigen recognized by an IgG1 murine monoclonal expressed by human renal cell carcinoma (RCC) but not detected in normal kidney. Expression in normal tissues is highly restricted and limited to bile duct and gastric epithelium. Clear cell RCC, the most frequent form of RCC, shows homogeneous expression of G250, whereas the less common forms of RCC and cancers derived from other organs generally do not express G250. The imaging and biodistribution characteristics of ^{131}I-labeled G250 were studied in 16 presurgical RCC patients. Antibody labeled with 10 mCi ^{131}I was administered intravenously 7 to 8 days before surgery at 5 dose levels, with at least 3 patients at each dose level. Clear tumor images were observed in 12 patients with G250-positive tumors, whereas 2 of 3 G250-negative tumor bearing patients did not show tumor images. The imaged lesions were confirmed at surgery and the smallest lesion visualized was 8mm in diameter. In several patients occult disease was imaged, e.g. extensive lymph node involvement, an adrenal metastasis diagnosed as an adrenal adenoma by MRI, and diffuse metastatic disease in a polycystic kidney, not recognized by MRI or CT scans.
The specificity of ^{131}I-mAbG250 localization was established by biopsy dosimitry, autoradiography and immunohistochemistry. ^{99m}Tc-human serum albumin blood flow studies demonstrated that ^{131}I-mAbG250 uptake was not the result of increased tumor blood pooling. Tumor:serum and tumor:normal kidney parenchyma ratios reached values as high as 175:1 and 285:1, respectively. The mean tumor:serum and tumor:kidney ratios in G250-positive tumors were 13.6 and 28.6, respectively. The liver was imaged at the lower dose levels, but was not visualized at the higher doses. Due to the saturability of G250 sites in the liver, tumor:liver ratios were dose dependent and ranged from 0.1 at the lowest dose to 92 at the higher antibody doses. The fraction of the injected dose localized in the renal tumors expressed as percent injected dose per gram of tumor (%ID/g) was as high as 0.117% (range 0.117-0.0005%). The mean %ID/g in G250-positive tumors was 0.014% In contrast, G250-negative tumors accumulated at least 10 fold less antibody (mean 0.0013%, range 0.002-0.0002% ID/g). These are the highest values, relative as well as absolute, reported for tumor biopsies obtained 8 days after intravenous mAb administration. Based on the specific localization and high accumulation, mAb G250 may have therapeutic potential, and may be a useful imaging agent for RCC patients.

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TUMOR ASSOCIATED ANTIGENS AND EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AS PROGNOSIS TOOLS FOR TRANSITIONAL CELL CARCINOMA (TCC) OF THE BLADDER

V. RAVERY[1], J.J. PATARD[1], J. BELLOT[2], S. GIL DIEZ[1], Y. FRADET[3], C.C. ABBOU[1] and D.K. CHOPIN[1]. (1) Service d'Urologie, Hôpital Henri Mondor, 94010 Créteil, France ; (2) Laboratoire d'Anatomo-Pathologie, Hôpital Henri Mondor, 94010 Créteil, France ; (3) Centre de Recherche en Cancérologie, Université de Laval, Hotel Dieu, Québec, Canada

Biological or molecular markers which may be of clinical relevance to dismember the spectrum of bladder cancer are still awaited. An immunohistochemical analysis was performed on 50 TCC (27 stage < T2 ; 23 ≥ T3 and 10 healthy urothelium), using monoclonal antibodies against the external domain of the EGFR. Monoclonal antibodies G4, E7 (DKC) and T43-T138-19A211 (YF) previously described were also used in the study. Immunohistochemistry was performed on fresh frozen section using an alkaline phosphatase method. Staining with each antibody was analyzed according to TNM classification. Results on initial tumor was compared to subsequent clinical evolution. Progression for TCC < T2 was defined as occurrence of muscle infiltrative disease or the need for radical therapy due to local progression. Progression for TCC ≥ T2 was defined as occurrence of patent metastasis or death from tumor. Pattern of immunostaining was in accordance with initial description of theses antibodies.Results indicate on this restricted panel of tumors that 2 antibody-makers have a potential pronostic value, comparing the progression rate survival using the log Rank test: the anti EGFR antibody ($p = 0.036$) and the T138 antibody ($p = 0.0041$). These data are in accordance with previous study using either T138 or EGFR antibodies. These markers may have some clinical relevance and are candidates for a multifactorial analysis on a larger panel of tumor.

Work supported by University Paris XII.

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PERMANENT PROPAGATION OF THE HUMAN PROSTATIC CARCINOMA CELL LINE LNCAP IN ATHYMIC NUDE MICE. Gert Jan van Steenbrugge, Xin Zhao, Connie J. van Uffelen, Eppo Mulder* and Fritz H. Schröder, Depts. Urology and *Endocrinology & Reproduction, Erasmus University Rotterdam, The Netherlands.

The LNCaP human prostate cancer cell line is an androgen dependent *in vitro* cell line. It was shown that 0.1 nM of the synthetic androgen R1881 optimally stimulates growth of LNCaP-FGC (FGC) cells, but that high concentrations (10 nM) of estradiol (E_2) were stimulatory for these cells, as well. *In vivo*, in athymic nude mice, LNCaP cells were shown to be poorly tumorigenic. It was attempted to improve the transplantability of the LNCaP cells by manipulation of the host connective tissue (by the neutral protease dispase) of the host (NMRI) nude mice, or by stimulating the process of angiogenesis (by prostaglandin-E_1). Although the (local) application of both of these compounds resulted in an substantial enhancement of the initial take rate (up to 100% in the PGE_1-treated mice) of the FGC cells, with this approach no permanent *in vivo* growth of the LNCaP cell line was achieved.

In addition, high dosages of E_2 were administered to tumor cell inoculated mice, using time release pellets of 15 mg. The initial take rate of cell inoculates of 10^7 FGC cells in E_2-supported female mice was 65%, whereas in control mice 25% of the tumors developed. Control tumors consisted of large hemorrhagic (cystic) nodules with mainly necrotic tissue, which was not transplantable. By contrast, E_2-supported mice developed solid tumors, of which the tissue was serially transplantable in E_2 substituted mice but not in control animals with placebo pellets. Tumor-bearing mice had pharmacological levels (approx. 20 nM) of circulating E_2, which was in the same range as the concentration found to be stimulatory for LNCaP cells *in vitro*. At present, the descending permanent tumor line is in its 15th transplant generation. In passage 2 to 10 of the E_2-supported FGC tumor line, an average take rate of 75% was reached, with tumors having a relatively short tumor lag phase (14 days) and a tumor doubling time of approx. 5 days. The serum of FGC tumor-bearing mice contained high levels of prostate-specific antigen (PSA) and a significant correlation was found between the circulating levels of PSA and the total tumor burden ($R=91$; $n=46$). Like the FGC cells in culture, the E_2-supported tumor tissue in nude mice expressed the androgen receptor. Application of (15 mg) pellets with dihydrotestosterone (DHT), resulting in high (supraphysiological) plasma levels of DHT in the host animal, seems not to result in a similar enhancement of the LNCaP tumor take. This preliminary result with DHT might be indicative for a specificity of estradiol in the observed growth of LNCaP in the nude mice. Whether this steroid directly influences the growth of these cells or indirectly, e.g. through affecting the remaining immunological system of the host animal, has yet to be determined. In conclusion, the permanently growing E_2-supported LNCaP-FGC tumor in athymic nude mice provides a valuable model for studies of human prostate carcinoma *in vivo*.

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RELATIONSHIP OF GRADE, AGE, METASTATIC STATUS AND SURVIVAL OF PROSTATIC CARCINOMA PATIENTS TO THE KI-67 AND PCNA EXPRESSION IN THE TUMOURS

Maureen E.Harper, Lindy Goddard, Eve Glynne-Jones & Keith Griffiths, Tenovus Institute for Cancer Research, Cardiff, CF4 4XX, Wales, UK,

Estimation of the growth fraction in 153 prostatic carcinomas was undertaken by assessing the Ki-67 expression in their tumours. The relationship of this parameter to various clinical characteristics of the patient and his tumour was examined. Correlation of Ki-67 expression with histological grade of the tumour was found ($p < 0.001$), high Ki-67 scores being associated with poorly differentiated tumours. No relationship with either the patients age at diagnosis or the primary tumour stage (T category) was seen. A correlation with the metastatic status was observed ($p < 0.05$), higher Ki-67 scores were associated with M1 disease. Life-table analysis of 86 patients showed a statistically significant difference in survival of patients whose Ki-67 expression was present in < 1% of the tumour nuclei when compared to patients in which the Ki-67 expression was 1% or greater.($p < 0.0001$).
Proliferating cell nuclear antigen (PCNA), an auxillary protien of DNA polymerase delta,is also a cell cycle associated protein which can be used to estimate the growth fraction of tumours. It has the advantage of application to routinely fixed wax-embedded tissue although Bouins fixation appears to be preferable to formalin fixed tissue, the former being used in this study on 102 prostatic cancer specimens. A relationship with histological grade was also seen with this antigen ($p < 0.001$), PCNA expression being usually more abundant in poorly differentiated tumours. No significant correlation was found however with the PCNA score and the patient's age, metastatic status, or primary tumour grade (T category). In 65 patients the survival of those individuals whose tumours expressed PCNA in < 10% of the tumour nuclei were compared with those patients whose expression was seen in 10% or more of the nuclei. Life table analysis of the two groups indicated that the patients with the lower PCNA score survived significantly longer than those with the higher PCNA scores, $p < 0.04$.
Comparison of Ki-67 expression in frozen sections and PCNA expression in wax-embedded tissue from the same tumour specimens obtained from 86 patients with prostatic cancer showed a significant correlation ($p < 0.001$), although the estimation of the growth fraction using Ki-67 scores was generally lower than that given by the PCNA scoring system. When multivariate analysis of the survival data for prostatic cancer patients was employed Ki-67 sores appeared to be a better prognostic indicator than the PCNA scores, although they could be substituted for one another. Ki-67 expression also appeared from Cox Analysis of the data to be an independent variable from that of histological grade and metastatic status.

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REGULATION OF INTRAPROSTATIC DHT CONTENT

Sabine Tunn, Ralf Nass *, Heike Weißer, Michael Krieg

Institute of Clinical Chemistry and Laboratory Medicine, University Clinic Bergmannsheil, Bochum, and *Medical Clinic "Innenstadt", Ludwig-Maximilians-University, München, Germany

At present it is unknown to what extent the intraprostatic enzymes are involved in the regulation of the actual endogenous concentration of 5α-dihydrotestosterone (DHT). To address this question, we compared the age-dependent activities of DHT metabolizing enzymes with endogenous androgen concentrations, being also associated with aging.
Potential activities (Vmax/Km) of 5α-reductase, 3α-HSORred/ox and 3ß-HSORred/ox were measured in mechanically separated epithelium and stroma of normal prostates and BPH from 30 donors, aged 15 to 95 years, by incubation with radioactively labelled substrate, extraction with ether, and separation of the metabolites by HPLC. The intraprostatic testosterone and DHT concentrations were determined by ether extraction, separation of the steroids by HPLC, and quantification by radioimmunoassay. The significance of age-dependent alterations was calculated by Spearman rank correlation coefficient. $P<0.05$ was considered significant.
The following significant age-dependent alterations were found: 1. Vmax/Km of 5α-reductase decreased with age in epithelium, but remained constant in stroma. 2. At a 2- till 4-fold lower level Vmax/Km of 3α-HSORred and 3ß-HSORred decreased with age in epithelium. Moreover, in stroma Vmax/Km of 3ß-HSORred increased with age. 3. At a rather low level Vmax/Km of 3α-HSORox decreased in epithelium, but increased in stroma. 4. The intraprostatic DHT-concentration decreased with age in epithelium, but remained constant in stroma.
In conclusion: Due to the similarity of age-dependent alterations of 5α-reductase activity and DHT content it appears that the potential activity of 5α-reductase determines the intraprostatic DHT content.

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ANDROGEN RECEPTOR MODULATION IN HUMAN PROSTATIC TISSUE DURING ENDOCRINE COMBINATION THERAPY.

Theodorus H. van der Kwast and Bernard Têtu. Dept. of Pathology, Erasmus University, Rotterdam, The Netherlands and Dept. of Pathology, L'Hôtel-Dieu de Québec, Québec, Canada.

The modulation of androgen receptor (AR) expression in non-malignant prostatic tissues was investigated by immunohistochemistry. Prostatectomy specimens from 7 patients treated for adenocarcinoma with combination endocrine therapy (with an LHRH-agonist and the pure anti-androgen drug hydroxy-flutamide) during three months were compared to specimens obtained from untreated patients. In control prostatectomy specimens most fibromuscular stromal cells as well as the secretory epithelial cells lining the prostatic glands displayed immunostaining for AR. Also nodules of fibromuscular hyperplasia were intensely stained for AR. Combination endocrine therapy led to a dramatic decrease of AR expression of both the stromal and glandular component of the prostates. This reduction in AR was variable as in a number of cases a few glandular epithelial cells and areas of stromal cells retained nuclear immunostaining for AR. The variability in AR down-regulation by androgen blockade, particularly within the stromal compartment, is compatible with the view that an alteration in the regulation of AR expression may underly benign hyperplastic disease of the human prostate.

Posters for plenary discussion

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GENETIC DIFFERENCES IN RESPONSE TO RAS + MYC-INDUCED CARCINOGENESIS IN RECONSTITUTED MOUSE PROSTATE

V.W. Merz[1], D. Kadmon[2], W.F. Flanagan[2], S. Egawa[2], P.T. Scardino[2], T.C. Thompson[2]. [1]Department of Urology, University of Berne, Switzerland; [2]Department of Urology, Baylor College of Medicine, Houston, TX, USA

Previous studies utilizing the mouse prostate reconstitution model system have shown that the ras and myc oncogenes together induce poorly differentiated prostatic adenocarcinomas. The induction protocol involves the introduction of the two cooperating oncogenes via a non-replicating recombinant retrovirus (Zipras/-myc 9) into both the epithelial and mesenchymal cells of the mouse urogenital sinus (UGS) and their subsequent growth in vivo for 4 weeks as renal subcapsular grafts. The virus produces only stable integrations of the proviral DNA in the genome of the primarily-infected cells and their progeny, without causing subsequent spread of the virus to neighboring cells. We now report that the induction of carcinogenesis by this protocol is strain specific. Whereas in the inbred C57/BL6 strain ras and myc induces carcinomas with high frequency (95%, n=24), in BALB/c inbred mice, the two oncogenes induce epithelial hyperplasia (100%, n=8), but never cancer. In all control experiments using a non-oncogenic recombinant retrovirus which carries the bacterial beta-galactosidase gene (BAG-alpha), normal prostatic differentiation was seen.
In order to test whether a longer growth period was necessary for subsequent genetic alterations to accumulate and convert the ras and myc-induced BALB/c hyperplastic glands to the malignant phenotype, Zipras/myc 9-infected UGSs were allowed to grow for 8 weeks instead of the usual 4-week period. The glands remained hyperplastic, but in no case did a carcinoma arise (n=5). Thus, the genetic differences between the resistant BALB/c and sensitive C57/BL6 appear to reside in the step to malignant conversion.
In summary, we have demonstrated a genetic difference in the response of prostatic epithelium to a specific combination of transforming genes. This model offers hope for the identification of heritable tumor suppressor and/or susceptibility genes specific to prostate cancer in humans.

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GENETIC ALTERATIONS OF PROTO-ONCOGENES C-Myc, CerbB-2/Neu and Int-2 IN PROSTATIC CARCINOMA.

G. FOURNIER (1), J.H. ABALAIN (2), A. LATIL (4), Y. AMET (2), A. VOLANT (3), Ph. MANGIN (1), H.H. FLOCH (2), R. LIDEREAU (4).
Services d'Urologie (1), de Biochimie (2), et d'Anatomo-pathologie (3) - Hôpital Morvan, BREST.
(4) Service d'Onco-virologie. Centre René Huguenin. SAINT CLOUD (FRANCE).

Various genetic alterations have been described in human solid tumors. Only a few studies have concerned prostatic carcinoma. Proto-oncogenes amplification (c-erbB-2/neu, c-myc, int 2) were observed in another hormone-dependant carcinoma (breast cancer). Amplifications of these proto-oncogenes were not observed in a previous study concerning prostatic carcinoma in early stage disease (specimens obtained from patients who underwent radical prostatectomy for stage B carcinoma). In our study, fresh tumor specimens were obtained from 15 patients with previously untreated prostatic carcinoma, respectively stage B in two cases, clinical stage C in 7 cases and 6 stage D (D1- one patient and D2-five patients). Specimens were obtained either by radical prostatectomy (2 cases), T.U.R. (6 cases), or trans-rectal needle biopsy (7 cases), and frozen immediatly in liquid nitrogen. A fragment of each sample was analysed by conventional histological methods to verify its malignant status. Genomic DNA was prepared from the frozen specimens and from blood lymphocytes of each patient and used for Southern blot analysis. Proto-oncogene amplifications (c-erbB-2/neu, c-myc, int 2) are not observed, either in our 2 patients with a localized disease (stage B) or in the 13 patients with a more advanced prostatic carcinoma (stage C and D).

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INCREASED EXPRESSION OF HMG-I(Y) IN HIGH GRADE PROSTATE CANCER DETERMINED BY RNA IN SITU HYBRIDIZATION .

Yahya Tamimi, Marion J. G. Bussemakers, Marilene Denyn, Rainy Umbas, Henk Van der Poel, Frans M. J. Debruyne, and Jack A. Schalken.
Urological Research Laboratory, Department of Urology and pathology, Radboud University Hospital, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands.

In a previous study using the Dunning rat prostate cancer model, we found increased expression of HMG-I(Y) in metastatic tumors when compared to non metastatic ones. Interestingly, other studies showed that this 12 KD non histon-chromosomal protein is also over-expressed in undifferentiated fast proliferating myeloid derived cells. Hence, HMG-I(Y) may be a candidate to characterise the aggressiveness of tumors. First, by Northern analysis we showed that HMG-I(Y) expression increases in high grade prostate tumors. These studies, however, require fresh material, and too short clinical follow-up is available. In order to be able to perform a clinical retrospective study, paraffin-embedded material should be used. RNA In situ Hybridization (RISH) allows evaluation of mRNA in such material. Likewise, we studied tumors from 50 patients with prostate cancer, and the microscopic analysis of each sample included 4 differents sections. i) Estimation of non specific hybridization was done on sections hybridized with sense HMG-I(Y) and 28s rRNA probes. ii) The estimation of the preservation of RNA was confirmed by hybridization with 28s rRNA. iii) Hybridization with anti-sense probes allowed the localisation of HMG-I(Y) mRNA in the expressing areas. High expression of HMG-I(Y) was observed in poorly differentiated and anaplastic regions, whereas in moderately differentiated lesions both weakly positive and negative ones could be found. Little or no expression was observed in well-differentiated glands. These data are confirmed by quantification using an image analysis system, showing that a good correlation of HMG-I(Y) expression with undifferentiated phenotype of prostatic tumors exists. Therefore we conclude that HMG-I(Y) expression is related to grade and the fact that archival material can be used makes retrospective studies possible.

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DECREASED EXPRESSION OF E-CADHERIN ASSOCIATED WITH PROGRESSION OF HUMAN PROSTATE CANCER.

Rainy Umbas[1], William B. Isaacs[2], Tilly W. Aalders[1], Bob S. Carter[2], Herbert F.M. Karthaus[3], H. Ewout Schaafsma[4], Frans M.J. Debruyne[1] and Jack A. Schalken[1].
[1]Department of Urology and [4]Pathology, University Hospital Nijmegen, Nijmegen, The Netherlands; [2]James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD, U.S.A.; [3]Department of Urology, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands.

E-cadherin is a Ca^{2+}-dependent cell adhesion molecule thought to play an important role in normal growth and development, via mediation of homotypic, homophillic cell-cell interaction. Recent studies suggest that E-cadherin may be important in neoplastic progression as well, particularly as a suppressor of invasion. We have previously demonstrated that the invasive phenotype of rat prostate cancer cells is associated with decreased expression of E-cadherin (Bussemakers, M.J.G., Van Moorselaar, R.J.A., Giroldi, L.A., Ichikawa, T., Isaacs, J.T., Debruyne, F.M.J., and Schalken J.A. Cancer Res., *in press*, 1992.). This is of particular interest since the locus to which the human E-cadherin gene is mapped (16q21) is frequently involved in allelic loss in prostate cancer. (Carter, B.S., Ewing, C.M., Ward, W.S., Treiger, B.F., Aalders, T.W., Schalken, J.A., Epstein, J.I., and Isaacs, W.B. Proc. Natl. Acad. Sci. USA, *87*:8751-8755, 1990 & Bergerheim, U.S., Kunimi, K., Collins, V.P., and Ekman, P. Genes Chromosomes & Cancer, *3*: 215-220, 1991.)
Impaired E-cadherin function is likely to be associated with aberrant expression of the protein. We therefore analyzed E-cadherin expression in situ by immunohistochemistry using anti-L-CAM (Uvomorulin), a monoclonal antibody against E-cadherin. We used snap frozen tissues of 84 human prostatic carcinoma specimens as well as 7 metastatic lesions in lymph nodes and 1 in testis. Twenty-three non malignant prostate specimens were also included in this investigation. In 40 out of 84 tumors, these studies showed reduced E-cadherin staining when compared to non malignant prostate which uniformly stained strongly positive at the cell-cell boundaries. Decreased levels of E-cadherin were also detected in 6 out of 8 metastatic deposits of prostate cancer. Whereas, there was a significant relation between decreased expression of E-cadherin and increasing Gleason score, the differential staining pattern within the groups of histologically similar tumors could be of even greater interest. Considering the biological function of this protein, it is tempting to speculate that these tumors have a worse prognosis. The value of this finding should now be tested in prospective and where possible retrospective trials.

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Integrin expression in human prostate cell lines PC3,LnCap, RII in vitro and grafted to the immunosupressed newborn rat.

B.Cuzin, N.Daemi*, M.F.Jacquier*, J.M.Maréchal, J.M.Dubernard, L.Rémy*.
Department of Urology and Transplantation, Herriot Hospital, Lyon
*Inserm U.218 Lyon.

