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Abstracts

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FLOW CYSTOMETRIC QUANTITATION OF THE "c-myc" ONCOPROTEIN IN PROSTATIC CARCINOMA.

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There is increasing evidence that the c-myc oncogene, plays an important part in DNA synthesis and the transformation of the quiescent cell into a stimulated form ready for cell division. The c-myc gene encodes a 62 kDa nuclear associated protein p62 to which a monoclonal antibody has been raised. We have carried out a pilot study on freshly fixed and archival prostate tissue to assess the significance of c-myc expression in prostatic cancer.

The c-myc encoded protein product p62 was assayed simultaneously with total DNA, using flow cytometry in nuclei extracted for archival TURP specimens and freshly fixed core biopsy material. The fluorimetric assays were carried out with a synthetic peptide induced mouse monoclonal antibody (MYC 1-CE10) for the protein and propidium iodide for the DNA. The technique of nuclei extraction was greatly enhanced by the use of a high velocity mechanical tissue disintergrator (cytolyser), which reduced the time of incubation and concentration of pepsin necessary for cell digestion.

Twenty specimens were analysed, 15 were archival (1986) and 5 were freshly fixed core biopsies, ranging from benign to Gleason 5+4. The levels of fluorescence for the c-myc protein were similar in both types of specimens. The most noticeable feature was that in both groups, the levels were highest in benign tissue, falling to the lowest levels in well differentiated tumours. This concurs with similar patterns seen in cervical and bladder neoplasia but is contrary to the patter of c-myc expression in ovarian carcinomas. Aneuploidy as defined by a distinct second peak separate from the diploid distribution, was not a significant feature of the DNA analysis, when compared to histological grade.

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PROSTATE-SPECIFIC ANTIGEN IN THE FOLLOW-UP OF THE PROSTATIC ADENOCARCINOMA TREATED WITH EXTERNAL BEAM RADIATION

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The optimal treatment of local prostatic cancer is a controversial issue. Radiation therapy, early endocrine manipulation, observation, reserving androgen deprivation for disease progression, radical prostatectomy or combinations of these techniques have been used. The local failure rates after radical prostatectomy have been reported to be 0-27 per cent in prostatic cancer without involvement of nearby structures and up to 43 per cent if the involvement has taken place. After external beam radiation and without additional hormonal therapy, local failure occurs in 5 to 40 per cent of the cases with involvement of nearby structures. The incidence of local failures after irradiation increases in less differentiated and bigger tumors. In monitoring responses and recurrences of prostatic cancer, prostatic acid phosphatase (PAP) has not clearly been correlated to tumor volume. Prostate-specific antigen (PSA) has been shown to be more sensitive than PAP in detecting prostatic cancer.

Serum levels of prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) were analyzed in 24 patients, treated with external beam radiation, in order to compare the usefulness of these markers during and after radiotherapy. PAP was only slightly positive in one patient (4%) after radiotherapy. His PSA level was highly elevated and he died of progressive disease. In the other 23 patients the cancer was in local control. However, serum PSA level was positive in five of these patients indicating that there may have been vital cancer cells still present. An alternative possibility is that there were metaplastic prostatic cells left after radiotherapy which secret PSA, as it has shown to be the case in prostatic hyperplasia. Before radiotherapy were increased PSA levels measured in three patients. In two of them the level declined to normal within six months after radiotherapy. The PAP levels were normal. It is concluded that PSA (positive in 25% of patients after radiotherapy) might be more sensitive than PAP (positive in 4%) in monitoring the effect of radiotherapy in prostatic cancer patients.

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PROGNOSTIC SIGNIFICANCE OF NUCLEOLAR ORGANIZER REGIONS DISTRIBUTION IN PROSTATIC CARCINOMA

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Since its first systematic description (CROCKER 1987) the evaluation of nucleolar organizer regions (NOR) in human tumor cells has proofed to be a useful tool for the estimation of the proliferative potential of neoplasms. Based on the argyrophilic staining of nucleolar proteins, which are linked specifically to sections of active transscrib ed rDNA,the number and area of NORS per cell seem to reflect both differentiation and malignant potential of cells. Fundamental questions concerning the method in itself (e.g.duration of staining) and the evaluation (e.g. counting and measurement) are not standardized yet (RUESCHOFF, J.Pathol. in Press). For the assessment of this new technique 57 punch biopsies of prostatic carcinoma (G1:11,G2:25,G3:21) as well as 10 biopsies of normal prostatic tissue were examined. Sections of 3µm thickness were stained with a slightly modified technique of PLOTON(1986). Determination of NOR number and area was performed in 100-200 tumor cells by using an image analysis system (Olympus CUE 2). Benign prostatic tissue exhibited fewer and larger NORS ($\bar{x}N=1.8$ +/-0.2, $\bar{x}F=0.391$ +/-0.139) than carcinomatous tissue ($\bar{x}N=3.5$ +/- 1.5, $\bar{x}F=0.076$ +/- 0.137). Malignant neoplasms showed evident variability in number and area of NORS per cell, ranging from 2 - 10 with a possible correlation to malignancy grade. These preliminary results indicate that NOR analysis is a powerful tool for determination of dignity and grade applied to prostatic carcinoma. The importance of both duration of staining and image analysis procedures for judgement of NOR Determination is stressed. The prognostic value as well as the therapeutic value is further evaluated in this study.