Integrins are a family of cell surface proteins that mediates cell adhesion and are involved in tumor cell invasion and metastases. Human prostate cell lines LnCap, RII (hormonosensitive) and PC3 (hormonoinsensitive) were grown in Mac Coy 5 A medium supplemented with 10%FCS on Labtek. Cultured cells were studied by immunolocalizationfor the presence of adhesion molecules such as: integrins $\alpha v\beta 3, \alpha 1, \alpha 2, \alpha 3, \alpha 5, \alpha 6, \beta 1, \beta 4$; cadherin and for the presence of laminin using indirect IF. LnCap and RII expressed a similar surface reactivity against $\alpha v\beta 3, \beta 1, \alpha 5, \alpha 6, \beta 4$, cadherin and a very weak staining for $\alpha 1, \alpha 2, \alpha 3$.PC3 expressed a strong reactivity against $\alpha 1, \alpha 2, \alpha 3, \alpha v\beta 3, \alpha 6, \beta 1, \beta 4$ a moderate staining against $\alpha 5$ and a weak staining against cadherin.None were colocalized with laminin.
LnCap cells were injected subcutaneously to immunosuppressed new born rats with matrigel and after 21 days have produced tumors without any lung metastases.These tumors showed the same pattern of expression of integrins as in the cultured cells. PC3 cells were injected without Matrigel to immunosuppressed newborn rats and have produce tumors and lung metastases (6/6). The tumors showed also the same pattern of integrin expression as in the cultured cells.The metastases seem to be more differentiated.
In conclusion these results showed that:1- Various integrins are expressed on the three human cell lines .The expression seems to be related to the invasivness and maybe to the hormonosensitivity. 2-our experimental animal model allows the growth of human prostatic tumors and lung metastases with a high rate of success.

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EFFECTS OF THE IMMUNOMODULATORY COMPOUND ROQUINIMEX ON PROSTATIC TUMOURS AND STEROIDAL AND PEPTIDE HORMONES.

Björn Forsgren, Kabi Pharmacia Oncology, Lund, Sweden.

The quinoline derivative roquinimex (Linomide^{R}) is an immunomodulatory compound with therapeutic effects in autoimmune disease and in experimental tumours, for example the Dunning R-3327 PAP, AT-1, AT-2, MAT-Lu and MAT-LyLu rat prostatic cancer sublines. Roquinimex inhibits the growth of these tumours in vivo as well as the metastatic spread of the two last-mentioned. Recent studies showed that roquinimex reduces the weights of testes, seminal vesicles and prostatic glands in rats, and decreases their plasma levels of testosterone, oestradiol and luteinizing hormone. In contrast plasma corticosterone is increased simultaneously with a slight increase in adrenal weight. Taken together these effects aroused an interest in roquinimex as an possible agent for treatment of prostatic cancer. The effects indicate that roquinimex affects the hypothalamus-pituitary-gonadal axis. Further studies on this mechanism of action are in progress. The possible connection between the immunological and hormonal effects will be touched upon.

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LOW DOSE CYCLOPHOSPHAMIDE INHIBITION OF DUNNING MAT LY LU GROWTH IN VIVO POTENTIALIZED BY POLYAMINE DEPRIVATION.

Y. BLANCHARD*, B. CIPOLLA, V. QUEMENER*, F. GUILLE, J.Ph. MOULINOUX* and B. LOBEL
Departments of Urology and Therapeutique anticancereuse*,
C.H.U. RENNES, FRANCE.

Polyamine deprivation combining a polyamine free diet, polyamine inhibitors DFMO and MDL 72527, neomycine and metronidazole for intestinal tract decontamination has been proved to significantly inhibit Dunning Mat Ly Lu tumor growth in vivo (Moulinoux et al Journal of Urology 1992, 146, 1408). In order to enhance this inhibition various chemotherapic regimens have been associated with a very significant impact on tumor growth but with also a very important toxicity (unpublished date). We have investigated the possibility of potentializing the effect of low dose cyclophosphamide by polyamine deprivation.
Material and Methods : 32 Adult Fischer-Copenhaguen F1 Rats were grafted subcutaneously with 2.10.6 Dunning Mat Ly Lu cells. When the tumors were palpable, the rats were randomly separated into 4 groups.
Group 1 : controls with a standard regimen chow (S.R.C.)
Group 2 : SRC + 20 mg/kg cyclophosphamide I.P. weekly,
Groupe 3 : Polyamine deprivation as already described 5 days a week,
Group 4 : polyamine deprivation 5 days/7 + 20 mg/kg cyclophosphamide I.P. weekly.
Results : Tumor volumes measured 32 days after tumor inoculation are for groupe 1 : 79 +/- 45cc, group 2 : 63 +/- 44cc, group 3 : 34 +/- 12cc and group 4 : 8 +/- 4cc realising a 90% volume reduction for polyamine deprived + cyclophosphamide treated rats compared to controls. Median survival tume is 34 days for group 1 and has not been reached for the other groups at the time of this abstract. Toxicity for group 4 rats is minimal.
Conclusions : Dunning Mat Ly Lu tumor growth inhibition in vivo by polyamine deprivation is confirmed. Low dose cyclophosphamide with standard chow is minimally effective on tumor growth. When associated to a polyamine deprivation both effects are significantly potentialised with minimal toxicity and survival should be improved.

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EFFECT OF FOCUSED EXTRACORPOREAL PYROTHERAPY ON THE METASTATIC BEHAVIOR OF THE DUNNING RAT PROSTATIC CANCER MODEL

N. BATAILLE[1], G. VALLANCIEN[2], E. CHARTIER-KASTLER[2], C. SCHATZ[1], S. GIL DIEZ[1] and D. CHOPIN[1]. (1) Service d'Urologie, Hôpital Henri Mondor, 94010 Créteil, France ; (2) Département d'Urologie, CMC de la Porte de Choisy et Centre d'Etude et de Recherches Appliquées, Fondation pour l'Avenir, 75013 Paris, France.

Pyrotech® is a device which is able to generate focused extracorporeal ultrasound waves. The device is composed of a therapeutic transducer coupled to a diagnostic transducer with the same focal lenght. Physical design of Pyrotech allows generation of necrosis effects: i.e., generation of high temperature, in a small volume, in a short period of time (≤ .5 sec). This system has been previously tested in vitro on human bladder carcinoma cell line 647V and in vivo on animal and human normal tissue. We have demonstrated (Vallancien et al, Eur. Urol., 1992) that a focalized necrosis can be obtained and that malignant cells can be destroyed. These tissue-effects are due to high temperature-generation and cavitation. Cavitation might be expected to have disruptive effects on cells within tissues and therefore, may promote dissemination of malignant cells via the blood or lymphatic systems. We have tested this hypothesis on the prostatic Dunning cancer model R3327 using a highly metastatic cell line (Mat Ly Lu) and a non-metastatic variant (G). The sublines were implanted subcutaneously in the leg and the tumor volume was determined twice a week by the equation $L \times W \times H \times \Pi/6$. Nodes and pulmonary metastasis were evaluated periodically on both treated (N = 30) and sham operated groups (N = 17) by groups of 3 animals at two weeks intervals. A local effect was obtained with G subline and a significant delay in growth with Mat Ly Lu. When a local effect was obtained there was a significant delay in occurrence of metastasis. In none of the treated animals we have observed a positive effect of the metastatic pattern when compared to controls. These data suggest that in addition to local effect there was no increase in the metastatic behavior of Dunning tumors treated with Pyrotech®.

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ANTITUMORAL EFFECTS OF LIAROZOLE ON THE GROWTH OF TRANSPLANTABLE R3327 PROSTATIC CARCINOMA IN RATS.

Gerda Smets, Robert Van Ginckel, Jean Van Wauwe, Marie-Claire Coene, *Frans C.S. Ramaekers, Marcel Borgers, Roland De Coster.
Janssen Research Foundation, Beerse, Belgium; *Department of Molecular Cell Biology & Genetics, University of Limburg, Maastricht, The Netherlands

The antitumoral activity of liarozole has been studied in the androgen-independent R3327 AT6 Dunning prostate carcinoma, grafted subcutaneously in (Copenhague x Fisher) F1 male rats. Liarozole is an imidazole-containing compound that inhibits the cytochrome P-450-dependent catabolism of all-trans-retinoic acid (RA). Oral administration of liarozole at dose levels of 80, 60, 30, 15, 7.5, 3.75 mg/kg twice daily for one month reduced mean tumor volume by 85 %, 84 %, 88 %, 75 %, 71 %, 84 %, respectively as compared with tumor growth in control and castrated rats. Levels of RA were measured in plasma and in tumors. The lowest dose of liarozole that induced detectable levels of RA in most of the animals was 30mg/kg for plasma and 3.75 mg/kg for tumor. In castrated as well as in control animals, plasma and tumor RA levels were almost not detectable (<0.5 ng/ml plasma or < 1 ng/g tumor tissue). Histologically, this Dunning subline is characterized by a keratinizing squamous carcinoma. The degree of keratinization was immunocytochemically detected with monoclonal anti-cytokeratin 56.5 kDa (CK10). Liarozole treatment resulted in dekeratinization, which was dose-dependent and at 80mg/kg of liarozole almost no keratinized squames could be found. This histological finding is probably related to the enhancement of RA levels in plasma and mainly in tumors, and supports the hypothesis that liarozole exerts its antitumoral activity through its inhibition of RA catabolism.

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EFFECTS OF CHEMICAL CASTRATION BY LHRH-ANALOG AND FLUTAMIDE ON LIPID METABOLISM

L. Denti, G. P. Ceda, G .Ceresini, G. Pasolini, *P. Cortellini, *S. Ferretti, A. Banchini and G. Valenti
Chair of Geriatrics and Gerontology, * Chair of Urology, University of Parma, Parma-Italy

The hormonal therapy of prostatic cancer can be accomplished by different protocols to achieve a chemical castration, but with different changes of endogenous sex steroid pattern.So far, to our knowledge, few data are available concerning the effects of such treatments on lipoprotein metabolism, which has been shown to be modified by the administration of exogenous estrogen and androgen compounds. The aim of our study was to investigate the effects of different therapeutical protocols on the lipid pattern, employing an LHRH-analog(Goserelin) and an anti-androgen non steroidal substance (flutamide), alone or in combination.Materials and methods. 36 patients, aged 62-81 yr., affected by prostatic cancer (C or D stage) were studied. Each of them was assigned to one of 3 groups, according to treatment: Group A) Flutamide 250 mg TID per os; Group B) Goserelin 3,6 mg, at 28 day intervals, subcutaneously; Group C) Flutamide plus Goserelin, at the same dosages as the two first groups. Before therapy and after 8 weeks, blood samples were collected for the determination of T, and E2 and for the assessment of lipid pattern Results E2 and T levels were significantly decreased after LHRH-analog administration, alone or with Flutamide; on the contrary Flutamide alone induced a significant increase of both steroids. An increase of HDL-cholesterol and Apo AI levels was documented in all groups, reaching the statistical significance only in group B and C, while total Cholesterol, LDL-Cholesterol, Triglycerides and Apo B were unaffected by treatment.The percentage increase of HDL-cholesterol documented in patients treated with flutamide was significantly higher in comparison with patients treated with flutamide plus LHRH-analog; as a consequence, Group B showed a more favourable LDL/HDL ratio.Conclusions Our data showed that chemical castration achieved by LHRH-analog, combined to a complete androgen receptor blockade by flutamide, can significantly affect lipoprotein metabolism by increasing HDL-Cholesterol and Apo AI levels.This effect is not so evident after LHRH-analog administration, in absence of Flutamide; this could be due to the persistence of adrenal steroids. HDL increase is more evident if a concomitant increase of Estrogen levels is associated to the blockade of testosterone action, as it can be achieved by Flutamide administration. In conclusion these data suggest that in man endogenous androgens, as well as exogenous androgen substances, affect HDL and Apo AI metabolism, by suppressing their circulating levels. However, their action is modulated by estrogens, that also in males, as it has been shown in women, can play a significant role in lipid metabolism.

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DOES BLADDER UROTHELIUM PLAY A ROLE IN THE INITIATION OF THE BCG-ASSOCIATED IMMUNE RESPONSE AFTER INTRAVESICAL INSTILLATION?

D. Schamhart, L. de Boer, P. Vos and K. Kurth
Dept. Urology, Univ. Amsterdam, The Netherlands

A BCG-initiated local immunological response is generally accepted causing the antitumor activity of (adjuvant) intravesical treatment of superficial bladder cancer by BCG (Bacillus Calmette-Guérin). Although knowledge about the involvement of cytokines and cell components of the immune system is rapidly increasing, substantial evidence concerning the mechanism and role of adherence of BCG to the bladder wall is still inadequate. One of the open questions is the possible (specific) interaction between BCG and normal bladder urothelium in the process of immune stimulation.
In the present study the effects of BCG on the gene expression of the human bladder cancer cell line T24 was investigated. For comparison several other bacteria and a bacterial cell wall skeleton preparation of Nocardia rubra [N-CWS] (Rubratin) were used. Incubation of subconfluent monolayer cultures of T24 cells (4 cm^2 dishes, 2 ml medium) in the presence of a dose of BCG ranging from 0, 5E4, 5E5 and 5E6 CFU/cm^2 resulted in a dose-dependent 24-h IL-6 secretion of 0.4, 2.7, 28.9 and 22.9 ng/ml, respectively. Comparable results were obtained with TNFα secretion, whereas no IL-1B was detected under these experimental conditions. In comparison, 24-h co-culturing in the presence of the highest used concentrations of Rubratin (15µg), Escherichia coli (2.5E8) and Streptococcus feacalis (2.5E8) showed an IL-6 secretion of 2.7, 0.0 and 0.9 ng IL-6/ml, respectively. In a next series of experiments, the relation between the period of presence of BCG and T24-associated IL-6 secretion was monitored (for 24 h) after co-culturing T24 cells with various concentrations of BCG (1E3-1E6 CFU/cm^2) for 0 and 15 min and 2 and 24 h. The results showed a strong dependency on the period of co-culturing: No induction of IL-6 secretion was found during 24 h after a 15-min BCG incubation, while the kinetics of IL-6 secretion after a 2-h incubation appeared to be intermediate compared to the continuous 24-h presence of BCG. Moreover, IL-6 secretion appeared to be detectable (detection limit 5 pg/ml) after 2-3 h. These latter results seem in agreement with those of Ratliff, showing substantial internalization of BCG into T24 cells after 2 h (personal communication).
In conclusion, assuming that the present results may be translated to the response of normal urothelium to BCG, it is tempting to speculate that a BCG-initiated response of normal urothelium requires a minimum period of time, being of clinical relevance. Currently experiments are in progress studying BCG-induced IL-6 secretion of T24 cells pretreated with anti-fibronectin, preventing phagocytosis of BCG.

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DIRECT CONJUGATION OF SULFONATED PHTHALOCYANINES VERSUS ENCAPSULATION INTO LIPOSOMES BEARING ANTIBODY FOR SELECTIVE PHOTOLYSIS OF HUMAN BLADDER CARCINOMA CELLS IN VITRO: EFFET OF TEMPERATURE AND URINE

J. MORGAN, H. LOTTMANN, J.J. PATARD, C. SCHATZ, C.C. ABBOU and D.K. CHOPIN, Service d'urologie, Hôpital Henri Mondor, 94010 CRETEIL, France

There is a need to improve photodynamic therapy selectivity and to better control targetting of tumor cells within specific tumor compartments. Selective in vitro phototoxicity of a human bladder carcinoma cell line 647V has been achieved by mean of targetting sulfonated phthalocyanines (AlSPc) with antibody. AlSPc were either directly coupled to antibody or encapsulated into small unilamellar vesicles bearing antibody (Ab-SUV). Monoclonal antibody E7 (IgM subclass) which recognized an antigenic determinant on 647V and absent on T24 (human bladder carcinoma cell line) and a control IgM antibody was directly coupled to aluminium tetra-2-sulphonyl chloride phthalocyanines AlSPc $(SO_4cl)_4$). Tetra AlSPc or mix of tri and tetra AlSPc were encapsulated into Ab-SUV. Sulforhodamine (RSC) and carboxyfluoresceine (CF) were respectively coupled to E7 or encapsulated into Ab-SUV. Immunofluorescence studies on living cells demonstrate specific cell surface localization of conjugates and internalization at 37°C. Phototoxicity was measured by MTT assay [3-(4,5dimethylthiazol-2-5-diphenyl-tetrazolium) bromide] after exposition to red light for a total of 3.6 J-cm^2. Selective significant AlSPc dose-dependent phototoxicity was observed with SUV-E7 or AlSPc-E7 in the range of 1 µM to 8 µM AlSPc at 4°C, 4°C plus 37°C and 37°C. Direct comparaison of the two kind of conjugates showed no difference in selective phototoxicity in the limit of the MTT assay. Free tetra AlSPc has no toxicitiy at any concentration, free AlSPc mix has significant but less toxicity at 8 and 4 µM. Significant increased of selective phototoxicity was observed at 4°C plus 37°C and 37°C, non-significant decrease of phototoxicity was observed in the presence of urine during illumination. SUV conjugates allow targetting of hydrophilic dye and to target 10 times more photosensitizer than antibody alone. These results suggests that targeted photosensitisers such as AlSPc could be used for mucosal directed therapy and may be useful for selectivly damage epithelial tumor cell compartment.

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THE ANTITUMOR EFFECTS OF LOW DOSE TNF-α WITH MULTIFOCAL HESW TREATMENT IN A RAT BLADDER CANCER

Cornel, Erik B., Cambeen Leon D.A.F., Smits, Geert A.H.J., van Stratum, Peter, Oosterhof, Gosse O.N., Debruyne, Frans M.J., and Schalken, Jack A. Department of Urology University Hospital Nijmegen, The Netherlands.

High Energy Shock Waves (HESW) in cancer therapy regained importance by the fact that they can potentiate the effect of cytokines to an extent where complete regression in established renal cancer xenografts can be provoked. In these studies however, cytokines were administered *peritumorally* in high doses and the HESW were given *unifocally*. In this experiment we treated another potential target for this modality, namely superficial bladder tumors. An appropriate model to study the antiproliferative effects of new treatment modalities in transitional cell carcinoma (TCC) is the syngeneic rat bladder tumor RBT323, which closely resembles human TCC. Moreover, this syngeneic model in immunocompetent animals enables us to study combined treatment strategies for efficacy and mode of action. Therefore we used this model to evaluate the antitumor effects of *systemic* low dose Tumor Necrosis Factor-α (TNFα) combined with uni- or multifocally HESW treatment.

Transplantation of the tumor was performed subcutaneously at the right hind limb by trocar pieces (20-30 mg). Tumors (140-170 mm^3) were exposed uni- or multifocally to 6000 or 4 x 1500 HESW (experimental electromagnetic generator Siemens), respectively, in three successive sessions with 12 hours intervals. Rats treated with TNF-α in combination with uni- or multifocal HESW treatment received TNF-α (17 µg/Kg rat) one minute before each HESW treatment systemically by a v.jugularis catheter.

Whereas 6000 HESW hardly influenced tumor growth, multifocal application of 4 x 1500 HESW showed a significant tumor growth inhibition in comparison with control tumor growth. The combined TNFα-HESW treatment (uni- as well as multifocal) resulted in a more pronounced antitumor effect. Several tumors treated with TNF-α and multifocal HESW showed complete regression. In both combination groups tumor growth cessation could be achieved, but after a 5 - 7 days these tumors started growing again.

Conclusion: Multifocal treatment schedules significantly enhances the antitumor effects of HESW compared to unifocal application. Futhermore, systemic TNFα cytokine therapy in combination with HESW results in enhanced growth inhibition of rat bladder cancer.

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ENHANCED IMMUNE RESPONSE TO INTRAVESICAL BCG BY LOCAL PENTOSAN POLYSULPHATE PRETREATMENT: A POSSIBILITY TO IMPROVE BCG THERAPY FOR SUPERFICIAL BLADDER CARCINOMA

L. de Boer[1], D. Schamhart[1], P. Vos[1], R. Vleeming[1], P. Steerenberg[2], K. Kurth[1]
[1]Dept. of Urol, Univ. of Amsterdam, The Netherlands
[2]Lab. for Pathology, Natl. Inst. of Public Health, Bilthoven, The Netherlands

Intravesical BCG therapy is effective in treatment and prophylaxis of superficial bladder cancer. The average complete response rate is about 60-70%. An immune response to BCG may be required to achieve an antitumor reaction although the actual relation with the clinical response is still vague. In some patients the initiation of the immune reaction may not occur due to insufficient adherence of BCG to the bladder wall. Enhancement of BCG adherence to the bladder wall, resulting in an increase of the immune response may improve intravesical BCG therapy.
Pentosan polysulphate (PPS) is a polysulphated polysaccharide with glycosaminoglycan (GAG)-like properties. In line with this PPS has a reported anti-adherence capacity for bacteria to the bladder mucosa, and it is applied as a drug to cure chronic- and radiation-induced cystitis. In in vitro experiments we have found PPS to bind significantly to BCG (3.6, 3.1, and 1.8 µg/mg dry weight for BCG RIVM, Pasteur and Connaught, respectively) in contrast to other, commonly found bacteria inducing cystitis (0.2, 0.3, 0.7, and 0.0 µg/mg dry weight for E.coli, S.faecalis, K.pneumoniae, and Proteus, respectively). Moreover, PPS binds to the bladder surface in vivo; after administration of 40µg, 80 µg, and 10 mg in appropriate volumes into rat, guinea pig and human bladders a binding capacity of 39.1±0.3 µg, 75.7±1.1 µg, and 4.3±1.8 mg PPS (n>5) was determined, respectively. Experiments with [3H]uracil-labelled BCG indicated an increased binding of BCG to the PPS-pretreated guinea pig bladder. Based on these results it was investigated in a guinea pig model whether the immune response to intravesical BCG could be increased by local pretreatment with PPS.
In 3 separate experiments guinea pigs were intravesically treated 6 times (once per week) with 5E7, 1E7, or 5E6 CFU BCG-RIVM, respectively, with or without pretreatment with 10 mg PPS in 1 ml for 0.5 h. In all experiments a significant immune response to intravesical BCG (+ or - PPS) was observed compared to controls (PPS+PBS). Depending on the dose of BCG an increase in several immunological parameters by PPS pretreatment was repeatedly observed; A) number of bladder wall infiltrates, 156% increase (BCG 14.4±13.9 vs. PPS+BCG 22.4±4.2, n=5), B) cell number in iliac lymph nodes, 154% increase (BCG 283±234 vs. PPS+BCG 435±359), C) weight of iliac lymph nodes, 127% increase (BCG 0.15±0.03 vs. PPS+BCG 0.19±0.10), D) PPD skin reaction, 116% increase (BCG 12.3±1.3 vs. PPS+BCG 14.3±2.4).
In conlusion, the enhanced immune response to intravesical BCG by PPS pretreatment may possibly be of future significance to increase the efficacy of intravesical BCG therapy. The optimal combination of PPS and BCG is currently under investigation.

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INTERIM RESULTS FROM PROSPECTIVE ANALYSIS OF THE VALUE OF KARYOMETRY FOR THE EARLY DETECTION OF BLADDER TUMOR RECURRENCES BY BLADDER WASHINGS

H.G. van der Poel, P. van Stratum, A.E. de Ruijter, G.O.N. Oosterhof, F.M.J. Debruyne, J.A. Schalken
Dept. Urology, University Hospital Nijmegen, The Netherlands.

The high recurrence rate (80%) of transitional cell carcinoma (TCC) of the bladder stresses the need for reliable follow up of these patients. Of the recurrent tumors 10-30% progress to invasive phenotype.

In an earlier study (van der Poel, 1991) we found the nuclear shape factor based on the Freeman difference chain code and the 2c Deviation Index to be of predictive value for the presence of tumor. In the present on-going second-phase study we analyzed the karyometric features of nuclei in bladder washings. Based on these features for each sample the chance of tumor was calculated. Bladder wash material from 5 institutes is immediately fixed in Carbowax and mailed to our lab. The karyometric analysis is done with the developed KARYOMETRY-system and for each sample a analysis report is returned to the clinician.

To date 925 samples from 410 patients have been analyzed. In 203 of these cases simultaneous tumor biopsies could be obtained. Tumor recurrence occurred in 48 cases during follow up. From analysis of the samples prior to tumor recurrence it appeared to the chance of tumor as scored by our system was significantly higher in recurrent tumors compared to patients without tumor recurrence during follow up. This was dependent on both tumor grade of the recurrent tumor and the time between the sample and tumor recurrence.

From these preliminary data and our experience with the logistic aspects of the study, we conclude that karyometric analysis of bladder washings is a useful and low cost screening tool for recurrent bladder cancer.

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IMMUNHISTOCHEMICAL DETERMINATION OF AGE RELATED PROLIFERATION RATES IN NORMAL AND BENIGN HYPERPLASTIC HUMAN PROSTATES

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

Benign prostatic hyperplasia (BPH) in men is a pathological condition associated with the presence of the testes and aging. Based on autopsy studies there is an age-dependent increase in volume of the prostate throughout the entire life of men. To study the question if this age-related growth of the human prostate may be based on an increase in proliferation rate, Ki-67 antibody labelling was used as this antibody recognizes one such protein that is present only in the nucleus of cycling, but not in resting, cells.
In prostates of 20 men with BPH removed by open prostatectomy (43-94 years old; mean weight 87 gm, range 30-200 gm) proliferation rates were determined immunohistochemically with the ABC (avidin-biotinylated peroxidase complex) method in epithelium and stroma. These results were compared to data obtained in 4 normal prostates (NPR) from organ donors (15-52 years old; mean weight 19.5 gm, range 9.5-33.5 gm). Proliferation rates were calculated in an area of approximately 5 mm^2 (approximately 9000 nuclei) in each specimen using a computer assisted image analysis-system.
Proliferation rate ± SD in epithelium of BPH (0.139 ± 0.083 %) was slightly higher compared to data obtained in stroma (0.121 ± 0.083 %). In NPR proliferation rate in epithelium (0.015 ± 0.006 %) and in stroma (0.003 ± 0.006 %) was 8 times and 40 times, respectively, lower compared to BPH. There was no significant correlation between proliferation rate and age in epithelium as well as in stroma of BPH. In addition no significant correlation between prostate weight and proliferation rates in both compartments could be demonstrated.
The results obtained indicate that the increase in BPH volume in aging men is not associated with an increase in proliferation rate.
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GROWTH FACTOR EXPRESSION IN EPITHELIAL OR FIBROBLASTIC CELLS CULTURE DERIVED FROM NORMAL PROSTATE OR BENIGN PROSTATIC HYPERPLASIA

F.DESGRANDCHAMPS*, O.CUSSENOT*, P.TEILLAC*, F.CALVO**, A. LE DUC*

(*)Dept of Urology, Pr A LE DUC, Hôp. Saint-Louis, Paris, France
(**)Pharmacology Lab., IGM, Hôp Saint-Louis, Paris, France

It's now widely accepted that factors other than androgen are involved in the normal and abnormal growth of the prostate, including benign prostate hyperplasia (BPH). Those growth factors have been suggested to be involved in the prostatic Epithelial-Stromal interactions, but very few is known on the prostatic cells responsible for their production.

Using a Northern blot analysis, we examined selective cultures of human prostatic epithelial or fibroblastic cells for expression of basic fibroblast growth factor (bFGF), transforming growth factor type beta1 (TGFß1), transforming growth factor type alpha (TGFα), and of its receptor, the epidermal growth factor receptor (EGF-R).

Those selectives cultures were derived from human adult or foetal prostates, and from BPH.

We have succeeded in identifying bFGF transcripts in normal adult prostatic epithelial cells, but levels of bFGF expression were significantly higher in normal adult and foetal fibroblasts than in epithelial cells.

TGFß1 mRNAs expression were detectable in fibroblastic cells as well as in epithelial cells, with no difference between BPH and normal prostates.

TGFα mRNAs expression were detectable in epithelial cells, with no difference between BPH and normal prostates, but were undetectable in fibroblastic cells.

EGF-R mRNAs expression were detectable in epithelial cells, and were depending on culture conditions in fibroblastic cells.

These findings point out the differences of growth factor production potentiality between the stromal and the glandular component of the prostate, and suggest that those growth factors could be locally produced in the prostate to be involved as paracrine or autocrine control of the prostatic growth.

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INCREASE LUMINAL CELL COMPARTMENT BUT INCREASE BASAL CELL PROLIFERATION AND EGF RECEPTOR EXPRESSION IN BENIGN PROSTATIC HYPERTROPHY (BPH) COMPARED TO NORMAL ZONAL-PROSTATIC TISSUE

S. GIL DIEZ[1], A. HIJAZI[2], V. RAVERY[1], J.J. PATARD[1], F. RAMAEKER[3], C.C. ABBOU[1], G. CARATERO[2] and D. CHOPIN[1].
1 Service d'Urologie, Hôpital Henri Mondor, 94000 Créteil, France ; 2 Laboratoire d'Histologie Embryologie, Faculté de Médecine de Purpan, 31073 Toulouse, France ; 3 Department of Molecular and Cell Biology, University of Limburg, PO Box 616, 6200 MD Maastricht, The Nertherlands

Evidences from animal studies have suggested a compartmental organization of prostatic epithelium precisely regulated during prostatic development and steady-state mature gland. We have studied basal and luminal cell using specific cytokeratin monoclonal antibodies and EGF-receptor using monoclonal antibodies directed against the external binding domain. Nine normal young adult glands were sampled according to McNeal zonal anatomy and 10 BPH. BPH specimen were all obtained by open surgical enucleation. From these 9 normal prostates we obtained 7 transition-zone (TZ) biopsies, 9 peripheral zone (PZ) biopsies and 6 central zone (CZ) biopsies. Immunohistochemistry and flow cytometry using DNA and antibody labelling was performed on all specimen.

CMF RESULTS	NORMAL PROSTATE TZ (7)	PZ (9)	CZ (6)	BPH (10)
RGE53				
% CF	29 ± 12	47 ± 17	34 ± 9	55 ± 17
FPP	22 ± 3	26 ± 6	23 ± 4	19 ± 2
RCK103				
% CF	42 ± 11	52 ± 20	42 ± 10	42 ± 15
FPP	18 ± 8	19 ± 6	17 ± 8	26 ± 7
EGFR				
% CF	31 ± 13	45 ± 18	40 ± 18	50 ± 19
FPP	10 ± 6	14 ± 9	18 ± 9	18 ± 7

% CF: percentage of fluorescent cells ; FPP: percentage of proliferative fraction

Immunochemistry demonstrate that RGE53 was limited to luminal cells. RCK103 stained strongly basal cells and faintly luminal cells. EGFR was detected on basal cells. Cytometry data indicate and increase proliferation population when antibody-defined cell compartment were used compared to total DNA profile ($p<0.05$). There is an increase in % CF for luminal cell when TZ was compared to BPH but FPP was identical. There was no increase in % CF for basal cell in TZ compared to BPH but in FPP ($p<0.05$). There was an increase of EGFR % CF and FPP in TZ compared to BPH. Cytometry and histochemistry data are compatible for phenotypic expression and cell localization of EGFR on basal cells. These data are in favor of an activation of basal cell during BPH. Since these cells are not expressing androgenic receptor, this activation is probably mediated by other paracrine or juxtacrine interactions involving EGF receptors. Basal cell may be an important mediator for stromal-epithelial interaction in BPH.

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TUMOR BLOOD FLOW REDUCTION INDUCED BY HESW TREATMENT AS MONITORED BY HOD EFFLUX DETECTED BY MAGNETIC RESONANCE SPECTROSCOPY

Smits, Geert A.H.J., Cornel, Erik B., *Heerschap, Arend, Oosterhof, Gosse O.N., Debruyne, Frans M.J., and Schalken, Jack A. Department of Urology and *Radiology, University Hospital Nijmegen, The Netherlands.

High energy shock waves (HESW) have recently been introduced as a new experimental anti-tumor modality. A temporary suppression of tumor growth has been demonstrated for different xenografts after local application of HESW. The mechanisms underlying this biological effect are not well understood. Histological examinations in various tumor models suggest that tumor necrosis is promoted by vascular damage. In a recent study we have shown that the application of HESW, focussed on the center of NU-1 human kidney cancer xenografts results in dose dependent temporary changes in the ^{31}P NMR spectrum of the tumor, similar to that produced by temporary ischemic inhibition of energy metabolism. In this present study we sought to corroborate the hypothesis that an important target of HESW involves the vascular functionality of the tumor. Tumor blood flow ml/100g.min (TBF) was measured by ^{2}H NMR monitoring the washout of $^{2}H_2O$.
NU-1 kidney cancer xenografts (250mm^3) were exposed to 0 and 800 electromagnetically generated HESW (Siemens Lithostar, P+=37.5 MPa, P-=5.2 MPa, 0.75 Hz). Each group consisted of at least five mice. NMR measurements were performed on a Bruker spectrometer equipped with a 4.7 T magnet employing a home-built $^{1}H/^{31}P$ or a $^{1}H/^{2}H$ double tunable two-turn surface coil. For ^{2}H NMRS 3 x 10μl $^{2}H_2O$ was injected at three different sites in the tumor. Within 90 seconds after injection, ^{2}H NMR measurements were obtained, and serial $^{2}H^{2}O$ NMR measurements continued for approximately 1 hour to follow $^{2}H_2O$ washout.
The control group, treated with 800 HESW adjacent to the tumor, showed a small, not significant increase in TBF. After exposure of the tumor to the same number of shock waves focussed on the tumor center, the mean TBF decreased to about 75% after 2 hours and 50% after 24 hours as compared to the mean pretreatment TBF. Levels in the treatment group were significant lower than in the control group (Wilcoxon; $p<0.005$, and $p<0.05$ respectively). Retreatment of these tumors with the same dose of HESW after normalization of TBF showed that the reduction of the TBF due to a second HESW treatment last longer.
Conclusion: This study further supports the hypothesis that the vascular functionality of the tumor is the primary target of HESW. Moreover, impaired TBF together with an altered energy metabolism and acidification of a tumor can provide a better rational for local enhancement of adjuvant therapies through HESW.

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THE GENERATION OF ANTI-IDIOTYPE MONOCLONAL ANTIBODIES FOR RENAL CELL CARCINOMA.

Hirotsugu Uemura, Wilhelmus P. Peelen, Erika Timmer, Frans M.J. Debruyne, Egbert Oosterwijk. Department of Urology, University Hospital Nijmegen, The Netherlands.

Anti-idiotypic antibodies (anti-Id) offer promise as 'vaccines' to various infectious agents, and they might be used to induce immune responses to tumor antigens as well. The potential application of anti-Id monoclonal antibodies (mAbs) that are the mirror image of human tumor-associated antigens (TAA) to implement active specific immunotherapy in patients with solid tumors has stimulated interest in the development of anti-Id mAbs elicited with antihuman TAA mAbs.
G250 is an as yet undefined cell surface antigen expressed by human renal cell carcinoma (RCC) but not detected in normal kidney. Expression in normal tissues is highly restricted and limited to bile duct and gastric epithelium. Expression in clear-cell RCC is homogeneous, whereas non-clear cell RCC show heterogeneous G250 expression or are negative. In general, tumors derived from other organs do not show G250 expression. Because of its expression in a high percentage of RCC, its limited heterogeneity, its high density on RCC cells, and its restricted distribution in normal tissues, this antigen has been successfully used as marker to visualize RCC lesions with radiolabeled mAb. These characteristics make this antigen a good candidate for anti-Id therapy.
For the isolation of anti-Id mAbs an IgG1 mAb recognizing G250 antigen (mAbG250) was cross-linked to Keyhole Limpet hemocyanin and used as immunogen. In four fusions, 20 hybridomas were isolated that reacted with $F(ab')_2$ fragments prepared from mAbG250 IgG1. Nine reacted with $F(ab')_2$ fragments prepared from IgG1 mAbG250, and $F(ab')_2$ fragments prepared from IgG2a mAbG250, indicating specificity for the mAbG250 binding region and not for the C_H2 region of IgG1. The 9 hybridomas were subcloned and tested in Western blots to determine the reactivity with reduced and non-reduced mAbG250 IgG and $F(ab')_2$. All nine anti-Id mAbs showed clear reactivity with mAbG250 in non-reducing gels, whereas no reactivity was observed in reducing gels, demonstrating that the anti-Id mAbs recognize a conformational epitope(s) comprized of the heavy and light chain, and not a structural epitope outside the mAbG250 binding site. Furthermore, all 9 mAbs were able to compete with native G250 antigen in a competitive ELISA. In summary, we have isolated 9 mAbs that appear to be true internal image anti-mAbG250-Id antibodies. We are performing cross-blocking experiments to determine whether these mAbs recognize different epitopes in the mAbG250 binding pocket. We have also initiated the functional characterization of these anti-Id mAbs, i.e. we will investigate whether they are able to induce antibody capable to compete with the parental mAbG250. Anti-Id mAbs that induce Ab3 will be tested for their therapeutic potential.

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BELLINI DUCT CARCINOMA OF THE KIDNEY

T. Prayer Galetti°, AO. Cavazzana*, N. Stella**, L.G. Spagnoli*, U. Santacatterina°, M. Dal Bianco°, P. Bassi° and F. Pagano°
° Ist. di Urologia - University of Padua - Italy
* II University of Rome - Italy
** Ospedale Civile di Vicenza - Italy

Bellini duct carcinoma (BDC) is an anusual and higly aggressive renal neoplasm whose histogenesis in still a matter of debate although a possible origin from collecting ducts has been proposed. A tumor cell line (>40 passages) was obtained from a BDC of a 57 year-old man who presented with a mass of the right kidney. The patient was submitted to radical nephrectomy. Pathological stage at surgery was pT3 N0 M0 G3. We observed a tumor relapse after 11 months from surgery. Time to death from BDR recurrence in the liver and peritoneum was 5 months despite sistemic and intraperitoneal chemotherapy at the recognition of relapse. In vitro, the tumor cells displayed different features from conventional renal cell carcinoma (RCC) when examined with phase contrast microscopy, scanning electron microscopy, and transmission electron microscopy. The cells were smaller than RCC cells, possessed long, slender cellular processes, and did not form glandular lumens . The expression of low (K18) and high molecular weight (K5, K8, K10) keratins was studied. The BDC tumor cells displayed strong positivity for keratins 5,8, and 18 but did not react with the anti-keratin 10 antibody. Cytogenetic analysis revealed a characteristic aneuploid karyotype: 53, XY, del (1) (p34), +iso (1q), +iso (5p), +4, +7, +8, -14, del (16)(q22), +17, -18, +20, -22. The positivity to high molecular weight keratins, normally expressed in the collecting ducts, supports the origin from the distal collecting system. Moreover, the absence of structural changes in chromosome 3 (usually present in conventional RCC) in conjunction with abnormalities involving the long arm of chromosome 1, as observed in malignant lesions of urothelial origin, seems to further substantiate this origin.

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HISTOPATHOLOGICAL CHARACTERIZATION OF SMALL "INCIDENTAL" RENAL TUMORS.

Miriam Konichezky, Eliahu Mukamel, Pepi Roizman, Ciro Servadio, Armand Abramovichi, Depts. of Urology and Pathology, Beilinson Medical Center, Petah Tigva, Israel.

Our previous study (1) has emphasized the importance of differentation between small renal cell carcinoma and adenoma by using routine hematoxylin and eosin staging. The aim of the present study was to further characterize the pathophysiology of the same renal tumors based on their oxidation capacity. Small renal tumors 2-3 mm in diameter were identified as described (1). All tumors were removed and stained routinely by hematoxilin and eosin and by anilins stain from the thiazin group. A positive staining reactivity was found in the normal proximal convoluted tubule which is known to have a high oxidative metabolism capacity, whereas the remaining parts of the nephron unit were not stained.
Small renal adenomas were positively stained and the small renal cell carcinomas were not stained. Renal oncocytomas showed stronger activity than adenomas. Our findings reconfirm that renal cell carcinoma and adenoma have a different biochemical capacity despite their common origin from the proximal convoluted tubles.

1. E. Mukamel, M. Konichezky, D. Engelstein, C. Servadio. Incidental small renal accompanying clinically over renal cell carcinoma. J Urol 140:22-24, 1988.

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INFLUENCE OF AGING ON ANDROGEN AND PROGESTERONE RECEPTOR CONTENT IN HUMAN BENIGN PROSTATIC HYPERPLASIA

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

The development of human benign prostatic hyperplasia (BPH) is somehow associated with androgens and aging. This is underlined by own studies showing significant age-dependent alterations of androgen metabolizing enzymes in epithelium and stroma of human prostate. The question arises whether similar age-dependent alterations regarding the androgen (AR) and progesterone receptor (PgR) in epithelium and stroma can be described.

We measured AR and PgR in mechanically separated epithelium and stroma from ten men with BPH, aged 60 to 95 years by a hybrid ligand method, using ^{3}H-R1881 as radioactively labelled tracer and 5α-dihydrotestosterone and triamcinolon-acetonide as competitor of specific AR- and PgR-binding, respectively. Whole cell homogenates of epithelium and stroma were incubated at 4°C for 72 h. Bound radioligands were separated by a hydroxylapatite batch absorption procedure. Maximum binding sites (Bmax) and dissociation constants (KD) were calculated by Scatchard plots. The significance of the differences between the means was calculated by students' t-test, the significance of age-dependent changes by Spearman rank correlation coefficient.

The main results were: 1. If relating the values on protein, Bmax of AR [fmol/mg protein±SEM] was found to be significantly ($p<0.001$) greater in epithelium (70±10) than in stroma (29±3). 2. However, Bmax of PgR was greater in stroma (7.5±0.6) than in epithelium (5.6±0.9). 3. The binding affinity (KD) of AR [nM±SEM] showed no significant differences between epithelium (2.9±0.3) and stroma (3.0±0.5). 4. The same was true for PgR in epithelium (1.9±0.4) and stroma (1.4±0.2). 5. In epithelium, Bmax of AR decreased significantly ($p<0.005$) with age, while in stroma no age-dependent alteration could be found. 6. The KD-values of AR in epithelium and stroma remained constant over the whole age range. 7. The PgR showed neither in epithelium nor in stroma significant age-dependent alterations of Bmax and KD.

In conclusion: BPH appears to be characterized by an age-dependent decrease of AR levels in epithelium, but no age-dependent alterations in stroma. Furthermore, PgR at a lower level remains constant over the whole age range. However, the impact of such age-dependent alteration of the epithelial AR level on BPH development is still unclear.

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EFFECT OF EXTRACTS FROM SABAL SERRULATA ON 5α-REDUCTASE IN HUMAN BENIGN PROSTATIC HYPERPLASIA

Heike Weißer, Sabine Tunn, Bert Behnke*, Michael Krieg
Institute of Clinical Chemistry and Laboratory Medicine, University Clinic Bergmannsheil, Bochum and * Pharma Stroschein GmbH, Hamburg, Germany

It is more than likely that 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 (DHT), is a most promising concept in the treatment of BPH. Besides synthetic steroids with proven inhibitory effects, various phytotherapeuticals are believed to possess also inhibitory effects on 5α-reductase. The aim of the present study was, therefore, to explore whether or not an extract from Sabal serrulata fruits (Strogen forte, Stroschein) is capable of inhibiting 5α-reductase in vitro.

Three BPH were separated mechanically in epithelium and stroma. 5α-reductase activity was determined by incubating epithelium and stroma homogenates with radioactively labelled testosterone (580 nM) alone or in the presence of one of the following Sabal serrulata compounds: a) unfractionated ethanol extract, b) three subfractions from the ethanol extract containing mainly either acid lipophilic compounds (e.g. fatty acids), phytosterols or hydrophilic compounds, c) lauric acid, myristic acid, palmitic acid and oleic acid which are known to be the main fatty acids of the Sabal serrulata extract. The androgen metabolites were separated by HPLC.