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DNA Flow Cytometry in Fine- Needle Aspiration Biopsies of the Prostate Carcinoma for Diagnosis, Prognosis, and the Study of Tumor Biology.

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Fine- needle aspiration biopsies from more than 500 newly detected prostate cercinomas were studied using flow cytometry. The tumors were subdivided according to their nuclear DNA content into diploid,tetraploid, non tetraploid aneuploid and tumors with several aneuploid cell populations.The results were related to tumor grade, local tumor stage and existence of metastases. The clinical and prognostic significance of this subdivision was further evaluated by follow-up of patients given various treatments and also patients given no treatment. We concluded from our quantitative cellular DNA measurements that prostate carcinomas initially are near diploid but undergo continous transformation into more malignant DNA poidy patterns concomittant with progression. In consequence with this concepta postate carcinoma may exhibit during this evolution coexisting tumor cell populations of various degree of ploidy. This is proven by morphometric and DNA measurements of identified cells on slides. The significance of tumor evolution and heterogeneity for therapy will be discussed.

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THE USE OF MONOCLONAL ANTIBODY Ki-67 FOR MONITORING THE EFFECTS OF ENDOCRINE THERAPY IN HUMAN PROSTATIC CARCINOMA.

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The feasibility of the monoclonal antibody Ki-67, that reacts with a proliferation-associated nuclear antigen in human cells, as a proliferation marker in human prostatic carcinoma (PC) was studied. Cytological and histological specimens obtained from 50 patients were immunohistochemically stained with Ki-67. Among the 31 patients that were proved to have a PC, the Ki-67 index varied from 0.3-13.3% (mean: 4.1) in cytological smears and from 0.8-14.6% (mean: 4.9) in cryostate sections from histological core biopsies. In 23 patients who underwent both a cytological and histological biopsy, comparison showed an individual histological Ki-67 index being 0.4-8.0 (mean: 1.9) times higher than the cytologically determined Ki-67 index. No correlation between the Ki-67 index and the histological tumor differentiation could be established. In 19 patients with benign prostatic hyperplasia (BPH), the Ki-67 index varied from 0-3.0% (mean: 1.2) and from 0-3.8% (mean: 1.2) in cytological and histological material, respectively. The differences in the observed Ki-67 index between benign and malignant prostatic tissues are of statistical ($p < 0.001$) and of clinical significance.
Nine patients receiving endocrine treatment or radiotherapy entered the follow-up protocol in which the Ki-67 staining procedure was applied to periodically taken cytological prostate biopsies; five of these patients are still on active follow-up. During the first month after start of therapy, in a group of 9 PC patients a statistically significant ($p < 0.05$) decrease of the Ki-67 index to 58% of the control values was found; at 2 and 3 months the proliferative fraction showed a further decline to 27% and 7%, respectively. The present results indicate the suitability of the Ki-67 antibody for estimating the proliferative cell fraction of the human prostate and its potential use as a marker for monitoring the effects of endocrine therapy in patients with prostatic carcinoma.
This study was supported by Schering AG, FRG and by the Dutch Cancer Society.

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Nuclear-DNA-Analysis: Relevance of ploidy, DNA-heterogeneity and phases of the cell cycle: an 8-year-study of 329 patients with prostatic carcinoma

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In 329 patients with locally advanced prostatic carcinoma, the ploidy, DNA, heterogeneity and phases of the cell cycle occuring in the tumours were determined by means of single-cell DNA cytophotometry, in order to establish further prognostic factors in addition to the ones known so far (stage and grade). The patients were followed up for a period of 1 to 8 years.