The main results were: 1. The ethanol extract was found to inhibit the 5α-reductase in epithelium and stroma. The mean inhibition was 24% and 50%, respectively. 2. This inhibitory effect is mainly caused by the fatty acids containing subfraction which showed a mean inhibition of 38% in epithelium as well as in stroma. Phytosterols and hydrophilic compounds showed no inhibitory effect. 3. The mean inhibition of the main fatty acids of the Sabal serrulata extract in epithelium and stroma was: 65% and 62% (lauric acid), 48% and 40% (myristic acid), 36% and 19% (palmitic acid), 30% and 29% (oleic acid), respectively. 4. The inhibitory effect of lauric acid on the 5α-reductase was dose dependent and not competitive. The mean IC50 values were 85 µg/ml and 125 µg/ml in epithelium and stroma, respectively.

These data indicate that the inhibitory effect of extracts from Sabal serrulata fruits on 5α-reductase is mainly caused by fatty acids of the acid lipophilic fraction, especially by lauric acid. As the integrity of the membrane environment consisting mainly of phospholipids is obligatory to keep the membrane-bound 5α-reductase in its active conformation, it is discussed whether those fatty acids inhibit the activity of 5α-reductase by modifying its microenvironment.

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IMMUNOHISTOCHEMICAL DISTINCTION BETWEEN AGGRESSIVE AND DORMANT PROSTATIC ATYPIA. CYTOKERATIN (Ck), CHROMOGRANIN A (Chr A), PsAP, PAP, NSE, ACTH and CEA EXPRESSION IN BENIGN, ATYPIC AND CARCINOMATOUS PROSTATE.

Snježana Frković-Grazio, Mladen Belicza, Ivo Kraljić, Zoran Čulig and Marko Tarle, Clinical Hospital Sestre Milosrdnice, Zagreb, Croatia.

Our unpublished studies indicate a remarkable level of prostatic atypia that becomes clinically detected cancer within 2 years after TURP. To distinguish a dormant from potentially aggressive atypical prostate we have studied 5 specimens of glandular BPH, 5 cases of atypical glandular prostate (AGP) that were altered to cancer and 10 well differentiated (G1) carcinomas after TURP. In 7 G1 tumors AGP cells were found in the proximity of cancer structures. Monoclonal Ab to Ck (56-64 kD) and Chr A were used together with polyclonal Ab to other markers, all purchased from Dakkopats. A positive stain for CEA was found (a) in the presence of inflammation but also (b) in secretory AGP cells adjacent to tumor regardless of inflammatory components and was 2-fold stronger than either in cancer or in AGP tissue. Basal layer was in a benign prostate much stronger Ck positive than in AGP. Secretory cells were in BPH weakly Ck positive while in those of AGP and G1 cancer the intensity was moderate. Chr A positive cells were more numerous in AGP than in G1 cancer. The expression of PSA and PsAP was gradually decreased with the elevation in Gleason's score. A high expression of Chr A was found to be a prerequisite for the occurrence of paraneoplastic syndrome. The only assessed case of NSE positive tumor was locally aggressive and gradually developed pseudo-sarcomatos cancer accompanied with a total loss in PSA and PsAP expression. According to the reported data we support the clinical application of the above markers as indicators of the conversion of AGP to the manifest prostate cancer, a loss of differentiation in glandular tumors, and the development of unusual prostate cancer.

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DNA CONTENT VERSUS PSA AND HISTOLOGIC GRADE IN PATIENTS SUBMITTED TO RADICAL PROSTATECTOMY FOR PROSTATE CANCER.

F. Zattoni, T. Prayer Galetti, F. Vianello, R. Bertoldin, M. Panozzo*, S. Blandamura**, G. Costantin**
Institute of Urology, University of Padua
* IST Section Biotecnology, Institute of Oncology, Padua ** 2nd Service of Cytodiagnostic, Hospital of Padua

Dna nuclear ploidy determined by flow cytometry was evaluated from prostate tissue in 51 patients with prostatic cancer who had undergone radical prostatectomy. DNA ploidy pattern was diploid in 46% and aneuploid in 54% of tumors. DNA ploidy was compared to histological tumor grading. Aneuploidy was found in 0% of the tumors with Gleason score between 2 and 4; in 62% between 5 and 7 and in 50% between 8 and 10. Our results suggest there is no relationship between the two compared parameters. No statistical relationship was observed between grade and S-phase. Mean PSA preoperatory value was higher in aneuploid tumors than in diploid one. Preoperatory PSA levels and DNA content seem to be a valuable tool to identifie a subset of patients with aneuploid tumor and elevated PSA at higher risk for an early tumor relapse.

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TISSUE MARKERS IN THE PROGNOSIS OF PROSTATIC CARCINOMA (CLINICAL STAGES C-D).

P.Belmonte,R.Zucconelli,G.Fiaccavento.
Servizio Autonomo di Urologia,Presidio Ospedaliero,Portogruaro(VE) Italy.

We evaluated the prognostic value of PAP and PSA on 38 patients with locally or disseminated prostate cancer.
The clinical stages were 16 C,5 D1 and 17 D2.
The pretreatment PSA levels were elevated in all D patients and in 87.5% of the C patients,while the pretreatment PAP levels were elevated in 45.4% of D-patients and only in 6.2% of C-patients.
The absolute values of both markers were higher in stage D than in stage C.
The decrease of PSA levels was statistically significant one month and more evidently six months,after the beginning of the endocrine treatment.
In the patients who progressed(18.4%)the PSA levels were left elevated(higher than 10.0 ng/ml).
The PAP levels didn't decreased equally significantly .
In our experience PSA evaluation has been a marker more reliable than PAP and a real prognostic factor of responsivenes to endocrine treatment and of disease progression.

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EPITHELIAL TISSUE MARKERS IN PROSTATIC CARCINOMA.

A.V.Bono(*), B.Frigerio(°), Degiovanni G.(°), C.Capella(°)
(*) Dept. of Urology, Regional Hosp. Varese
(°) Dept. of Human Pathology, University of Pavia at Varese, II Faculty of Medicine.

A variety of exocrine and neuroendocrine substances, or "markers", may be shown by immunichemistry and use of lectins both in the normal prostate and in the prostatic carcinoma (PCs). Recently, in addition to the well known prostatic acid phosphatase (PAP) and prostatic specific antigen (PSA), pepsinogen II (PG2) and lectins have been proposed as markers of PCs. Histologically proved PCs were obtained from a series of 89 consecutive patients affected by PCs who underwent either trans urethral resections (TURP) for advanced PC or radical prostatectomies (RP) for stage B disease. The WHO classification was used and to study glycidic substances, the lectin reactions were employed: Ulex Europeaus (UEA-I) and Concanavallin-A (Con-A). PAP, PSA Leu-7 antigens and PG2 were detected using immunohiostochemical techniques (ABC). To demonstrate the endocrine cells the Grimelius (GRIM) reaction was used.

The histochemical and immunohistochemal results showed that PG2 and UEA-I, which are markers usually present in the central zone of the normal prostate, were widely expressed in the low stage, well differentited (mostly G1) PCs coming from patients submitted to RP. On the contrary these markers were poorly expressed in high stage PCs of patients who underwent TURP. The GRIM, Leu7 and CON-A and PAP, PSA immureactivities were present in all types of tumors, irrespective to the stage, grade and type of surgical treatment.

From the present data therefore PG2 and UEA-1 appear to be related to a favorable stage and low malignancy grade of the disease and could probably represent a reliable biological markers.

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SILVER STAINED NUCLEOLAR ORGANIZER REGIONS IN CARCINOMA OF THE PROSTATE

Rengin AHISKALI[1], Yusuf ALİCAN[2], Ferruh ŞİMŞEK[2], Gülsün EKİCİOĞLU[1], Sevgi KÜLLÜ[1], Atıf AKDAŞ[2]

MARMARA UNIVERSITY, SCHOOL OF MEDICINE, DEPARTMENTS OF [1]PATHOLOGY, [2]UROLOGY, ISTANBUL, TURKEY

INTRODUCTION

Nucleolar organizer regions are DNA loops encoding ribosomal RNA and are detectable by silver staining techniques. *1
Silver-stained nucleolar organizer regions (AgNORs) were studied in patients with advanced carcinoma of the prostate (P.Ca) and we here in report our preliminary results.

MATERIAL AND METHODS

Paraffin sections of the specimens from 19 patients with advanced P. Ca were evaluated by a one-step silver staining technique*2 in terms of number of AgNORs in malignant tissue. Data of this evaluation was further compared to tumor grade and outcome of the disease.

RESULTS

The mean number of AgNORs in patients with grade I and grade II P.Ca were fairly close to each other (8.89+0.25 and 8.66+0.10, respectively). Grade III tumors had more AgNORs when compared to other grades (mean10.47+0.20) but this difference was not statistically significant. Further analysis of data of AgNORs counts by stratification of the patients according to their prognosis was not conclusive.

CONCLUSION

Our preliminary results indicate that AgNORs technique should still be regarded as experimental and certain conclusions awaits further investigation.

REFERENCES

*1.Cheville,J.C.; Clamon,G.H.; Robinson,R.A.: Silver-stained nucleolar organizer regions in the differentiation of prostatic hyperplasia, intraepithelial neoplasia, and adenocarcinoma. Mod Pathol 3:596-598(1990)
*2.Contractor,H.; Ruschoff,J.; Hanisch,T; et al: Silver-stained structures in prostatic carcinoma: evaluation of diagnostic and prognostic relevance by automated image analysis. Urol Int 46:9-14(1991)

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^{1}H MAGNETIC RESONANCE SPECTROSCOPIC CHARACTERIZATION OF FOUR HUMAN PROSTATE CANCER CELL LINES

Cornel, Erik B., Smits, Geert A.H.J., *Heerschap, Arend, de Ruyter, Anja, Oosterhof, Gosse O.N., Debruyne, Frans M.J., and Schalken, Jack A. Department of Urology and *Radiology, University Hospital Nijmegen, The Netherlands.

Magnetic resonance spectroscopy (MRS), is a relatively new non invasive method which can monitor tissue metabolism and enables longitudinal investigations of concentrations of various metabolites and may thus be utilized to evaluated tumor behaviour in vivo. Recently, we have shown that MRS is capable of detecting differences in metabolic content of human prostate tissue samples which were correlated with different tissue type (BPH versus prostate cancer). Within the Dunning R-3327 tumor model system we have demonstrated that MRS can be used to discriminate between differentiation grade and metastatic potential, whereas androgen sensitivity could not be correlated with any metabolite detectable by MRS. In this study we attempted to identify metabolites which could be of use in demonstrating tumor heterogeneity in human prostate cancer. Futhermore, we were interested whether these metabolites correlate with any of the ratio's of metabolites found in our Dunning MRS study. Moreover we were interested in the amount of citrate in these human prostate cell lines, which was low or not detectable in our human MRS study.
Perchloric acid (PCA) extracts of the following human prostate cancer cell lines were analyzed by ^{1}H MRS, performed on a Bruker 500 Mhz spectrometer: LNCaP, PC-3, DU-145 and TSU. Five different passages of each cell line were investigated. The cells were cultured under standard culture conditions. Cells were harvested for PCA extraction when the cells, cultured in six flasks of 162 cm^2, achieved 70 to 80 % confluency. The total amount of viable cells was always between 40 and 60 million cells.
The ^{1}H spectra obtained for the four human prostate cell lines showed several differences. Citrate could be demonstrated at low, but detectable levels in spectra of the hormone dependent, moderately differentiated LNCaP cells. Also the alanine / lactate ratio in the LNCaP cells was higher in comparison to the other cell lines. The metastasizing cell lines TSU and PC-3 showed a relative high level of choline, a finding which is in contrast to our Dunning MRS study.
Conclusion: These results indicate that subtle differences between cell lines with different biological behaviour can be measured by MRS. Whether in vivo prostate MRS will be usefull in detection of tumor heterogeneity remains to be investigated.

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MORPHOLOGIC EFFECTS OF CASTRATION IN THE DUNNING R-3327 PROSTATIC ADENOCARCINOMA VERSUS NORMAL RAT PROSTATE.

Patrick Westin, Anders Bergh, Jan-Erik Damber, Dept of Pathology and Urology & Andrology, University of Umeå, Umeå, Sweden

INTRODUCTION

Apoptosis and programmed cell death occur in the normal prostate after castration but whether prostatic tumours respond in the same way is less studied. Copenhagen/Fishers rats with implanted hormone dependent Dunning R3327 tumours were castrated and after 3, 7 and 14 days respectively, sacrificed and fixed by vascular perfusion. From each rat was taken tumours and normal prostate for quantification of tumour volume, epitehlial cell numbers and -sizes, using stereological methods. The number of mitotic and apoptotic epithelial cells was also counted.

RESULTS

In the normal ventralprostate, castration resulted within three days in a significant decrease in organ volume and epithelial cell numbers and sizes, mitotic index as well as in cell death (apoptosis).
In the Dunning tumour growth rate and mitotic index and eventually the epithelial cell size were reduced, but there were neither any significant changes in epithelial cell numbers, nor any signs of epithelial cell death.

CONCLUSION

It is concluded that the castration induced inhibition of tumour growth can not be explained by tumour cell death. The reason why this high differentiated, androgen-dependent tumour respond different than the normal prostate is unknown.

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ESTROGEN RECEPTORS IN NORMAL AND NEOPLASTIC PROSTATE TISSUES AND CELLS.

L. Castagnetta [1,2], G. Carruba [1], M. Lo Casto [2], A. Cassetti [1], L. Comito [1], C. Pavone [3] and M. Pavone-Macaluso [3].
[1] Hormone Biochemistry Laboratory - University of Palermo - Italy; [2] Experimental Oncology and Molecular Endocrinology Sections National Institute for Cancer Research of Genova - Palermo Branch - Italy; [3] Institute of Urology - University of Palermo - Italy

Introduction

Prior to the advent of antiandrogens, patients with prostate cancer (PCa) were often treated with estrogens. It is generally assumed that the action of estrogens was merely due to inhibition of testosterone production. A direct effect of estrogens on prostate cancer cells was often postulated, especially to account for the alleged clinical results of high-dose estrogens in hormone-resistant tumours. However, such a direct effect, possibly mediated through specific receptors, is still the object of debate. Many reports have demonstrated that several, if not all, normal and pathologic (BPH, PCa) prostate tissue contain androgen receptors, but only few data are available in the literature with regard to other steroid receptors. Most reports do not provide information on the distribution of estrogen receptors (ER) within the cellular compartments and do not differentiate between type I and II receptors. In this study, preliminary evidence of high affinity, low capacity ER in BPH and PCa tissues, as well as in long term PCa cell lines (PC3, DU145 and LNCaP) is presented.

Results

20 tissue samples from BPH and PCa patients were studied. In about 50% of all tissues, both soluble (s) and nuclear (n) ER were shown. These binding sites were characterized as type I sites, i.e. high affinity (K_d < $5.5x10^{-10}$M), low capacity. From the affinity constant characteristics it was demonstrated that these binding sites are superimposable to those present in other endocrine-related tumours, such as breast or endometrial cancers. The presence in both soluble and nuclear cell fractions suggests an unimpaired mechanism of action of ER. Moreover, 3 different cell lines, all derived from PCa tissues and most commonly used in *in vitro* studies, showed to be endowed with type I ER_s and ER_n: in fact both LNCaP and DU145 cell lines exhibited site I in both s and n fractions, whereas PC3 cells displayed ER_n only, suggesting an impaired function of mechanism of action. These observations, although preliminary, deserve more extensive studies and legitimate the hypothesis that estrogens may play a role in the growth processes of neoplastic and hyperplastic prostate epithelial cells. It is of interest to note that these cell lines show, at short term, a different response to transforming growth factors (TGF) alpha and beta, exhibiting a inverse correlation between response to steroids and to GFs. Preliminary data seem to indicate a certain ability of some GFs to modulate estrogen metabolism in these cell lines.

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PLASMINOGEN ACTIVATORS AND INHIBITOR EXPRESSION ARE CORRELATED WITH THE CELLULAR TYPE AND ITS LOCALIZATION IN THE HUMAN PROSTATE.

Desruisseau Sylvie[1], Ragni E.[3], Martin P.M.[2]. 1-INSERM U. 270, 2-Laboratoire de cancérologie : Faculté de Médecine Nord, Bd P. Dramard, 13326 Marseille Cedex 15. 3-C.H.U. Nord (service d'Urologie) Marseille.

The expression of plasminogen activators (PA) and their inhibitors plays an important role in normal cellular processes (differentiation, cell migration during development, etc) in which they are in equilibrium. In pathological processes, the cells secrete more proteases implicated in the degradation of the extracellular matrix allowing the invasion of normal tissue.

Fibroblasts and epithelial cells from different segments of normal, tumoral and hyperplasic human prostate (previously cut according to the Mc Neal model) were obtained and grown in different culture conditions. Single cells or organoids were obtained after a collagenase dissociation or culture explants of tissue. Epithelial cells were cultured in RPMI medium supplemented with 3% fetal calf serum (FCS) and different growth factors and hormones; whereas fibroblasts were initially cultured in RPMI supplemented with 20% FCS and then along the last week with 2% FCS. Secretion of u-PA, t-PA and PAI-1 as well as their enzymatic activities were measured in culture media and cellular extracts of fibroblasts and epithelial cells, using commercial kits. We have shown that periferic fibroblasts secreted more PA and PAI-1 than central fiboblasts; moreover periferic segment is more involved in the tumor processes than central prostate which is the hyperplasia seat. So the variations of expression of PA and inhibitor could be correlated with the involvement of the different segments in the tumoral or hyperplasia development.

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TPS SEROTEST AS A NONSPECIFIC MARKER OF STAGE DO-D2 PROSTATE CANCER.

Marko Tarle, Ksenija Kovačić, Maja Kaštelan,
Nuclear Medicine and Oncology Clinic, University Hospital, Zagreb, Croatia.

TPS is a monoclonal assay for the measurement of the M3 epitope of TPA in serum and has been proposed as an indicator of the tumor proliferation rate. We have serially assessed in a retrospective study serum PSA, PAP and TPS (donated from Beki Diagnostics AB, Bromma, Sweden) concentrations together with respective scintigraphic bone images in 29 patients with hormonally treated Stage Do-D1 prostate cancer, 19 treated subjects with Stage D2 disease and in 8 healthy controls. In some of these subjects NK activity was also measured. As long as tumor cells are not present in the bloodstream TPS level remains normal (<80 U/l) regardless of serum PSA and PAP values and the outcome of the treatment. In Stage D2 patients numerical values reflect both tumor healing and progressive processes by their respective normal and elevated concentrations. Nevertheless, the direct numerical correlation between PSA and TPS level is rather poor (r=0.05) possibly indicating both contribution of selected tumor cell subpopulations to the TPS level elevation and/or the discrepancy between PSA level and the outcome of hormonal treatment. In untreated M+ patients NK activity vs TPS values correlate well (r=0.75) together with NK activity vs PSA and TPS vs PSA values (r=0.5 and 0.88, respectively) in M- subjects. Untreated diabetes, arthritis, severe rheumatism and prostatic inflammation elevate TPS remarkably (treated diabetes to some extend only). TPS level was found to be normal in healthy age-matched controls.
We advocate the preliminary assessment of TPS in monitoring Stage D2 prostate cancer patients as clinically efficacious and cost-effective addition to other procedures applied in monitoring protocols. Prospective study is now in progress to prove the reported data.

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CAN PSA LEVELS PREDICT THE LOCAL STATE OF PROSTATIC CANCER FOLLOWING RADIOTHERAPY TREATMENT?

Zvi Leib*, Uri Gabbay**, Jacquline Sulkes**, Harry Winkler*, Ciro Servadio*. Department of Urology* & Epidemiology**, Beilinson Medical Center, Petach Tikva, Israel

The PSA levels of 39 patients with clinical stage C prostatic cancer, were followed at 3 month intervals over a period of 6-24 months post-treatment. Prostatic volumes (measured by transrectal ultrasound) and biopsies were performed pre-treatment and at 6, 12, 18, 24 months post-treatment. 22 patients received only 70 Gy radiotherapy while the other 17 received 70 Gy radiotherapy in conjunction with 8 1-hour transrectal hyperthermia treatments (using the PROSTATHERMER™ from Biodan Medical Systems). The clinical aspects of the treatment regimens are discussed elsewhere.
The objective of this study was to determine the correlation coefficient during the follow-up post treatment period between: 1) PSA levels and prostatic volume and 2) PSA levels and the probable positive cancer cells in biopsies.
Before treatment, there was a strong positive correlation between PSA levels and prostatic volumes which was statistically significant ($r=0.51$, $p=0.0013$, $n=39$).
At 6 months post-treatment, as PSA level decreases, the volume increases ($r=-0.15$, $p=0.56$, $n=17$). For longer followup periods up to 18 months, the negative correlation coefficient becomes stronger, and the same inverse relationship is maintained.
At 6 months post-treatment, there was a weak positive correlation between PSA levels and a positive biopsy, which was not statistically significant. This trend is maintained for longer followup periods.
We attempted to determine whether the percentage change of PSA (between pre-treatment and post-treatment levels) was more significant than the absolute values. However, no significant correlation was identified.
When the treatments for the two patient groups were compared relative to PSA levels, those patients who received hyperthermia had lower PSA than the ones without.
These preliminary findings raise questions about the suitability of PSA level as the sole predictive measure for post-treatment followup of local prostatic cancer.

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FIRST EVALUATIONS ABOUT SOME "INTEGRINS" AND ONCOGENES PRODUCTS' STUDY IN PROSTATIC CANCER.

D. Fontana, G. Fasolis, L. Gubetta*, F. Porpiglia, E. Leonardo*, L. Rolle, R. Tarabuzzi, S. Cappia*.
From the Division of Urology, Department of Clinical and Biological Sciences - University of Torino. (Italy); * Pathology Service of St. Luigi Gonzaga Hospital, Orbassano (Torino), Italy.

We activated an open perspective study to evaluate the possible interaction of some "integrins" and oncogenes' products within the development of prostate cancer for trying to identificate some possible new prognostic factors instead of those normally used.
We made trans-rectal prostatic biopsy on to 18 patients suffering from advanced prostatic carcinoma (stage C: 8 patients - stage D: 10 patients). Using different monoclonal antibodies we determinated the following "integrins" or adherence cellular receptors: intracellular adhesion factor (ICAM) - laminine (LAM) - fibronectine (FIBR). We also determinated the following oncogenes' products: EGF-R , c-ERBB-2, c-RAS, p 53.
The results, in this preliminary stage, pointed out the presence of: ICAM in 25% of patients, LAM in 50%, FIBR in 33%, EGF-R in 91%, c-ERBB-2 in 75%, c-RAS in 0%, p53 in 8%.
The oncogene c-ERBB-2 results more expressed in D staged neoplasia (100% of patients) than in C staged neoplasia (60%).
We didn't observe any difference concerning "integrins" or the other oncogenes' products.
Only the follow-up on a huger number of patients may demonstrate the real significance to refer to these new possible prognostic factors.
This study is ongoing.

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SOME ONCOGENES' BIOLOGICAL PARAMETHERS EVALUATION INTO BLADDER CARCINOMA.

D. Fontana, M. Bellina, L. Gubetta*, C. Scoffone, E. Leonardo*, M. Colombo, G. Del Noce, S. Cappia*.
From the Division of Urology, Department of Clinical and Biological Sciences - University of Torino. (Italy); * Pathology Service of St. Luigi Gonzaga Hospital, Orbassano (Torino), Italy.

Our knowing about molecolar biology now make possible to assert that neoplastic disease is the consequence of genic alteration and trasformation of cells once normal.
Oncogenes are the activated expression of protooncogenes, that are physiologically present in normal cells. Their activation brings to neoplastic trasformation. In particular, about the bladder carcinoma, some oncogenes identification plus the study of proliferative activity and the typical prognostic factors make possible to acquire important anathomo-clinical paramethers. We evaluated 99 patients suffering from bladder carcinoma with different grading and staging, at the first diagnosis who underwent to T.U.R. with a follow-up from 3 to 38 months ($\bar{x}$= 12,9). Every patient was evaluated by immunohistochemical method, observing the share of neoplastic cells into cells activity (Ki-67), into proliferation (PCNA); we also observed the presence of the following oncogenes' products: c-RAS, c-ERBB-2, p53. Therefore we correlated these biological determinations with the usual anathomo-clinical paramethers (G, T, recurrence time, multiple foci). Referring to the obtained results we suggest to use the considered biological paramethers for a more punctual prognostic evaluation and a better therapeutic approach of bladder carcinoma.

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FLOW CYTOMETRIC DEOXYRIBONUCLEIC ACID STUDIES AND AgNOR COUNTS IN PATIENTS WITH TRANSITIONAL CELL CARCINOMA OF THE BLADDER

Çiğdem ATAİZİ[1], Yusuf ALİCAN[2], Özdal DİLLİOĞLUGİL[2], Müjdat BAŞARAN[3], Gülsün EKİCİOĞLU[1], Yalçın İLKER[2], Sevgi KÜLLÜ[1], Atıf AKDAŞ[2]
MARMARA UNIVERSITY, SCHOOL OF MEDICINE, DEPARTMENTS OF [1]PATHOLOGY, [2]UROLOGY, [3]PEDIATRICS, ISTANBUL, TURKEY

INTRODUCTION

Flow cytometry appears to be a promising technique as a diagnostic method, which may influence the therapeutic approach in transitional cell carcinoma (TCC) of the bladder.
The number and distribution of silver-stained nucleolar organizer regions (AgNORs) seems to correlate with growth fractions of the cells and may also have potential diagnostic and prognostic value in different neoplasms.
In this study, we report the results of combined flow cytometric analyses and AgNOR counts in a group of patients with TCC of the bladder.

MATERIAL AND METHODS

DNA content of paraffin embedded archival tumor tissues of 37 patients with a pathologically proven diagnosis of TCC of the bladder were measured on a Beckton Dickinson flow cytometer (FACScan). Preparation of the samples were performed according to a modified method of Hedley and associates. AgNOR staining of the same tissues were carried out according to Ploton et al.

RESULTS

A positive correlation was observed in the histological grade in relation to ploidy of tumors and aneuploidy increased by tumor grade (grade I 66.7%, grade II 69.2%, grade III 73.3%). Reccurrence rates were also higher in aneuploid tumors.
Differences among mean AgNOR numbers of different groups defined by DNA content, histological grade, clinical and pathological stage and disease outcome were not statistically significant by student's t test.

CONCLUSION

In addition to the conservative histological grading systems, DNA content of the tumors should also be measured for decision of more aggresive treatment in some cases.
According to our preliminary results, we could not find any correlation between AgNOR counts and clinical-pathological parameters.

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QUERCETIN BINDS TO TYPE II ESTROGEN BINDING SITES AND INHIBITS BLADDER CARCINOMA CELL PROLIFERATION

A. Capelli, E. Macrì, A. Rinelli, N. Maggiano, M. Piantelli, F.O. Ranelletti°, E. Alcini°°, M. Giustacchini°°, L.M. Larocca.
Depts. of Pathology, °Histology, and °°Surgery, Univ. Cattolica del S. Cuore, Largo F. Vito 1, I-00168 Roma, Italy.

Five cases of human bladder transitional cell carcinoma were investigated for the presence of estrogen receptors. We found that these tumors specifically bound estradiol. This binding almost exclusively resulted from the presence, in all the cases tested, of type II estrogen binding sites (type II EBS). Type II EBS display lower affinity but higher capacity for the ligand than classical estrogen receptors. Owing to the relatively low binding affinity (Kd 12-20 nM) of type II EBS for estrogens, it is hard to imagine that these sites could be occupied by estrogens in vivo. A solution of this paradox has partially been realized by the observation that type II EBS in rat uterus are occupied by a ligand with growt inhibitory activity. Thus the physiological function of type II EBS might not be to bind estrogen but rather to bind the type II ligand. Although this ligand has not been structurally identified, some evidence suggests that it may be a plant flavonoid-like molecule, probably of dietary origin. Quercetin (penta-hydroxy-flavone) is able to inhibit the binding of tritiated estradiol to type II EBS with a potency similar to that of diethylstilbestrol. In order to evaluate the Q sensitivity of proliferating tumor cells we used bromodeoxyuridine (BdU) uptake which was then identified immunocytochemically. In vitro incubation of small fragments of tumors with 10 µM Q produced a significant reduction in the number of BdU labelled cells in the 3 cases tested. Furthermore, the rhamnosyl-glucoside of Q (rutin) and hesperitin-7-rutinoside (hesperidin) that do not compete with estradiol for type II binding, do not inhibit the proliferation of tumor cells. The growth inhibitory properties of Q, together with the presence of type II EBS in bladder carcinoma suggest that this substance could be of some therapautic potential.

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NU-20M, A NEW HUMAN RENAL CELL CARCINOMA CELL LINE WITH HIGH METASTASIZING CAPACITY IN THE NUDE MOUSE MODEL.

A.J.M.C. Beniers, H. Uemura, F.M.J. Debruyne, E. Oosterwijk, J.A. Schalken.
Dept. of Urology, Academic Hospital, Nijmegen, The Netherlands

A cell line with high spontaneous metastasizing capacity in nude mice was developed from the NU-20 human renal cell carcinoma (RCC) xenograft, a granular cell tumor originating from a T2 primary tumor with predominantly granular cells. NU-20 xenograft passage no. 9. was cultured at very low numbers of cells in a 75 cm^2 tissue culture flask. Subsequent cloning resulted in the NU-20M cell line. The human origin of this line was confirmed by chromosomal analysis.
Intravenous (i.v.) injection of 1.10^6 NU-20M cells resulted in extensive pulmonary lesions and death in 10 days. Intraperitoneal (i.p.) or subrenal capsule injection of the same number of cells killed the mice within 14 days because of i.p. tumor mass. Mice injected subcutaneously (s.c.) with $0.5.10^6$ cells had to be sacrificed within 14 days because of rapidly growing s.c. tumors weighing > 5g. Multiple lung metastases were found at that time. In addition, approximately 50% of mice suffered from axillary lymph node metastases.
NU-20M lung and lymph node metastases were transplanted s.c. into nude mice and gave rise to two sublines: NU-20M-L (lung) and NU-20M-N (node). The in vivo doubling time of the NU-20M, NU-20M-N and NU-20M-N tumors is approximately 24 hours. Cell lines of the NU-20M-L and -N xenografts were made as described for the NU-20M line. Morphology of the M and M-L lines is the same with about round shaped granular cells sometimes with pseudopodal extensions whereas cells of the M-N cell line forms more elaborate pseudopodal extensions. Adherence of this cell line to the culture flask is also much more prominent than the M and L lines which easily form floating (but living) cells. S.c. injection of as little as 2000 cells of the L and N lines induce tumor formation. Even at these low cell numbers, tumor take is 60% at day 14 post injection. Lung metastases could be found as early as 7 days after s.c. transplantation of tumor pieces of only 1 mm^3. All lines gave rise to lung metastases and on day 14, pulmonary lesions were found in 80- 100% of the mice. In comparison with the NU-20M-L line, NU-20M-N showed more extensive nodal disease which indicates that the selection procedure has resulted in cell lines with different characteristics. These cell lines may provide a reliable model to study RCC metastases and efficacy of new therapies.

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NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED TRANSITIONAL BLADDER CANCER FOLLOWED BY PRESERVATION OF THE ORGAN

S.Crispino*,E.Scardino,V.Franchini,M.Andres,R.Musci,F.Rocco

* Divisione di oncologia,ospedale S.Gerardo,Monza
Clinica Urologica II,università di Milano

40 patients affected with locally advanced transitional carcinoma of the bladder were treated with CDDP 70 mg./m2 i.v. day 1,and MTX 40 mg./m2 i.v. days 8 and 15;the cycle being repeated every 3 weeks.35 patients were males and 5 females with an average age of 63 (36-70) and an average performance status of 100 (80-100). The average number of cycles was 3 (1-5).
After the chemotherapy and an accurate restaging (CT,cistoscopy, mapping and bioptic T.U.R.),10 patients (25%):4 in CR and 6 in PR with minimal residue,underwent a conservative treatment: a T.U.R. in 6 and a partial bladder resection in 4.
After an average follow-up of 36 months (60-24+),9 patients are alive and disease free (8/9 with bladder).
Three years after the T.U.R. one patient developped a primitive pulmonary neoplasm and died without evidence of bladder disease.
1 patient,a year after the partial resection,had a cerebral recurrence which was treted with a metastasectomy and RT. The patient is presenty disease free in the bladder.
1 patient has an endovesical recurrence (Cis) treated with a BCG course and who has been in CR for a year.
1 is alive with NED but without bladder due to a recurrence in the bladder that resulted in a cistectomy.
These results suggest that,in selected cases,a T.U.R. or a partial bladder resection after neoadjuvant chemotherapy seem to valid options for bladder preservation.

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FLOW CYTOMETRY STUDY OF DNA CELL CYCLE AND CELL PROLIFERATION THROUGH A NUCLEAR ANTIGEN (Ki67) AS PROGNOSTIC PARAMETERS FOR THE EVALUATION OF PRIMARY BLADDER CANCER.

G.Fischetti, P.Scialpi, P.Cinti*, E.Renna Molajoni*, R.A.Chiellino, A.Furbetta. Department of Urology "U. Bracci"-*Laboratory of Immunology (II Clinica Chirurgica) - Policlinico Umberto I 00161 - Rome

Nowadays histopathology represents the only method to evaluate solid tumors and bladder neoplasms as well. Traditionally cell cycle analysis has been performed by evaluation of DNA histogram;it defines number of cells in G0/G1, S and G2/M. The evaluation of the S phase fraction (SPF) represents an useful tool for the prediction of bladder tumor spreading, the risk of pelvic lymph node metastatsis and metastasis in distant sites,as the highest values of SPF have been associated with a worste prognosis.Study was aimed at defining either the DNA ploidy pattern of bladder tumors and cell proliferative activity with the Ki67 index. Ki67 is a nuclear antigen defined by a monoclonal antibody and expressed in actively cycling cells but not in resting G0 cells; the Ki67 levels can be shown to increase during the S phase and to be maximal at G2/M.In bladder carcinoma the Ki67 index would appear to be related to the biological aggressiveness of the tumor. 24 samples (histologically confirmed) out of 20 patients undergoing TUR (n=12) and surgery (n=8) for bladder carcinoma, were submitted to biological study (DNA analysis). 10 µL FITC conjugated Ki67 MoAb were added for 30 m' to cellular suspensions, previously fixed with 70% ethanol and preserved at -20 °C. The nuclei were stained for 30 m' in 50 µg/ml propidium iodide solution, in the dark. The measurement of nuclear DNA content was undertaken by flow cytometry (FACS-can Becton Dickinson). Aneuploidy, SPF, and Ki67 index were related to clinical stage and histological grade. Patients with invasive disease (n=7) had higher SPF and Ki67 index. This approach could offer more accurate prognostic informations in early stage disease than TNM stage and histological grade.

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DETECTION AND QUANTIFICATION OF ACIDIC AND BASIC FIBROBLAST GROWTH FACTOR (FGF) mRNA BY POLYMERASE CHAIN REACTION AND FGF RECEPTORS BY CROSS-LINK IN BLADDER CANCER

S. GIL DIEZ[1], S. PALCY[3], A. DELOUVET[2], J.J. PATARD[1], C.C. ABBOU[1], J.P. CARUELLE[3], J.P. THIERY[2], D. BARRITAULT[3], F. RADVANYI[2] and D. CHOPIN[1]. (1) Service d'Urologie, Hôpital Henri Mondor, 94010 Créteil, France ; (2) Laboratoire de Physiopathologie du Développement, 75230 Paris Cédex 05, France ; (3) Laboratoire de Biotechnologie des Cellules Eucaryotes, Université Paris XII, 94010 CRETEIL, France

Increase aFGF immunoreactivity has been demonstrated in bladder cancer compared to normal urothelium by our laboratory by means of immunochemistry (Urol. Res., 1992, 20: 211) and competitive enzyme immunoassay (J. Urol., 1989, 141: n°4, A594). These data have suggested the possible involvement of aFGF in the biology of urothelial transformation. To further study the putative FGF autocrine loop in human bladder cancer FGF receptors were analysed by cross-link on purified membranes preparations and FGF mRNA by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) on total RNA obtain by cesium chloride gradients. Results indicate a modulation of FGF receptor during bladder transformation with a decrease of bFGF binding sites and an increase of aFGF binding sites in bladder tumors compared to normal urothelium. A semi quantitative analysis of FGF mRNA by RT-PCR using TFIID as an internal control shown no difference between normal and neoplastic urothelium. Increase detection of aFGF-like proteins in bladder cancer may be related to post-transcriptional modifications and may affect regulation of FGF receptors. This hypothesis is being investigated further.

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GENEEXPRESSION DURING PROGRESSION OF SUPERFICIAL BLADDER CANCER.

Hans-J. Knopf, Reindert J. v. Moorselaar, Frans M. Debruyne, Jack A. Schalken, Urological Research Laboratory, University of Nijmegen, Nijmegen, The Netherlands

STUDY OBJECTIVE A major problem in the treatment of superficial transitional cell carcinomas (TCC) of the bladder is the fact that 10 - 20% of patients progress to a more aggressive state. There are no specific markers available which can predict the clinical course of superficial TCC.

MATERIALS & METHODS We used 2 spontaneous arisen bladder tumors of ACI-rats which were serial transplantable (RBT-157 and -323). As model representing the progression of bladder cancer, we used 16 passages (P) of the RBT-323 and 6 P of the RBT-157, resp.. BIOLOGICAL PROPERTIES: Tumor doubling time: *RBT-157*: 11 days (P1) - 9.5 days (P6). *RBT-323*: 13 days (P2) - 4 days (P15)., Histology: *RBT-157*: grade II in all P. *RBT-323*: progression from grade II to III. Metastasis: *RBT-157*: from P2 on (avérage 29%; > 50% in P4-6). RBT-323: from P5 on (average 62%; > 90% in P8-16). We tested 12 genes (v- + c-Ha-ras, c-myc, c-fos, c-fms, neu, v-raf, EGFR, p53, HMG-I, E- + P-cadherin) on mRNA-level (Northern blot analysis) so as to study the possible relationship between gene expression and the progression of TCC.

RESULTS In RBT-157 we found a constant expression of v-Ha-ras, p53, E- and P-cadherin in all passages. No expression of the remaining genes. In RBT-323 we detected a constant increase of v-Ha-ras from P1 to 16, an increasing expression of p53 in the first passages with a remarkable decrease in the late passages, a slight expression of neu only in the highly metastatic passages and an inconstant expression of E- and P-cadherin. No expression of the remaining genes.

CONCLUSIONS We present a rat-bladder-tumor-model with local and systemic progression. In the aggressive RBT-323 we observed a different expression of genes. This expression pattern seems to correlate with the local and systemic progression of the primary superficial tumor. In RBT-157 we did not find any correlation of progression and gene expression. So in both tumor lines different pathways are necessary for progression of primary superficial bladder tumors. Further studies are necessary to look for mutations of the tested genes and immunohistochemical studies are required to compare mRNA- and protein-levels .

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METACHRONOUS BLADDER TUMORS IN PATIENTS WITH UPPER URINARY TRACT TCC -

Eliahu Mukamel, Dan Simon, Miriam Konichezky, Ciro Servadio.
Institute of Urology and Dep. of Pathology, Beilinson Medical Center, Patah Tigva, Sackler Faculty of Medicine, Israel.

Fifty-five patients who underwent nephroureterectomy for upper urinary tract Transitional Cell Carcinoma (TCC) were followed between 2 to 5 years for tumor appearance and recurrence in the bladder. The follow-up was performed by repeated cystoscopies, urine cytology and intravenous pyelograms. The following data were collected on every patients from the hospital charts: tumor grade, tumor location (calyx, pelvis, ureter), multifocality, timing of tumor appearance and recurrence in the bladder, tumor progression and patients survival. Of the 55 patients, 30 developed metachronous bladder tumors, 28 (93%) of these tumors appeared within two wears following the nephroureterectomy. Two out of 5 (40%) patients with grade I tumors, 17 of 34 (50%) patients with grade II tumors, 7 out 10 (70%) patients with grade III tumors and 4 of 6 (66%) patients with grade IV tumors developed metachronous bladder tumors. None (0%) of the patients who had a single calyceal tumor developed bladder tumors, whereas 11 of 19 (57.8%) patients with a single pelvic tumor and 10 out 15 (66%) patients with ureteral tumors developed bladder tumors. Seven out of 8 (87.5%) patients with multifocal calyceal and renal pelvic tumors developed metachronous bladder tumors. Of the 30 patients who developed metachronous tumors 18 had one tumor recurrence, 5 had two recurrences and 7 had 3 recurrences. An upstaging of the recurrent bladder tumors was noted in two patients. All 30 patients were treated with intravescical thiotepa or BCG instillations. A significaltly higher 5 year survival was noted for patients without metachronous bladder tumor compared with those with bladder tumors. Our data suggest that recurrent metachronous bladder tumors in patients with upper tract tumors in an unfavourable prognostic indicator.

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THE INTRAOPERATIVE RADIOIMMUNODETECTION OF CEA:A NEW METHOD IN THE SURGICAL TREATMENT OF INVASIVE BLADDER CARCINOMA

Siracusano S,Tanda F*,Arru A,Bosincu L*,Azzena M. D**,Madeddu G**,Trombetta C and Belgrano E**
Department of Urology,Department of Pathology*,Institute of Nuclear Medicine**,University of Sassari,Sassari,Italy

Introduction
The radioimmunodetection surgery has been show to be a valid aid in cancer surgery of the colon and rectum and other neoplasms (1).In this study we aim to show the possible application of this technique in surgical treatment of bladder carcinoma.

Materials and Methods
The positive expression of CEA was noted be the immunoistochemical method on the bioptic multifocal specimen performed on the patient with bladder carcinoma (T3 NO MO).Anti-CEA monoclonal antibodies marked with I 125 were administred twelve days prior to the operation.The intraoperative detection of radioactivity emitted by marked antibodies was carried out by the Neoprobe 1000.Antibody activity was intraoperactively evaluated in common iliac lymphonodus sites,hypogastric and obturator,as well as the external surface of the small pelvis thus confirming that complete radical surgery was performed.

Results
The intraoperative detection of radioactivity did not show significant differences between the lymphonodus sites examined and the areas of the pelvic wall which were used as control.However the presence of marked positivity was found (+1.5) corresponding to the bladder neoplasm.The monitoring performed in the small pelvis after the removal of the above-mentioned anatomical structures confirmed the absence of other tumor sites.The results obtained by intraoperatively were confirmed by those obtained by histology and immunoistochemistry.

Conclusion
These data,referring to a single case reported for the first time in the Literature,suggest the usefulness of this technique for the eventual use of CEA in oncological Surgery for invasive carcinoma.

References
1.Sickle Santanello BJ et Al
radioimmunoguided surgery using the monoclonal antibody B72.3 in colorectal tumors. Dis.Col.Rect. 30:63,1987.

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SERUM FERRİTİN AS MARKER SUBSTANCES İN TÜMOR OF TESTİS

Y,ÖZGÖK.,B,SEÇKİN.,D,ERDURAN.,A,PEKER.,K,KARADEMİR.,Ç,HARMANKAYA.
GÜLHANE MİLİTARY MEDİCAL FACULTY
DEPARTMENT OF UROLOGY ETLİK/ANKARA/TURKEY

Ferritins are iron binding proteins present in most cell and serum.Farthermore ferritins are plentiful rediculoendotelial system and malign cell

34 patients were studied betweenI986 andI992 at the GülhaneMilitary Medical Faculty department of urology.Their age are between 20 and 32

In 9 patients with seminoma and 23with nonseminomatous germ cell tumors of the testis.

Serum levels ofCEA,AFP,hCG,LDH, and ferritin were measured preoperative,postoperative and during menagement.

We followed them postoperative periodicaly every three months

We compared as preoperative,postoperative,during the chemotherapy and during tumor recurrens according to pathological staging.

Serum ferritin levels were foundelevated after orchiectomy in the serum of patients with seminomatous and nonseminomatous tumors.There was found a decrease associated with response to therapy and increasing with relaps of tumor.But ve found increasing associated with chemoterapy regimens containing platinium.It may be platinium toxicity.That is,Ferritin is an exellent marker of toxicity of the chemotherapy with platinium.

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HYPERCALCEMIA IN RENAL CELL CARCINOMA: A POSSIBLE CAUSATIVE ROLE FOR INTERLEUKIN-6.