253 (76,8 %) of the 329 patients had a T3 N0 M0 tumour while 76 patients (23,1 %) were in stage T3/T4 N + M1. Cytological malignancy grade I was found in 11,8 % of the patients, 64,3 % of them had malignancy grade II, and 23,8 % of the patients had a grade III carcinoma. Analysis by single-cell DNA cytophotometry showed a rate of aneuploidy of up to 71 % and a rate of diploidy of up to 23,8 % for the higher grades of malignancy, i.e. grades II and III. The rate of diploidy for malignancy grade I, on the other hand, was 68 %, and the rate of aneuploidy was 21 %. The differences are significant ($p < 0,001$).

A significant correlation was found between the results of DNA cytophotometry and the clinical course of the disease. Patients with diploid tumour-cell nuclei developed no metastases and no local tumour progression for up to eight years, whereas patients with aneuploid tumour-cell nuclei showed metastatic proliferation and local tumour progression within periods between 8 and 22 months. The patients died of the tumour disease 18 months after primary diagnosis on the average.

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FLOW CYTOMETRY AND BIOCHEMICAL MARKERS IN FINE NEEDLE ASPIRATION BIOPSIES AS OBJECTIVE METHODS FOR DETERMINATION OF MALIGNANCY POTENTIAL IN PROSTATIC CARCINOMA. Reinhard Stege, Barbro Lundh, Kjell Carlström, Bernhard Tribukait and Michael Hasenson. Karolinska Institutet, Stockholm, Sweden.

We have previously shown (The Prostate 14, 1989, in press) a highly significant correlation between cytological grading and the amount of prostatic acid phosphatase (PAP) or prostate specific antigen (PSA) assayed directly in prostatic tumour cytosols from fine needle aspiration biopsies. To further evaluate the clinical importance of tumour cytosol PAP/PSA assays, we have analysed PAP and PSA i cytosols in an enlarged clinical material of 133 biopsies and compared the results with flow cytometry data as another objective method for determining malignancy potential. PAP and PSA values are expressed relative to DNA content and are given as geometric means:

G	Diploid	Tetraploid	Aneuploid
0	32	1	0
1	10	3	3
2	29	5	14
3	12	2	22

	Diploid		Tetraploid		Aneuploid	
G	PAP	PSA	PAP	PSA	PAP	PSA
0	7.40	5.38	1.87	4.16	-	-
1	1.58	2.28	0.22	0.60	3.40	5.76
2	0.14	0.26	0.22	0.21	0.06	0.11
3	0.13	0.17	0.01	0.01	0.05	0.05

PAP and PSA values were clearly associated with flow cytometry data as well as with cytological grading. There was also a clear correlation between PAP and PSA values and T staging. PAP and PSA in tumour cytosol and flow cytometry may be valuable objective complements to cytological grading in the assessment of malignancy potential in prostatic carcinoma.

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KERATIN ANTIBODIES AS DIFFERENTIATION MARKERS IN PROSTATIC EPITHELIUM

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Androgens might play a role in the differentiation pathway of prostatic epithelium. This could be of great importance for processes involved in neoplastic progression of prostatic epithelial cells.

In the discrimination of differentiation stages antibodies against keratins have proven to be very instrumental. We, therefore, used these antibodies to study both primary, as well as recurrent prostatic human tumors. The well differentiated prostatic tumors were positive for both basal and luminal cell type keratins. Moderately differentiated as well as anaplastic prostatic tumors were only positive for luminal cell type keratins. An interesting group of tumors was that of tumors that recurred after androgen ablation therapy. Again the anaplastic tumors were solely positive for luminal cell type keratins. However, a number of moderately differentiated recurrent tumors were found that were only positive for basal cell type keratins, indicating that this subpopulation selectively overcame the androgen withdrawal.

Also human prostate cancer Xenographts revealed tumors (PC-3 and PC-135) that were positive for basal cell type keratins besides tumors that were positive for luminal cell type keratins. Similar results were obtained in Dunning R-3327 derived rat prostatic cancer sublines. The well differentiated H-tumor, that resembles in its keratin expression pattern the normal prostate, gave rise to a poorly differentiated, androgen independently growing tumor, HI-F, in which most cells were exclusively positive for basal cell type keratins. However the anaplastic highly metastatic MATLyLu tumor that arose indirectly from the H tumor is exclusively positive for luminal cell type keratin.

These studies revealed different pathways in the tumor progression of prostatic cancer using antibodies directed against keratins.