M.G. Weissglas[1], D.H.J. Schamhart[2], P. Vos[2], C. Löwik[3], S.E. Papapoulos[3], K.H. Kurth[2]
Dept. of Urology[1] and Endocrinology[3], State University of Leiden and Dept. of Urology,University of Amsterdam[2], The Netherlands.

Hypercalcemia is a complication of renal cell carcinoma (RCC). Rise of serum calcium is thought to be due to production of a humoral substance by the tumor, that promotes bone resorption. Interleukin-6 (IL-6) is a multifunctional cytokine with effects on many cellular targets, including bone cells. In vitro, IL-6 has been shown to promote osteoclastic bone resorption.
A renal cell carcinoma, derived from a hypercalcemic patient was established as a transplantable tumor line in nude mice. After transplantation the tumor induced a severe cachectic condition in the animals starting from day 16 , resulting in a weight loss of more than 25 % and death within 30 days. Tumor bearing animals developed hypercalcemia after day 16 with a mean value of 3.81 mmol/L versus 2.54 in controls. Levels of human IL-6 (Elisa), not detectable in controls and tumor bearing animals at day 10, rose from day 17 after transplantation (32 pg/ml) to levels of more than 1900 pg/ml 28 days after transplantation. There was no detectable circulating mouse IL-6 in controls as well as in tumor bearing animals. Human Tumor Necrosis Factor α and Interleukin-1 were not demonstrable in the circulation of the animals. Further evidence of IL-6 secretion by the tumor could be obtained by the demonstration of IL-6 production in culture of tumor cells and the expression of IL-6 mRNA by the tumor. In bone of tumor bearing mice a significant increase in osteoclasts could be demonstrated compared to controls, indicating increased bone resorption.
In conclusion, in this animal model of RCC we found hypercalcemia,increased bone resorption and cachexia together with significant production of human IL-6 by the tumor. These findings suggest that IL-6 may be responsible for these paraneoplastic symptoms in RCC.

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EPIDEMIOLOGICAL CONSIDERATIONS ON BLADDER TUMORS BASED ON THE DISTRIBUTION OF QUANTITATIVE DNA ALTERATIONS.

Franzolin N., Lotto A., Grassi D., De Siati M., *Azzolina L.S., *Marchioretto F., *Tridente G.
Divisione di Urologia, Thiene(VI), *Istituto di Immunologia e Malattie Infettive, Università di Verona.

During a 3 years clinical activity at the Urology Div. in the U.L.S.S. n.6"Alto Vicentino" of the Veneto Region, 129 cases of bladder tumors of various stage and grade have been recorded from an afferent population pool of 160,000, with an incidence ratio of 1/1240 (0.08%). The male/female ratio was 4/1 and the mean age 66y.(range 35-89y.). These basic data make evident the high incidence of bladder tumors on the local population. Since Jan.1992 we have begun to analyze bladder tumors for DNA content by flow cytometry. Sofar we have studied biopsies from 23 pts. with tumor grades G1-G3 and stages Ta-is-T3. Identical tumor samples have been also morphologically examined by the pathologist. DNA histograms have been acquired in a Profile II Analyzer(Coulter Corp.) and the cell cycle distribution calculated with the Multicycle program. The cells of 13 biopsies contained diploid DNA with increased S and/or G2 phases to various degrees, and 10 contained aneuploid DNA. It is remarkable that all the latter tissues had hyperploid DNA(indices between 1.3 and 3.4), that correlates with unfavourable prognosis, according to the most recent literature.We are now working on the hypothesis that the population localized in this community has remained relatively homogeneous, manteining peculiar genetic traits that predispose to this type of tumor, under the influence of local risk factors.To this purpose, the study on the bladder tumor incidence will be extended to a larger patients population with an adeguate follow-up.

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LOW DOSE vs STANDARD DOSE BCG THERAPY OF SUPERFICIAL BLADDER CANCER: A PHASE III COMPARATIVE TRIAL

Pierfrancesco Bassi, Claudio Milani, Tommaso Prayer Galetti, Agostino Meneghini, Antonio Garbeglio, Gianluca Drago Ferrante, Massoud Gholamalipour, Nicola Piazza, A. Calabrò and Francesco Pagano (Dept. of Urology, University of Padova - Italy)

In a previous report[1] we demonstrated that a low dose BCG regimen can decrease the toxicity and achieve significant response rates if compared with standard dose regimens.

We report the results of an ongoing prospective randomized phase III trial designed to compare the effectiveness and the toxicity of low dose (75 mg) and standard dose (150 mg) intravesical BCG regimens. From 1989, 87 patients with multifocal superficial bladder cancer were randomized to receive 75 mg (31 pts = Ta - T1; 11 pts = Tis) or 150 mg Pasteur BCG (31 pts = Ta - T1; 14 pts = Tis) in a 6 - week course therapy. An additional course and a maintenance course were administered in non responder and in responder patients, respectively. Complete response was defined as negative histology and washing cytology.
In prophylaxis group (papillary tumors: TUR + BCG) 90.5% and 85% complete responses were achieved with 75 mg and 150 mg BCG regimens, respectively. 90% and 78.5% complete responses were observed in therapy group (Tis: only BCG) with 75 mg and 150 mg BCG, respectively. Tumor progression was experienced in 2% and 6% of patients, respectively. Cystitis and fever were more frequent in standard dose group (cystitis: 31% vs 54%; fever: 14% vs 26%). No major complications were observed.

In conclusion, the incidence of side effects was higher in standard dose group than in low dose group, as well as the effectiveness was similar in both groups.

1. F. Pagano, P. Bassi and Coll.: A low dose Bacillus Calmette-Guerin regimen in superficial bladder cancer therapy: is it effective?. J. Urol. 146:32-35, 1991

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THE ROLE OF ALFA 2b INTERFERON IN SUPERFICIAL BLADDER CANCER RECURRENCES' PREVENTION

Abbolito A., Amoroso G., Cappucci M., Cefaloni A., Gentile G., Lentini M., Maione G., Marzano D., Moretti G., Pisi F., Provinzano V., Vermiglio M.

USL RM29 Ospedale di Frascati, 00044 Frascati (Roma)

Among BRM's (BIOLOGIC RESPONSE MODIFIERS), BCG and Interferons have been the most used agents to prevent recurrences of superficial bladder cancer. The good results of BCG treatment are now widely documented as well as its adverse reactions and complications so that it is not the first choise drug for all patients and its use is limited to the most aggressive tumors.

Interferons' treatment, on the other hand, is free from adverse reactions or complications, its patient's compliance is very good but we still do not know very much about its efficacity, alone or in combination with other drugs such as chemiotherapic agents.

Our group began to use alfa 2b interferon in patients with superficial bladder cancer (Ta-T1 and G1-G2) on 1988 and has treated more than 350 patients in different multicentric trials.

Considering our results we can conclude that interferon is certainly useful in prevention of recurrences of superficial bladder carcinoma, it is less active than epirubicine if administrated alone, it is more active if sequentially given with epirubicine and that when locally administrated is free from adverse reactions or complications.

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CAN SUPERFICIAL TUMOR RELAPSES AFTER INITIAL SUCCESSFUL RESPONSE TO BCG BE EFFECTIVELY RESCUED BY A FURTHER BCG THERAPY?

Pierfrancesco Bassi, Claudio Milani*, Nicola Piazza*, Tommaso Prayer Galetti, Agostino Meneghini*, Antonio Garbeglio*, Massoud Gholamalipour, Gianluca Drago Ferrante*, Francesco Pagano (Dept. of Urology, University of Padova - Italy)

The effectiveness of Bacillus Calmette Guerin (BCG) therapy of superficial bladder cancer is well known. Nevertheless some patients that initially responded to intravesical BCG recur later. To date no reports on the effectiveness of a rescue BCG therapy of tumor relapses after initial response to BCG are available.

To verify the responsiveness to further BCG therapy, we treated in a prospective study 20 consecutive patients (15 pts = Ta, T1; 4 pts = Tis; median time to relapse: 31 mos) who previously successfully responded to BCG, with a 6-week course of BCG therapy (150 mg, Pasteur strain).
Six-teen (84%) of them achieved a complete response, 2(10%) did not responded and one patient (6%) experienced tumor progression (follow-up: 6 to 26 months; median: 13 mos). BCG adverse effects are comparable to those previously reported. No major complications were observed.

In conclusion, a rescue BCG therapy of tumor relapses after initial successful BCG treatment seems effective as well as whorwhile of further investigations.

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RADIOCHEMOTHERAPY (RT/CT) AND ORGAN PRESERVATION IN THE MANAGEMENT OF INVASIVE BLADDER CARCINOMA (BC)

L. Luciani, E. Menichelli, P. Campanini, S. Maluta*, G. Fellin*
Departments of Urology and *Radiotherapy, Santa Chiara Hospital, Trento, Italy

Successful application of new post-cystectomy diversion techniques does not seem to have limited the greatly felt need for organ preservation in patients with BC.

Patients and method: between January 1989 and March 1991 24 patients with invasive BC (4 T2, 19 T3, 1 T4) and residual tumor after transurethral resection underwent CT (two courses of MCV: Methotrexate 20 mg/m^2, Cisplatin 70/m^2 and Vinblastine 3 mg/m^2) followed by 40 Gy to the pelvis and two additional doses of Cisplatin (CDDP). In 4 of 12 patients who presented mono- or bilateral hydronephrosis pre-therapeutic transcutaneous nephrostomy was performed. Cystoscopy, biopsies and urinary cytology were first obtained after MCV courses and then after RT plus CDDP.

If the latter biopsies were negative 24 Gy were delivered to the primary plus and additional dose of CDDP (total RT dose: 64,8 Gy in 36 fractions). In case of positive biopsy radical cystectomy was performed.

Results: seventeen patients were evaluable: 9 had negative biopsies after the first radio-chemotherapeutic course (MCV x 2 and 40 Gy plus CDDP) and completed their RT-CDDP treatment. One of them experienced local recurrence and 1 developped distant metastases. The remaining 8 patients showed positive biopsies: 5 of them underwent radical cystectomy and 3 completed RT-CDDP course but no further complete responses were observed. Two patients died with disease and 7 are alive free of disease and retain their functioning bladder; 8 patients underwent surgery (1 of these is alive with evidence of disease) - median follow-up 17 months -.

Complications included vomiting (grade 2: 8 pts; grade 3: 4 pts) and leukopenia (grade 1: 8 pts; grade 2: 5 pts).

Conclusions: our preliminary results suggest that in some patients with muscle-invasive carcinoma bladder preservation may be attempted by combined radio-chemotherapy under careful follow-up.

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SUPERFICIAL BLADDER CANCER: B.C.G. IMMUNOTHERAPY ASSOCIATED WHITH TIMOPENTINA STIMULATION.

Morzenti S. - Ospedale S. Biagio, Clusone (BG).

The aim of B.C.G. immunotherapy in reducing recurrencies in superficial bladder cancer is widely accepted. The effect of B.C.G. intravescical administration can be observed as erosion of the epithelium, followed by epithelial dysplasia, but also as a local aspecific inflammatory infiltration, whith strong recruitment of immune cells. To try to reduce the incidence of the most common side effects characterizing the B.C.G. induced cystitis (severe dysuria, haematuria, tenesmus, iperthermia), and expecially to avoid the dangerous diffusion of the infection, we combine the intravescical B.C.G. administration whith Timopentina immunomodulation. We usually start the immunotherapy after trans uretral resection of the tumour whith Timopentina administration twice a week, for one month. Then the patient undergoes bladder instillation whith B.C.G. 10^6 saline solution (50cc), to be kept inside the bladder for two hours, and then cystoscopy, or cromocystoscopy, and urinary citology are carried out: if they are negative, immunotherapy is continued monthly for further 12 - 18 months.
We have included in this trial ten patients (six females, and four males) whith non invasive bladder urotheliomas, and one patient (female) whith carcinoma "in situ" of the bladder. Up to date, no side effect has been observed in all patients, recurrencies occurred in four cases (diathermocoagulated during cistoscopy, thanks to theyr small dimensions). Onlythe carcinoma "in situ" demonstrated unfavourable increasing, but immunotherapy is still performed, according to the wishes of the patient, who rejects cistectomy.
Thought it is not certainly adfirmable that Timopentina immuno stimulation allows B.C.G. intravescical therapy to reduce recurrencies of superficial bladder tumours, the results of this experience demonstrated, in our opinion, that its most common side effects can in this way be avoided.

References: Anglis J. et Al.: Complications of topical administrations for urothelial cancer. Br J Urol, 1987, 59: 547-549.
Cumming J.A., Webb J.N.: Intravescical B.C.G. therapy for bladder cancer. Br J Urol, 1989, 16:401-404.

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SYSTEMIC NEOADJUVANT CHEMOTHERAPY CMV AND M-VAC. THE RESULTS COMPARISON.

Andrzej M. Stokłosa[1], Jerzy Świerż[2], Ewa Koźmińska[1], Bronisław Stawarz[2], Jan Szymanowski[1]
1. Dept. of Urology, Bielański Hospital, Warsaw, POLAND
2. Clinic of Urology, Central Clinical Hospital of the Military Medical Academy, Warsaw, Poland

We report the results of our attempt to compare the effectiveness and toxicity of neoadjuvant chemotherapy CMV and M-VAC in two groups of patients with infiltrating bladder cancer. Group A (Bielański Hospital) consisted of 44 pts. and was treated by CMV multidrug regimen (cisplatin, methotrexat, vinblastin), group B of 34 pts. (Military Medical Center) was treated by M-VAC (methotrexat, vinblastin, cisplatin, adriblastin). The medication consisting of two courses of neoadjuvant chemotherapy was delivered prior to the radical cystectomy. The response to the chemotherapy was assessed according to the WHO's standards. Complete clinical /CR/ response was observed in 29% and 21% of pts. in group A and B respectively, partial clinical /PR/ response in 27% and 35% in group A and B respectively, cancer stabilization /STAB/ in 24% and 29% respectively. Progression /PROG/ was observed in 15% of both groups of patients. The morbidity due to chemotherapy was significantly lower in CMV group (48,8% vs 94%; p= 0,0015). Radical cystectomy underwent 21 of 44 pts. (49,5%) in group A , and 11 of 34 pts. (32%) in group B. Radical surgery was performed only in pts. with CR, PR and STAB. The remaining pts. were given other treatment modalities. The observation time was 1-51 months. The pts. with CR did significantly better than pts. with PR and STAB.
We consider the CR to neoadjuvant chemotherapy as a good prognostic factor. The effectiveness of the chemotherapy was similar in both group, but toxicity was much lower in CMV group (p= 0,0015).

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GENERATION OF TUMOR INFILTRATING LYMPHOCYTES (TIL)FROM RENAL CELL CANCER

N. Thiounn ; C. Mathiot ; T. Flam ; M. Zerbib ; B. Debré ; W. Fridman ; C. Peyret
Paris. France

The metastases of renal cell cancers raise difficult problems for treatment. This explain the interest arising from the new therapeutic approach of adoptive immunotherapy which offers appealing propects.
Adoptive immunotherapy consists of the transfert of immunologically competent cells, such aslymphokine activated killer cells (LAK) or tumorinfiltrating lymphocytes (TIL). Experimental studies haveshown that the cells with the hightest cytolytic activity are TIL.
19 tumor specimens were cultured with recombinant IL2 in order to produce tumor-infiltrating lymphocytes.The cells were cultured in a serum-free media with 200 units IL2/ml. 14 of the 19 samples developped TIL (74%). At day 30, 3 cultures among 10 tested expressed CD3+ CD8+ phenotype. The TIL from these 10 cultures exerted non MHC cytotoxicity when tested at day 30.
The reproducibility of the development of TIL from renal tumors allows to design therapeutic trials for metastatic renal cancer.

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SUBCUTANEOUS RECOMBINANT INTERLEUKIN 2 (rIL-2) AND ALPHA INTERFERON (aIFN) IN METASTATIC RENAL CANCER.

C. Graiff, M. Amichetti, S. Maluta.
Div. Radiation Oncology, S.Chiara Hospital, Trento, Italy.

From July 1990 to December 1991 eleven previously untreated patients (pts.) with metastatic renal cancer were treated with a combination of rIL-2 and aIFN. There were 7 males and 4 females with a median age of 51 years (range 37-69). Site of metastatic disease was: lung (3 pts.), lung and bone ± nodes (5 pts.), bone (2 pts.), and liver (1 pt). Pts. with CNS involvement were excluded from the study. rIL-2 was given subcutaneously on an outpatient basis at a dose of 5 million U/m^2 every 12 hours for 5 days per week up to 6 consecutive weeks per cycle. aIFN was given subcutaneously at a dose of 5 million U (day 1-3-5 every week). If no progressive disease occurred the treatment was continued up to a maximum of 6 cycles. Median number of delivered cycles was 2 (range 1-6). Overall response rate was of 27% (2 PR, 1 SD). No CRs were observed. According to the involved sites, responses were observed at lung (2 PR) and bone (1 SD). Duration of response ranged from 3 to 11+ months. Treatment related toxicity was limited to WHO grade I malaise, anorexia, nausea and hypothension with the exception of fever of grade III. The treatment proved to be feasible on an outpatient basis as a home therapy, with a favourable toxicity profile in comparison with the reported side effects of intravenously administered rIL-2. The combination of the two cytokines do not seem to increase the overall response rate observed with rIL-2 or aIFN alone.

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RESULTS OF SUBCUTANEOUS IL-2 AND IFN-ALPHA 2b THERAPY VERSUS SUBCUTANEOUS IFN-GAMMA THERAPY IN METASTATIC RENAL CELL CARCINOMA

S. Möllhoff, M. Goepel, G. Emmerich, H. Rübben
Dept. of Urology, University of Essen Medical School, Essen, Germany

In a randomized phase II study we evaluated the response and side effects of a combined administration of recombinant Interleukin-2 (rIL-2) and Interferon-alpha 2b (IFN-alpha 2b) versus Interferon-gamma (IFN-gamma) in patients with metastatic renal cell cancer.
Patients in group A received 200 mcg. IFN-gamma sc. weekly. In group B patients were treated with a combination therapy of rIL-2 and IFN-alpha 2b over 6 weeks.
Up to now 40 patients were treated, 20 patients in each group. Toxicity of IFN-gamma treatment was absent. The therapy with rIL-2 and IFN-alpha 2b led to sideeffects grade 3 (WHO): Fever, chivering, fatigue and weight-loss. Follow-up after 6 months (1-8 months) showed stable disease in 10 patients and progression in another 10 in group A. In group B there were 3 complete remissions, 2 partial remission, and 10 patients with progressive disease .
The clinical outcome showed no remission rate in group A but 25% objective response rate combined with sideeffects grade 3 in group B.

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STAGING OF UROLOGIC CANCER AND ADRENAL MASSES

E. Menichelli, G.L. Failoni, M. Niccolini, L. Luciani
Departments of Urology and Diagnostic Radiology, S. Chiara Hospital, Trento, Italy

Introduction. With wider use of Ultrasonography (US) and Computed Tomography (CT) an increasing number of adrenal masses is now being detected. The adrenal offers many hospital factors for metastatic disease (vascularity, nutritional substances, concentration of steroids, steroid receptors...) and since the presence of metastatic adrenal lesions in oncologic pts can influence prognosis and treatment planning, it is necessary to obtain a definitive diagnosis of true nature of the mass. Aspiration Cytology (AC) has been used in the diagnostic evaluation of retroperitoneal tissues and organs but has surprisingly had limited use in the study of adrenal pathology. We report our experience with AC in the diagnosis of adrenal metastases from urologic cancer. **Patients and methods.** A series of 18 pts with urological neoplasms and adrenal masses underwent transcutaneous CT/US guided or intraoperative AC using modified Chiba needle*. Transcutaneous biopsy was performed through posterior translumbar or anterior transabdominal approach. **Results.** 2 adrenal adenoma and 11 metastases were identified. In 3 pts cytological specimens resulted inadequate and in 2 pts negative results were found. In 4 pts 2 passes were necessary (Table). No complications were encountered.

PTS	AGE	SEX	NEOPLASM	ADRENAL MASS	PASSES	NEEDLE GUIDANCE	CYTOLOGY
1	59	M	Left RCC	Bilateral	2	CT/US	+/+
2	66	M	Prostate	Bilateral	1	CT	+
3	72	M	Bladder	Left	1	CT	+
4	70	M	Bladder	Left	2	CT	inadeq.
5	63	M	Left R pelvis	Right	1	CT	+
6	48	M	Bladder	Right	2	CT	inadeq.
7	66	M	Bladder	Left	1	CT	adenoma
8	43	M	Right RCC	Left	1	US	+
9	57	M	Right RCC	Left	1	intraoperative	+
10	54	M	Right RCC	Left	1	US	+
11	78	M	Bladder	Left	2	US	+
12	72	M	Prostate	Right	1	CT	+
13	65	M	Left R pelvis	Right	1	US	adenoma
14	75	M	Prostate	Left	2	CT/US	inadeq.
15	46	M	Right RCC	Left	2	CT/US	+
16	64	M	Bladder	Right	1	US	-
17	61	F	Bladder	Left	1	US	+
18	72	M	Left RCC	Right	1	US	-

RCC = Renal Cell Carcinoma

Conclusions. Good results have been reported with percutaneous adrenal biopsy in selected patient populations but the procedure is not widely used in the staging of urologic cancer. Our experience emphasizes the feasibility and diagnostic value of AC in detecting these still unusual metastases in the pre-therapeutic evaluation of pts with urological neoplasms.

* Cyto-aspir (patent pending)

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MALIGNANT PHEOCHROMOCYTOMA IN INCIDENTALLY DISCOVERED 23 ADRENAL TUMOURS: REPORT OF 2 CASES

E. Maestri*, M. Franzini*, S. Binacchi*, S. Passerini*, L. Mariani*, F. Piscioli°, A. Capitanio°, G. Verrico°.

* General Hospital of Guastalla (Reggio Emilia),
° General Hospital of Rovereto (Trento)

INTRODUCTION.

Incidentally discovered adrenal masses (IDAM) represent a diagnostic challenge. The morpho-functional evaluation and the successive follow-up lead in most patients to the diagnosis of "non functional - non evolutive adrenal masses", not requiring surgery. Most series report low incidences of neuroectodermic tumours and among them a high proportion of benign lesions.
The authors report their experience of two malignant pheochromocytomas presenting as IDAM (among 23).