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THE PREDICTIVE VALUE OF FLOW CYTOMETRIC DNA MEASUREMENT IN THE MANAGEMENT OF TESTICULAR SEMINOMA

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Paraffin-embedded archival specimens from 97 patients with testicular seminomas treated at the Mayo Clinic between 1960 and 1980 were analyzed by flow cytometry. Ninety-three percent were of the classic histologic subtype, 4% were spermatocytic, and 3% showed anaplastic features. The patients were followed for a median of 11.4 years. All patients underwent radical orchiectomy followed by radiation therapy. Forty-eight percent of tumors showed DNA diploid patterns and 52%, showed DNA aneuploid patterns. All ana-plastic seminomas exhibited DNA diploid patterns. The relationship of DNA ploidy to postoperative tumor progression was studied for patients with no distant metastases at diagnosis or followed for at least 2.5 years. None of the patients with diploid tumors evidenced progression. In contrast, the testicular seminomas of all patients that subsequently showed progressive disease were DNA aneuploids ($p<0.04$). Furthermore, none of the patients with diploid tumors died of testicular seminoma. However, only 88% of those with aneuploid tumors survived tumor death at 5 and 10 years after treatment ($p<0.02$). It appears from this data, that ploidy pattern may have an important role in the study and management of patients with testicular seminoma.

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A NEW DEVICE FOR NUCLEI EXTRACTION FOR FLOW CYSTOMETRY.

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Methods of preparing samples for flow cystometry, whether from archival or fresh material, are well described. However there are problems when dealing with very fibrous tissues (e.g. prostate) in extracting the cells and consequently their nuclei. This usually requires prolonged incubation with pepsin (0.5 mg% of 1.5 hours) in acid saline, which in turn can lead to digestion of cellular and nucleic proteins. We have adapted a new instrument, the cytolyser, a high velocity mechanical tissue disintergrator which forms a tissue suspension of particles 10 microns in size. It consists of a 5 mm rotary blade spinning at 40,000 r.p.m. mounted on a hand held motor. Using the cytolyser in a quantitative flow cytometric study of c-myc in prostatic carcinoma, we were able to reduce the incubation period to 30 minutes, and reduce the concentration of pepsin to 0.1 mg%. With this technique, we were able to increase our cell sample by 300% and record uninformly higher antibody levels (up to a 5 fold increase) in all our samples. This was irrespective of whether the tissue was freshly fixed whole specimens, or 40 micron sections from archival wax blocks. We suggest that using this device greatly facillitates the extraction of nuclei for flow cystometry.

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NUCLEAR MORPHOMETRY ANALYSIS OF BLADDER CANCER CELLS AND HISTOPATHOLOGICAL GRADING.

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Nuclear image analysis of 16 transitional cell carcinoma specimens and 5 normal bladders was performed on Samba 200 System - Tissues were obtained at transurethral resection, radical cystectomy or cadaveric organ procurement. All samples were frozen in liquid nitrogen and kept at - 80°C until use. Feulgen staining was performed on imprint smears from unfrozen samples. The histological classification according to the WHO classification was obtained on paraffins sections of the same specimen ; G1 3 cases ; G2 6 cases ; G3 7 cases. Fifteen morphonuclear parameters were analysed for each nucleus with Samba 200 software to study 3 classes of parameters : nuclear site, densitometry, chromatin texture. Non parametric statistical analysis indicates a significative difference between normal bladder, grade II, grade III TCC and nuclear surface, integrated optical density and chromatin texture. Thse results suggest that Samba 200 analysis may be of value to dismember the spectrum of urothelial tumors.

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Prognostic relevance of DNA ploidy and proliferative activity in urothelial carcinoma of the renal pelvis and ureter.

A study on a follow-up period of 6 years

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In 55 patients with urothelial carcinoma of the renal pelvis or ureter, the ploidy, the DNA heterogeneity and the counts of cell-cycle phases in the tumour were examined by means of single-cell DNA cytophotometry in order to find more prognostic factors than those already known (stage and grade). Follow-up periods ranged from 1 to 6 years. At the time of first diagnosis, 42 (76,3 %) of the patients had tumours of the renal pelvis, 13 (26,6 %) of them had ureteral tumours. 23 (41,3 %) patients were in stage pT1 N0, 15 (27,2 %) in stage pT2 N0, 12 (21,8 %) in stage pT3 N0, and 5 (9,1 %) were in stage pT3 N+.

The histological malignancy grade most frequently seen in the patients examined - i.e. in 50 % of cases - was malignancy grade II. 25,4 % of the patients had grade III tumours whereas only 23,6 % had grade I tumours. With malignancy grade I, DNA cytophotometry showed DNA frequency peaks to be in the diploid range while tumours with malignancy grade II showed heterogenous DNA patterns. 72,7 % of the patients with malignancy grade III showed aneuploid DNA values; 25,7 % of them had polyploid DNA values. Diploid frequency patterns were found only in 2,6 % of these cases. For malignancy grades II and III, the proliferation rate of the tumour cells was statistically significantly higher than for malignancy grade I.