CASES REPORT

Case 1: T.D. (woman age, 65) was referred for IDAM discovered during an ecograghic study performed for cholelithiasis. She was followed for mild hyperglicemia and hypertension since 10 years but no paroxysm was described. The tumour produced mainly NA (1000 mcg/24 hrs.). The surgical removal of the left adrenal tumour (6x9 cm.) leaded to the regularization of PA and glycemic values but NA excretion did not reach normal levels. The MIBG scan showed 3 minimal metastatic deposits along the lumbar aorta, not discovered by CT scan and radiometabolic administration of MIBG was performed in November 1991 with reduction of NA excretion.
In the case 2 R.L. (male, age 65) an abdominal echography was performed for routine evaluation after trauma. The patient was normotensive and no diabetes mellitus was found. The echo and CT scans showed a right adrenal mass of 12 x 9 cm. Urinary catecholamines and VMA excretion was normal. The histologic examination suggested the diagnosis of malignant pheochromocytoma. The immunocytochemical stains confirmed the histological diagnosis: were strongly positive for S-100, Neuron-specific-enolase and negative for cytokeratins. The MIBG scan showed metastasis along the lumbar aorta, not discovered by CT scan and the patient radiometabolic administration of MIBG was performed in July 1992.

DISCUSSION

Our experience of two malignant (and one benign) pheochromocytomas in a series of 23 IDAM, points out a relatively high incidence of these tumours among IDAM and warrants a more complete evaluation (MIGB scan at least), in order to exclude this diagnosis also in patients with normal catecholamines excretion.

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FLUTAMIDE VERSUS ORCHIDECTOMY IN PATIENTS WITH METASTATIC PROSTATE CARCINOMA.

L. BOCCON-GIBOD, Paris ; G. FOURNIER, Brest ; P. BOTTET, Caen ;
C. MALLO, Levallois, France

Flutamide has been widely used in combination with orchidectomy or LH-RH analogs in treatment of metastatic prostate carcinoma. Very few data are available concerning its use as monotherapy.
In this pilot study we compare the efficacy and safety of flutamide (F) with that of orchidectomy (O) considered the reference treatment. 104 men with newly diagnosed untreated stage D2 prostate carcinoma were randomized and allocated to a 250 mg TID dose regimen of F (n=54) or bilateral orchidectomy (n=50). Patients (Pts) charateristics were comparable in both groups. Pts were clinically, hematologically and biochemically controlled at months 3, 6, 12, 18, 24. Clinical response was evaluated according to the NPCP criteria. Moreover sexual scrore before and during treatment was evaluated by questioning Pts in terms of libido, erection and sexual intercourse.
Up to now, the median follow-up is 17 months. Progression-free survival is similar in both arms (p=0.97) : 18 progressions after a mean time of 276 days in arm O and 24 progressions after a mean time of 267 days in arm F. PSA returned to normal values at month 3 in 32% of Pts of arm O and 11% of Pts of arm F (P=O.O3) but at month 6 this difference was not significant (P=0.46) since 34% of Pts had normal PSA in arm O vs 26% in arm F. In arm F, the mean increase in testosterone plasma levels from baseline was 53% at month 3 and 23% at month 6 remaining inside normal limits. Sexual consequences of treatments were impossible to assess statistically because most of the Pts had a null initial sexual score.
Adverse reactions in arm O consisted of gynecomastia in 4 Pts, hot flushes in 13 Pts, and in arm F of 1 diarrhea leading to termination of F, 10 cases of gynecomastia, 1 case of hot flushes.
To date there is no difference between both treatment arms in terms of progression-free survival.

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A PHASE 2 OPEN STUDY DETERMINING THE EFFICACY AND TOLERANCE OF CASODEX IN PATIENTS WITH ADVANCED PROSTATE CANCER.

A. Bono - on behalf of all participating investigators.

INTRODUCTION: CASODEX, a pure potent non steroidal antiandrogen with a plasma half life suitable for once daily oral administration, has been shown to be very well tolerated with fewer side effects compared with other antiandrogens. The most commonly reported side effects, prompted by direct questioning, were breast tenderness (63,4%), breast swelling (52,5%) and hot flushes (23,6%).
MATERIALS AND METHODS: A 50 mg once daily oral dose was evaluated in 267 pts, of whom 20 underwent detailed assessment of endocrine function and another 20, detailed assessment of cardiac safety.
RESULTS: Objective response rate (partial response by protocol criteria) was 55,5% (95% confidence limits 49,5% to 61,6%). Subjective response rate by protocol criteria was 56,1% (95% confidence limits 43,8% to 68,3%).
The product limit survival estimate of the median time to treatment failure in all patients was 35,6 weeks.
Of sixty-nine patients receiving second line hormonal therapy after progression, 42 were assessable for subsequent response: 4 had partial response, 10 had stable disease and 20 progressed further. Endocrine analysis showed Week 4 testosterone and Day 8 and Week 4 luteinizing hormone to be higher than pre-treatment (0,1% significance level).
Cardiac safety analysis showed one statistically significant change (5% level) from pre-treatment to Week 12 in one patient, not considered clinically relevant.
There were 38 patients withdrawn because of adverse events. Other than the pharmacological effects associated with antiandrogenic activity, all other adverse events each occurred in less than 5% of patients.
CONCLUSION:
CASODEX was well tolerated and effective in patients with prostate cancer, giving objective response rates comparable with conventional hormonal therapy.

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OCTREOTIDE IN ADVANCED HORMONE-INDIPENDENT PROSTATE CANCER .

Gaetano Scipioni,Enzo Mastroberardino,Daniela Granchelli,Maria P. Di Cretico and Luciano Vincenti
Department of Urology "S. Liberatore" Hospital-Atri - (TE)-64032-Italy

Several neuropeptides,including somatostatin (SMS), have been shown to act as mitogenic growth factors(GF) in neoplastic progression phenomena of hormone-resistant prostatic adenocarcinomas (PC).Prostatic neuroendocrine cells are of major prognostic importance in PC,appearing more reliable in predicting patients'(pts) survival than do conventional (Gleason) histological grading systems:a significant correlation between survival and the absence of neuroendocrine cells in PC was demostrated (Cohen,R. J. et Al.,1990)and Abrahamsson et Al. (1989)showed that there is an inverse relationship between the degree of tumor differentiation and the extent of neuroendocrine differentiation. Recently,Kadmon et Al. (1991)reported that plasmatic chromogranin-A level was elevated in 48% of 25 pts with stage D2 prostate cancer and suggested that this marker can be used to monitor the clinical course of these pts.Somatostatin(SMS) has inhibitory effect on Growth Hormone,Prolactin,GFs(IGF-1,EGF,PDGF and FGF) and tumor cell proliferation "in vitro".
We teated with a SMS-analogue,octeotide acetate,8 patients with metastatic(D2)prostate cancer(PC)relapsed following complete androgen blockade(IInd line therapy);all pts. underwent prostate needle biopsy and/or TUR-P,and specimens were submitted to immunoperoxidase staining for Neuron Specific Enolase(NSE) and Chromogranin-A (Ch-A),two neuroendocrine cell markers.The pts. with positive immunohistochemical staining of the specimens,received octreotide subcutaneously at a dose of 0.1 mg t.i.d.(minimal administration 4 weeks).
The mean age of the pts. wsa 69.1 years,the performance status(PS) ranging 0-2 (ECOG)and they were treated for an average period of 126 days.
Results:3 pts. achieved an objective Partial Response (NPCP criteria) and 4 pts. were stabilised;main response duration is 84 days.Side effect only include mild diarrohea in the few first days of treatment(3/8 pts. 1-2 WHO grade).
On the basis of our initial experience,believe that SMS-analogues may have therapeutic implications in the management of patients with prostatic carcinoma.

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Parenteral estrogen treatment. The Swedish experience.
Stege. R, Pousette Å, Henriksson P and Carlström K.

Dept of Urology, Clinical Chemistry, Cardiology and Obstetrics and Gynaecology, Huddinge University Hospital, Karolinska Institutet, S-14186 Huddinge

During nearly half a century surgical castration and estrogens were the main treatment modalities of prostate cancer. The oral administration of estrogens, however, is combined with highly increased morbidity and/or mortality, mainly due to arterial ischaemic events and tromboembolies. The mechanism behind these complications seem to be mediated by disturbances of the liver metabolism, e.g. the coagulation system. Oral estrogen administration causes extremely high estrogen concentration in the liver due to the first liver passage. By administrating the estrogens strictly on the parenteral route (Estradurin) in order to avoid the first liver passage the above mentioned liver mediated side effects do not occur. (Prostate 10, 1987). In several pharmacokinetic studies with Estradurin we could show preserved gonadotropin inhibition resulting in testosterone decrease to castration level (Am J Clin Oncol. 11, 1988; Prostate 14, 1989; Urol Int, 45, 1990). In an open study only 4 of 53 patients (8%) treated with 240 mg Estradurin monthly showed at 3-years follow up cardiovascular complications compared to a 40 % incidence in patients with oral estrogens (Scand J Urol Nephrol, suppl 135, 1991). The tumour effects were comparable to other established hormonal treatments. An ongoing randomized study of Estradurin vs. surgical castration will be presented.

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ESTRAMUSTINE PHOSPHATE IN ESCALATING DOSE IN PREVIOUSLY UNTREATED METASTATIC PROSTATE CANCER.
A multicenter study.

M. Brausi, A. Reich*, F. Micali°, M. Benelli°°
Dept. of Urology, Ramazzini Hospital, Carpi (Italy); *Dept. of Urology, Santa Chiara Hospital, Trento (Italy); °Institute of Urology, University of Perugia (Italy); °°Dept. of Urology, Misericordia Hospital, Prato (Italy)

Time to disease progression and toxicity of Estramustine phosphate were investigated in 115 previously untreated patients with grade G1-G2 and G3 metastatic prostate cancer, participating in an open multicenter clinical trial.

Patients and method. Estramustine was administered orally in a 5 day escalating dose (840 mg/die max) with dose reduction (420 mg/die) in case of complete response (CR) or partial response (PR). Time to progression and survival were evaluated.

Results. After 2 months 67 patients (58,3%) achieved a PR. Forty-two patients (36,5%) had stable disease (SD) and 6 patients (5,3%) progressed (PD). After a median follow up of 536 days 87% of the patients were still alive. Thirty-one patients /28%) developed PD in a median of 592 days.

Toxicity. Four patients (3,48%) discontinued the treatment because of adverse events (AE). Forty-three patients (37,4%) reported nausea and vomiting, 19 patients (16,5%) peripheral edema.

No statistically significant differences in time to progression were seen between G1 - G2 and G3 tumors.

Conclusion. This could suggest the use of Estramustine phosphate as a first line treatment in metastatic prostatic cancer patients with less favourable prognostic factors.

Late results will be presented.

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CLINICAL/PATHOLOGICAL STAGING AND RADICAL RADIOTHERAPY (RRT) IN THE MANAGEMENT OF APPARENTLY LOCALIZED PROSTATIC CARCINOMA.
A study of 190 patients (1978-1989)

L. Luciani, G.L. Falloni, S. Bosetti, U. Graffer, G. Fellin*
Departments of Urology and *Radiotherapy, Santa Chiara Hospital, Trento, Italy

RRT is widely used in the treatment of clinically localized prostate cancer but few Authors support the concept that pelvic lymphadenectomy (PL) may be required since nodal metastases have highly prognostic significance. Even the value of lymphangiography and CT scan, followed or not by aspiration cytology, is often questioned.

Patients and method: from 1978 to 1989 190 pts with biopsy proven prostatic adenocarcinoma underwent RRT (10 MeV linear accelerator) after clinical (95 pts) and/or surgical staging (95 pts). They were given 70 Gy in 35 fractions (200 Gy per fraction and 5 fractions per week in 7 weeks) and had fields limited to the prostate if nodes were negative. N+ pts had fields extended to the pelvis (50 Gy with additional boost of 20 Gy on the prostate). Tumor control was assessed by periodical clinical, biochemical, radiological and radionuclide scan studies follow-up and post-RRT prostatic biopsy (2 yrs or more) - median follow-up (all pts group): 60 months -.

Results: 5- and 10-year disease specific and disease free survival (DSS/DFS) rates and the significance of differences among various groups of pathologically staged patients are reported in the table (Kaplan-Meyer method and log-rank test):

TNM	N°	% 5-yrs DSS	% 10-yrs DSS	p	% 5-yrs DFS	% 10-yrs DFS	p
N-	65	86.9	72.7	< 0.007	82.5	64.5	< 0.0001
N+	30	61.8	41.5	"	34.6	6.9	"
G_{1-2}	56	80.1	69.9	N.S.	69.6	42.6	N.S.
G_3	39	76.2	47.0	"	61.1	27.0	"
T_{1-2} N-	36	89.5	75.7	"	86.2	79.5	<0.05
T_3 N-	29	83.4	68.2	"	77.3	46.8	"
All pts	95	78.4	62.3	-	66.2	37.2	-

Conclusions: the knowledge of true stage of patients with clinically localized prostatic carcinoma may improve the results of RRT and the interpretation thereof. The therapeutic role of PL is as yet undetermined.

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PROGNOSTIC FACTOR IN PROSTATE CANCER: NOR AS A NEW METHOD FOR QUALITY OF LIFE EVALUATION.

Marandola P., Orlando G., Bianchessi I., Vicini D., Mirando P., La Marca F., Derenzini M.*, Trerè D.*, Lardennois B.**, Speroni A.

School of Urology, Pavia University, Italy
* Experimental Pathology Department, Bologna University, Italy.
** CHRU, Urology Department, Reims France

The prognosis in prostate cancer is a unresolve problem. It is a widely diffused opinion that moving backword in time the moment of the diagnosis of cancer of prostate, so that the tumor is detected earlier than normal, means that the treatment would be more effective than the one adopted in the usual times of diagnosis. With modern oncology concepts of diagnostic phase are changing in the way that a correct and valuable diagnosis is based upon a rational staging and a reliable prediction of the evolution of the cancerous disease. Theoretically the mortality rate of prostate cancer can be reduced by the prevention programs and by the improvement of treatment methods, but the "earlier" diagnosis is certainly an easier and less expensive strategy to achieve the same objective. The Authors have started a multicentric protocol in Italy and France based on a computerized record and a telematic network. The preliminary results of the AgNOR proteins fixed in alcohol on 30 cases of adenocarcinoma of prostate are significant. The Ag-NOR have been stained with silver technique set up by Ploton and Derenzini while the quantitative index has been evaluated by a semi-automatic system part commercially available, partly modified by the Authors. The conclusions:

a) the Ag-NOR index is a simple and reproducible method;

b) the Ag-NOR staging system corresponds to the Gleason's grading;

c) the Ag-NOR elevation is a reliable marker of increased cell proliferation and is more simple of Gleason's determination.

d) the patients quality of Life is a fundamental parameter in the activity of physician. The attention for prognostic factors allows better considerations for the therapeutic strategy in a view of a better quality of life of the patient.

The patient quality of Life must be the greater important parameter in the therapeutic choice.

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FLOW CYTOMETRY ON FINE NEEDLE ASPIRATES OF RENAL TUMOURS

G. Bianchi, P. Beltrami, C. Tallarigo, N. Franzolin, L.S. Azzolina[1], G. Mobilio.

Cattedra e Divisione Clinicizzata di Urologia, [1]Istituto di Scienze Immunologiche, Università degli Studi di Verona, Italy

Flow cytometry (FCM) of DNA has been performed by numerous investigators on renal tumour tissue. The results obtained are controversial, since not all investigators have demonstrated a statistically significant correlation between presence of aneuploid cell populations and patient survival. The most important reason is probably the different number of samples analyzed for each tumour.

In fact, the renal tumours are known to be composed of a heterogeneous cell population and with only a limited number of samples it may prove difficult to detect the significant cell population as regards prognosis, i.e. the aneuploid population.

Therefore most investigators agree that is essential to perform the flow cytometry analysis on a large number of tumour tissue samples.

In our study we decided to do the DNA analysis on tissue samples and on needle aspiration samples drawn from the surgically removed tumour mass and to determine the reliability of this type of cytometric analysis compared to that of FCM performed in multiple tumour tissue samples.

FCM analysis was performed in 60 renal tumours removed surgically.

Analysis of our results in tissue samples yielded 32 diploid and 28 aneuploid tumours.

The DNA analysis carried out on needle aspirates revealed 23 diploidies and 37 aneuploidies.

In our study FCM DNA analysis on fine needle aspirates is capable to detected aneuploid population at least as frequently as on tissue samples.

The needle-aspirate cell sampling technique and DNA analysis of these samples by flow cytometry may simplify sample preparation procedures. What is more, pre-operative FCM on needle aspirate may provide an important additional parameter to aid us in the choice of conservative-type surgical treatment, given that the only criterion currently available is the size of the tumour.

Oral communications: Molecular oncology and endocrinology II

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The Apoptosis-Suppressing Oncoprotein, *bcl-2* is Expressed in Germinal Layers of the Human Prostate Gland and In Various Developmental Stages of Prostatic Oncogenesis. Marc Colombel, Fraser Symmans, Sixtina Gil, Dominique Chopin, Mitchell Benson and Ralph Buttyan. Columbia University, New York, NY, U.S.A. and Centre Hospitalo Universitaire Henri Mondor, Creteil, France.

The oncoprotein, *bcl-2*, is unique in both its site and mode of action. Originally identified from studies of a translocation (14:18) associated with the development of follicular B-cell lymphoma, the protein encoded by this gene is bound to the inner membrane of mitochondria and, when overexpressed in transformed lymphocytes, these cells are extremely long lived and resistant to apoptotic stimuli such as steroids and radiation. An earlier report had examined the expression of this protein in several normal tissues and had identified constitutive expression in cell layers considered germinal centers.

Using a monoclonal antibody to *bcl-2* in an immunostaining procedure and *in situ* hybridization with labeled *bcl-2* riboprobe, we studied expression of this gene in a series of 43 human specimens obtained through surgical and biopsy procedure for prostate cancer. In fetal prostate, anti-*bcl-2* identified expression in the germinal epithelium of the prostate buds. This pattern is maintained in adult normal and hypertrophic glands by the basal cell layer. In contrast, intraepithelial neoplasia (PIN) demonstrated *bcl-2* expression in basal cells as well as the dysplastic cells. Immunostaining for *bcl-2* was observed in many of the invasive adenocarcinomas, most consistently in well differentiated tumors. Staining patterns became heterogeneous in higher grades of prostate cancer and were undetected in several specimens of poorly-differentiated tumors removed from untreated patients for local disease. In contrast, tumors and metastatic lesions obtained from hormone-refractory prostate cancer patients demonstrated consistent immunostaining in surviving glandular structures, cancers in primary tissue and in bone marrow and soft tissue metastases. The results of our survey identified striking patterns of expression that suggest that *bcl-2* might be important for prostate development, the early pathway to prostate carcinogenesis and also for apoptotic resistance in hormone-refractory prostate cancers.

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Soluble Tumor Necrosis Factor Receptors (TNFR p55 and p75) in prostatic cancer patients

P.R.Huber[1]), H.P. Schmid[2]),H.Gallati[3]),A. Maurer[4]). M.Thein[4]);Kantonsspital Basel,Hormone Laboratory[1]), Urology Dept[2])and F.Hoffmann-La Roche &Cie, Basel, Pharma Research[3]), Roche Diagnostic Systems[4])

Introduction:

Tumor necrosis factor α and ß are multipotent cytokines mediating inflammatory responses and exhibiting cytotoxic effects against tumor cells. Both TNF's react with cell surface receptors to trigger cellular signals. These receptor proteins are shed into circulation and can be detected in both serum and urine. Patients and methods:

TNF receptor concentrations (p55 and p75) were measured in serum of healthy controls (Blood donors), in patients with **benign hyperplasia of the prostate (BPH)** and in patients with **prostate carcinoma (P-CA)** of various stages and receiving different treatments at the time of venipuncture. TNFR p55 and TNFR p75 were estimated using an enzyme immunoassays based on the bead and on microtiter plate technique respectively (Roche,Basel,Switzerland) using highly specific monoclonal antibodies against human recombinant TNFR p55 and TNFR p75 with sensitivity of 100 pg/ml. The tracer used was rs-TNF-POD conjugate Therefore only the reactive soluble receptor was detected.

	TNFR p55 (ng/ml)	TNFR p75 (ng/ml)
Controls (N=23;N=17)	1.2±1.0	0.8±0.5
BPH (N=34;N=11)	3.6±1.9*)	2.9±1.2*)
P-CA(N=34;N=13)	2.9±1.6*)	1.9±0.9*)

*) significance p<0.05

No correlation of the TNFR-concentration to the concomittantly measured PSA concentration in either BPH nor P-CA sera exists. Comparing the serum concentration in identical sera of the TNFR p55 and TNFR p75 of BPH patients a good correlation (R=0.713;p<0.01) exists. The same is true for prostate carcinomas of lower degree of staging (TNM-system) (R=0.7;p<0.02). Higher staged and/or metastasising carcinomas of the prostate are associated with higher concentrations of TNFR-concentrations. A big caveat is indicated with respect to kidney function as a malfunctioning kidney tends to increase TNFR concentrations leading to misinterpration of results. Conclusions: Surveying the scattering of the TNFR concentrations with respect to the PSA concentration it appears that there exists a tendency of increased (above normal) TNFR concentrations to be associated with lower concentrations of PSA (<14 µg/L) in BPH, and prostate cancer of lower TNM-staging. It will be important to further break down the data available to establish whether the TNFR's will be of predictive value for further outcome of the prostatic lesions.

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ANDROGENIC CONTROL ON EPIDERMAL GROWTH FACTOR, ITS RECEPTOR AND ANDROGEN RECEPTOR IN HUMAN PROSTATE CANCER CELL .

E.Petrangeli, * L.Ravenna, °C.Lubrano , *A.Vacca, °F.Sciarra, §G. D'Eramo, *M.P. Felli, *A.Gulino, *L.Frati and §F.Di Silverio.
Inst. Tecnologie Biomediche, C.N.R., * Dpt. Medicina Sperimentale , ° V Clinica Medica , § Patologia Urologica , University "La Sapienza", Roma, Italy.

The mechanism by which androgens regulate prostatic growth has been only partially elucidated. Detection of epidermal growth factor (EGF) and its receptor (EGF-R) strictly related to the AR content in the human prostatic tissue, suggested that this growth factor may be involved in mediating androgen proliferative effect.

To investigate in more detail the complex interaction between androgens and EGF in the human prostate , we analyzed AR, EGF, and EGF-R gene expression during androgen and antiandrogen treatment in the human prostatic hormone-dependent cell line LNCaP. The cells were cultured in the RPMI 1640 medium supplemented with 5% FCS. 5 days prior to the experiments cells were grown in medium supplemented with 5% dextran-coated charcoal stripped FCS (DCC-FCS). Androgen R1881 (1 nM) and antiandrogen OH-flutamide (100 nM) treatment was prolonged for 7 days.

Immunoreactive EGF (irEGF) , measured in the conditioned medium, was significantly increased in serum stripped conditioned medium (8.5 + 2.1 pg/ 10 cells) with respect of FCS conditioned medium (4.2 + 1.8 pg/10 cells) and was strongly increased after 48 hours R1881 treatment (5.5 fold). This androgenic effect was completely counteracted by the simultaneous addition of OH-flutamide. EGF binding capacity ,evaluated on cellular membrane pellets, was lower in the 5% DCC-FCS (55.8 fmol/mg protein), than in the 5% FCS cultured cells (91.3 fmol/mg protein). Androgen treatment enhanced EGF-R levels within 24 hours (117.0 fmol/mg protein). When the treatment was prolonged, EGF-R capacity decreased to that of the untreated control. The simultaneous androgen and OH-flutamide treatment increased EGF binding capacity that remained elevated till 7 days .The EGF-R opposite behaviour with respect to irEGF levels may be explained by receptor internalization in the presence of increased EGF availability.

Northern blot analysis demonstrated a 5 Kb EGF mRNA, more evident in the cell cultured with 5% DCC-FCS ,that did not reflect the large increase of irEGF achieved during androgen treatment.Then , the irEGF rise could be a consequence of a translational or posttranslational control.

The AR mRNA levels considerably increased when the cells were grown in the medium supplemented with stripped FCS. The R1881 treatment induced a rapid decrease in AR mRNA transcription. OH-flutamide counteracted in part the androgenic AR down-regulation.

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AN IN VITRO MODEL OF UROTHELIAL OVERGROWTH BY TRANSFORMED UROTHELIAL CELLS

Johanna M.J. Rebel, Willem I. de Boer, Cornelia D.E.M. Thijssen, Marcel Vermey, Ellen C. Zwarthoff, Theodorus H. van der Kwast. Dept. of Pathology, Erasmus University, Rotterdam, the Netherlands

The major problem in handling patients with bladder cancer is the high frequency of tumor recurrence. One explanation for this phenomenon is the implantation of neoplastic cells on damaged urothelium at the time of local resection of the tumors. In order to get insight in the process of intra-epithelial tumor overgrowth at the expense of regenerating urothelium we designed an experimental model in vitro. Bladder tissue fragments derived from adult mice were allowed to grow on transwell membranes. The outgrowth of the explants was monitored by daily measurements. Within two weeks the transwell membrane (465 sq mm) was covered by a confluent epithelial cell layer. In this primary urothelial outgrowth multilayering of the epithelial cells occurred, analogous to the in vivo situation. Transformed urothelial cell lines were generated by transfection of spontaneously immortalized mouse urothelial cell lines with the polyoma large and small T genes or other oncogenes. After transfection some of these cell lines were endowed with tumorigenicity in nude and syngenic mice. To investigate the tumor expansion at the expense of the primary urothelium we inoculated transformed urothelial cells aside the bladder outgrowth. Preliminary results show that some of our transformed cell lines (as well as the human T24 bladder carcinoma cell line) are able to overgrow the bladder outgrowth, while in due course non transformed (control) cell lines are replaced by the primary urothelial culture. This model seems therefore to be suitable to study mechanisms of overgrowth of tumor cells at the expense of regenerating urothelium.

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GROWTH FACTORS AND EXTRACELLULAR MATRIX REGULATE UROTHELIAL CELL PROLIFERATION IN VITRO.

Willem I. de Boer, Johanna M.J. Rebel, Marcel Vermey, and Theodorus H. van der Kwast. Dept. of Pathology, Erasmus University, Rotterdam, The Netherlands.

Several studies showed that growth factors EGF, TGFa, and bFGF are present in urine of non-patients or patients with bladder cancer. We investigated whether growth factors modulated proliferation of urothelial cells in vitro, and whether different extracellular matrix (ECM) substrata affected this modulation under serum-free conditions. Using three mouse urothelial cell lines we found that EGF, TGFa, and bFGF, but not aFGF, indeed stimulated proliferation of two non-tumorigenic cell lines g/G and NUC-5. This stimulation was inhibited by TGFß in g/G which compares with other normal epithelial cells. IGF-I, IGF-II, or insulin were needed for this stimulation indicating their role as true cell cycle progression factors. The tumorigenic cell line NUC-1 proliferated autonomously, which could be enhanced by IGF-I, insulin, and although less effective, IGF-II, presumably via the IGF type I receptor. Other growth factors barely affected NUC-1 proliferation. Culturing cells on fibronectin or collagen type IV generally enhanced proliferation compared to culturing on plastic. Growth factor mediated proliferation of g/G and NUC-1 was preferentially enhanced in the presence of collagen type IV. Firstly, we conclude that several different growth factors are involved in urothelial proliferation regulation. Secondly, growth factor modulated proliferation can be affected differentially by extracellular matrix substrata. We are presently investigating effects of these and other growth factors and ECM substrata on differentiation and proliferation of primary cultures of urothelial cells.

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CHARACTERIZATION OF THE HUMAN ENZYME STEROID 5α-REDUCTASE 1 SYNTHESIZED IN A BACULOVIRUS EXPRESSION SYSTEM. Catherine Iehlé, Sylvie Délos, Odile Filhol*, Pierre-Marie Martin. Laboratoire de Cancérologie Expérimentale, Faculté de Médecine Nord, Bd Pierre Dramard, 13326 Marseille, France; *INSERM U244, CENG, 85X, 38041 Grenoble, France.

In the human prostate, the microsomal enzyme steroid 5α-reductase (5αR) catalyses the conversion of testosterone (T) into the more potent androgen, dihydrotestosterone (DHT), which is a factor regulating cell growth in this tissue. Two distinct cDNAs coding for 5αR in human prostate have been characterized. Enzyme 5αR1 shows a maximum activity at basic pH whereas 5αR2 has an acidic pH optimum.

For further caracterization of physical properties of the enzymes and the production of antibodies, large amounts of protein are required. We report here the expression of the human steroid 5αR1 in a eukaryotic expression system : the baculovirus-directed-insect cell expression system. The full lenght cDNA (as previously published by S. Andersson and D.W. Russell, *P.N.A.S.*, 1990, 87, 3640) was ligated into the baculovirus transfer vector pEV55 to yield pEV55 h5αR1 plasmid. Transfer of the 5αR coding region into the *Autographa californica* nuclear polyhedrosis virus (AcNPV) genome was accomplished by homologous recombination following cotransfection of pEV55 h5αR1 and wild type AcNPV DNA into Sf9 insect cells. Limiting dilution for three rounds of visual screening and dot-blot hybridization with the entire cDNA were used to identify and isolate the corresponding recombinant viruses. Sf9 cells were infected with these viruses and homogenates used in 5αR activity assays by HPLC showed that we produced a catalytically active enzyme. No endogenous 5αR1 activity has been found in Sf9 cells. The recombinant enzyme showed an apparent Km for T of 4.9 µM, a Vmax of 2.64 nmol of DHT/min/mg of protein and a specific activity of 3.4 nmol of DHT/min/mg of protein. Recombinant 5αR1 activity was inhibited by specific 5αR inhibitors like MK906 and 4-MA.

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IS THERE A HETEROGENEOUS GROUP OF 5α-REDUCTASES IN HUMAN PROSTATE ?

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

Previous enzyme kinetic studies underlined the possibility that in epithelium and stroma of the human prostate at least two distinct 5α-reductases might be present. The purpose of the present study was to clarify if there are not only different 5α-reductase enzymes in epithelium and stroma, but in addition different 5α-reductases converting either testosterone (T) into the most potent androgen 5α-dihydrotestosterone (DHT) or Δ-4-androstenedione (Δ-4-A) into 5α-androstanedione (5α-A).
Both, the amount (Vmax) and the substrate affinity (Km) of 5α-reductase were determined in mechanically separated epithelium and stroma of normal prostatic tissue (NPR) and benign prostatic hyperplasia (BPH), and the results were correlated with the age of the donors. Whole cell homgenates of epithelium and stroma were incubated with radioactively labelled T or Δ-4-A as substrate and the metabolites were separated by HPLC. The significance of the differences between the means was calculated by students' t-test, the significance of age-dependent changes by the Spearman rank correlation coefficient. $P<0.05$ was considered significant.
The main results were: 1. Using T as substrate, in NPR (n=10) the mean Km (nM±SEM) and mean Vmax (pmol/mg protein • h±SEM) were significantly lower in epithelium (Km: 26±5; Vmax: 29±5) than in stroma (Km: 78±6; Vmax: 81±15). In BPH (n=20), the mean Km and Vmax were also significantly lower in epithelium (Km: 30±3; Vmax: 28±3) than in stroma (Km: 186±14; Vmax: 174±12). 2. Using Δ-4-A as substrate, in NPR (n=3) the mean Km and Vmax were nearly identical in epithelium (Km: 93; Vmax: 55) and stroma (Km: 74; Vmax: 86). In BPH (n=10), the mean Km and Vmax were significantly lower in epithelium (Km: 83±9; Vmax: 75±10) than in stroma (Km: 407±74; Vmax: 430±75). 3. In stroma, using T as well as Δ-4-A as substrate, Km- and Vmax-values correlated positively with age. 5. In epithelium, using T as substrate, the Km-values correlated positively with age, whereas the Vmax-values remained constant. However, using Δ-4-A as substrate, no significant age-dependent alterations of Km or Vmax were found.
The present differences with respect to the amount (Vmax), substrate affinity (Km), compartimentalization and age-dependent alterations suggest that in human prostate there is a heterogeneous group of 5α-reductases. This heterogeneity of 5α-reductase must be taken into consideration when 5α-reductase inhibitors are used in the conservative treatment of BPH.

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ANDROGENIC REGULATION OF ENZYME ACTIVITY AND RATE OF TRANSCRIPTION OF PROSTATIC NUCLEAR-ASSOCIATED CASEIN KINASE II (PK-N2).

S. Yenice, S.A. Goueli, A.T. Davis, and K.Ahmed. V.A. Med. Ctr. and U. of Minn, Minneapolis, MN 55417

Our previous work suggested that androgen effects on nuclear protein phosphorylation are largely mediated via a regulation of the nuclear messenger-independent casein kinase II or NII (PK-N2) which appears to mediate most of non-histone protein phosphorylation in the nucleus (Goueli and Ahmed, Cell. Molec. Biochem., in press). We have now utilized ArgArgArgGluGluGluThrGluGluGlu as th specific substrate to determine the precise changes in PK-N2 activity in response to altered androgenic status in the animal. PK-N2 activity present in prostatic nuclei isolated from animals with varying androgenic status showed a progressive decline starting at 6 hr post-orchiectomy. By 72 hr following androgen withdrawal the nuclear PK-N2 activity was less than 25% of that of the normal controls. Changes in the rate of transcription of nuclear RNA in a nuclear run-off assay for this enzyme using a cDNA probe for the a-subunit of casein kinase II were also examined. Hybridization analysis the newly transcribed ^{32}P-labeled RNA suggested that there was no change in the hybridizable RNA at 12 hr postorchiectomy, but a progressive decline was noted in preparations from animals orchiectomized for periods of 24-72 hr. Androgen administration reversed these changes. The results suggest that following androgen withdrawal the activity of PK-N2 is lost more rapidly than what would be indicated by the presence of the message. This may result from an effect of the androgen on this enzyme at the translational as well as transcriptional level. [Supported in part by research grant CA-15062 awarded by the N.C.I., D.H.H.S., and by funds from the U.S.D.V.A.]

Workshop: Image analysis of urological tumours

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CORRELATION BETWEEN DNA CONTENT AND OUTCOME IN PROSTATE CARCINOMA

B. Tribukait.
Department of Medical Radiobiology, Karolinska Institute,
Box 60212, S-104 01 Stockholm, Sweden

In a prospective study the cellular DNA content of tumors was measured based on fine needle aspirates of the prostate. 287 untreated patients under active surveillance and 309 hormonally treated patients were followed for a minimum of 10 years. 506 patients with cytologic benign prostate lesions served as a control group. The subdivision of tumors into diploid, tetraploid and aneuploid enabled further characterization of cytologically defined tumors. Significantly better survival for untreated patients over hormonally treated patients was found when comparing patients of same stage, grade and tumor ploidy. The reason was the adverse effect of androgen deprivation on tetraploid and aneuploid tumors. This was considered due to elimination of hormone dependent diploid tumor parts in aneuploid tumors leading to growth advantage for hormone independent tetraploid and aneuploid cell lines. Multivariate analysis confirmed the high prognostic value of tumor ploidy in prostate carcinomas.

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PREDICTING TUMOR PROGRESSION IN STAGE $T_{1,2,3}N_0M_0$ RENAL CELL CARCINOMA AFTER TUMOR NEPHRECTOMY

H.G. van der Poel, P.F.A. Mulders, G.O.N. Oosterhof, F.M.J. Debruyne, J.A. Schalken.
Dept Urology, University Hospital, Nijmegen, The Netherlands

Approximately 40-60% of patients with renal cell carcinoma (RCC) are free of metastases at time of diagnosis. In these patients, tumor nephrectomy could be a curative treatment, for all tumor material can be resected. However, 50% of these patients will develop recurrences and/or metastases during follow up. In the present study we analyzed prognostic features for the population of low-stage tumors in order to define high risk groups for tumor recurrence. The prognostic factors could serve in the selection of patients with low-stage RCC eligible for adjuvant chemo or immunotherapy.

In a population of 121 RCC-patients, material of 56 patients with low-stage ($T_{1,2,3}N_0M_0$) tumors was studied with at least 3 year follow up. In an earlier study (van der Poel, 1992) we found karyometric features describing tumor heterogeneity of additional predictive value of survival in patients with RCC. Likewise this study, in the present analysis several histological slides were studied karyometricallyIn, case of the presence of morphologically different tumor areas, to obtain a measure for tumor heterogeneity. Karyometric features comprised nuclear size, shape, chromatin pattern and DNA-content. Moreover, for each patient 8 clinical features were documented.

In a univariate Cox's model the following features were significantly correlated with tumor progression: Karnofsky score, nuclear shape (Freeman difference chain code features), nuclear size, and differences in chromatin patterns. In a multivariate Cox's model the presence of differences in chromatin pattern as measured with Markovian texture features were the best predictors of tumor progression.

We conclude that karyometric analysis is a powerful tool for predicting tumor progression in patients with low-stage renal cell carcinoma. Tumor heterogeneity in nuclear chromatin patterns quantitated by karyometric analysis was of most value. These findings are particulary valuable for stratifying RCC-patients for adjuvant treatment modalities.

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EVALUATION OF DNA IMAGE CYTOMETRY ON 84 HISTO-CYTOLOGICALLY INVESTIGATED BLADDER TUMORS.

J. Assailly*, A. Vieillefond*, G. Benoit**, D. Schoevaert*, E. Martin*
* Service de Cytologie et d'Anatomie Pathologique, ** Service d'Urologie, Hôpital Bicêtre, 94273 LE KREMLIN BICETRE - FRANCE

Despite numerous studies on the bladder cell populations, we still do not have clear criteria for setting the threshold between benign and malignant bladder tumors. The goal of this study was to evaluate DNA content as a diagnostic aid in relation to histology and cytology both.
Conventional urine cytologic and DNA examinations were performed on 84 bladder tumors histologically investigated the same day.
Cellular DNA content was determined by image analysis (SAMBA 2005 cytometer) on routinely processed Feulgen stained cytocentrifuged preparations of urine sediments. From DNA histograms parameters range values (% of aneuploïd cells - % of hyperploïd cells >5c and % of proliferative cells), four DNA tumoral diagnostic classes were proposed: HP (High Positive), PO (Positive), S (Suspect of tumor process), N (Negative - Diploïd).
DNA measurements were positive (HP-PO) or tumor suspect (S) for 93% of patients with histologic invasive and not invasive bladder tumor identified the same day, while only 28 % of these patients were diagnosed tumoral with conventional cytology. Comparison with grade indicated an higher percentage of positive DNA for upper grade (Grade III: 79%; Grade II: 65%; Grade I: 50%). Furthermore, we obtained 52% of positive DNA for negative cytology and 2% of negative DNA for positive cytology.
These results evidenced interest of DNA measurements as a fuller information to histology and cytology in routine analysis.

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PROGNOSTIC EVALUATION OF MORPHONUCLEAR PARAMETERS IN SUPERFICIAL AND INVASIVE BLADDER CANCER. Marc Colombel, Yvan de Launoit, Claude Abbou, Robert Kiss and Dominique Chopin. Departement d'Urologie C.H.U. Henri Mondor, Creteil, France.

Computerized image analysis systems have been demonstrated to be useful and reliable for measuring morphometric parameters from histological and cytological samples. We previously demonstrated that SAMBA 200 (TTITN, France) provided quantitative analysis of morphonuclear parameters that correlated with grade of bladder tumors. We present the evaluation of these parameters as prognosis indicators for the progression of superficial and invasive bladder tumors.

Our survey included 46 patients presenting with untreated superficial (28T1, 7G1; 14G2; 7G3) and invasive (7T2;7T3;4T4, 1G1; 4G2; 13G3) bladder cancer. Feulgen stained imprints were processed for morphonuclear analysis using a SAMBA 200 computerized image analysis system (TTITN, France), providing the measurement of optical density (Integrated Optical Density, Surface, Mean Optical Density), textural (Long Run Lenght, Short Run Length Distribution, Run Length Percentage, Grey Level Distribution) and contrast (Contrast, Energy) parameters. Statistical analysis were performed with a particular interest for the occurrence of relapse and progression in both groups: superficial and invasive bladder cancer in function of the value of morphonuclear parameters.

In the group of superficial disease, our results indicated that the risk of recurrence and progression correlated with the value of optical density, and textural and contrast parameters. In addition, this relationship is time dependent as demonstrated by progression and recurrence free curves using the Kaplan Maeier method. Similar results were obtained in the invasive tumor group when evaluating the progression risk in regard to the value of the same parameters.

In conclusion, morphonuclear analysis provides valuable parameters for the prognosis assessment of bladder cancer and appeared to be predictive of recurrence and progression. We believe that image analysis is perfectly appropriate for the measurement of such parameters in the case of bladder cancer cells. However, we still have to demonstrate the superiority of this method on the prognosis parameters currently used in bladder cancer. Further studies are in progress.

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GRADING OF TRANSITIONAL CELL BLADDER CARCINOMA BY TEXTURE ANALYSIS.

P-U. Malmström, H. Choi, E. Bengtsson, T. Jarkrans, J. Vasko, K. Wester & C. Busch
Dept. of Urology and Pathology, Centre for Image Analysis, Uppsala University, Sweden.

In an attemt to create an image analysis based malignancy grading of bladder carcinoma we carried out a texture analysis of histological sections. The textural feature used was based on the spatial gray-tone co-cccurrence probability matrices for step-lengths 3,4 and 6 pixels.The digitized images were obtained from 5microm thick Feulgen stained parraffin wax sections. A standard monochrome video camera was used on a microscopewith an overall magnification of 400X. All the image processing was carried out on an EPSILON workstaion from IMTEC using the EGO software system.
A total of 197 primary tumors were analyzed. The obtained features were compared through multivariate statistical methods to the subjective grading carried out by the pathologist. Using a two-stage hierarchical classifier we obtained 82.7% agreement between the subjective and computerized classification. This was verified through the jack-knifed classification technique. In a comparison directly with patient prognosis the computerized grading seems to give better division between the grades than does the subjective grading.

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LABELING INDEX IN SUPERFICIAL BLADDER CANCER

F. Melone, M. Balzi, E.Zanieri, S. Bianchi, P. Mauri, G.B. Muraro, O. Gazzarrini, A. Becciolini.
Dept. Urology INRCA, Dept. Clinical Physiopathology, University, Florence Italy

Likelihood of recurrences in superficial bladder cancer ranges between 30 and 90% and the possibility of tumor progression between 5 and 50%. Because of this unpredictable behavior, there is an urgent need for stratifying patients into groups according to prognosis. In the analysis of predictors which could accurately reflect the malignant behavior of the tumor, the grade of anaplasia and the stage are considered. However, their prognostic value is not clearly established. The study deals with the in vitro determination of 3H-Thymidine Labeling Index (TLI) in 74 bladder transitional carcinomas pTa (46) and pT1 (28). Patients (87% males, aged between 35 and 83 years) underwent TUR of the tumor and intravescical treatment with BCG. The TLI presents a different behavior in pTa and pT1 tumors. The mean value in pTa group is significantly lower than in pT1 (2.54% vs 7.77%, $p(t)<0.001$). Tumors in pTa stage do not show differences of TLI regarding patient age and grade of differentiation G1 and G2. A preliminary analysis of pTa group shows that TLI, unlike the grade of differentiation, identifies 2 classes of patients with different rate of recurrences (36% vs 9.5%). In pT1 tumors TLI does not show differences with patient age, whereas it correlates with the grade of differentiation. Proliferative activity is not able to distinguish tumors with significantly different rate of recurrences and progression. The close relationship with the grade of anaplasia lead to the hypothesis of a role of both parameters on prognosis. Nevertheless a preliminary analysis of pTaG2 tumors shows a different incidence of recurrences and progression depending on the levels of TLI. An increased number of patients and a longer follow up will allow the evaluation of TLI prognostic significance in a multivariate analysis of survival. The identification of a more sensitive indicator of the malignant potential of disease could be usefull in planning therapy of bladder cancer.

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