The determination of tumour heterogeneity and tumour-cell proliferation by means of DNA cytophotometry affords valuable clues as to prognosis.

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THE RELEVANCE OF FLOWCYTOMETRIC DNA MEASUREMENTS WITH REGARD TO TUMOR PROGRESSION IN PATIENTS WITH BLADDER CARCINOMAS.

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Patients with transitional cell carcinomas are often confronted with recurrent or progressive disease. Several prognostic factors have been suggested in the literature. Among these tumor stage (T category) and tumor grade (G category) are most often implicated. Others have also shown that the DNA-content of the tumor cells is related to both tumor stage and grade. Much less evidence has been presented on the independent predictive value of DNA mesurements only and its relevance to tumor progression.

A large number of patients (i.e. more than 100) with papillary transitional carcinomas have entered the EORTC 30791 and 30832 trials and were frequently reexamined by cystoscopy. From the paraffin blocks of these tumors the nuclei were extracted as described by Hedley. Nuclei were also isolated from bladder biopsies from other patients. These patients also underwent frequent cystoscopies. Succesfull DNA measurements were performed on nuclei extracted from 449 paraffin blocks. These blocks were derived from 227 patients. The DNA-content of the isolated nuclei was measured with ethidium bromide as chromofore on a fluorescence activated cell sorter (FACS II).

The patient's records were examined for the occurrence of progression, recurrence and/or disease free survival. Tumor progression was defined as an increase in T category or death due to distant metastases.

The results show that a DNA-index of 1.5-1.9, i.e. between triploid and tetraploid, is associated with a four times higher chance to cause progression of a bladder cancer. The tetraploid tumors behaved intermediately. Bladder cancers selected for tumor grade, especially grade II, present the same pattern. This indicates that DNA-index alone is also a independent variable for tumor progression.

The results also suggest that polyploidization and subsequent chromosome loss may be important events during tumor development or progression. As such DNA-measurements of bladder tumors may be helpfull in choosing a therapy regimen and in predicting the prognosis.

Key words: Transitional cell carcinomas, DNA-index, progression.

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PRELIMINARY REPORT ON ARGYROPHILIC STAINING OF NUCLEOLAR ORGANIZER REGION-ASSOCIATED PROTEINS (Ag-NORs) IN CELLS OF BLADDER WASHOUTS

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Intra nucleolar non-histon-proteins related to the loops of rDNA transcribing for ribosomal RNA are specifically stained by the Ag-Nor-technique (GOODPASTURE a. BLOOM, 1975). This number and/or area of Ag-NORs visualize activity and proliferation of a cell (CROCKER et. al, 1988). In other urological tumors (e.g. testis, prostate) our group could confirm a relation between tumor grading and Ag-Nor area and number. In a pilot study Ag-NORs in 60 bladder washouts were evaluated by image analysis (Olympus Cue 2) and compared to the histological findings.

As shown in the table there is a strang correlation between total Ag-NOR area per cell and malignancy. Further experience will reveal if Ag-NOR-technique enhance diagnostic accuracy of urinary cytology.

Histology	Normal	GO	G1	G2	G3
$\bar{x}$ Tot.Area (μm^2)	0.63	1.01	1.52	2.2	4.01
s	0.1	0.6	0.7	0.4	0.7

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FLOWCYTOMETRY AND URINARY CYTOLOGY IN TRANSITIONAL CELL CARCINOMA (TCC) OF THE BLADDER.

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The prognostic value auf flowcytometric DNA analysis was studied in 56 newly diagnosed cases of TCC. Flowcytometry was done several times during a follow-up of more then two years (mean 4 0 months). T-stage of euploid an aneuploid G-2 TCC is shown in Table 1

	euploid	aneuploid
T_a	12	13
T_1	11	13
T_2	3	4
n	26	30

Progression of disease was not seen in diploid cases. 4 out of 12 tetraploid cases showed progression of disease after a mean interval of 27 months, 9 out of 18 non-tetraploid cases showed progression after a mean interval of 11 months.

The recurrence rate/year in euploid cases was 0.16 and in aneuploid cases 0.7 demonstrating a significant difference (p 0.01).

Urinary cytology and flowcytometry each were able to detect histologically proven TCC in 64 % of 273 cases. Combination of both methods increased the diagnostic yield to 76 %. The increase of diagnostic yield was especially seen for well differentiated TCC. Flowcytometric DNA analysis is done routinely since 1976 in this institution. Correlation of prognosis with ploidy level in G-1 and G-3 TCC is well known. Non-tetraploid G-2 tumors demonstrated to bear a high risk to progress during the first year after primary diagnosis.

The combination of flowcytometry with urinary cytology is able to reduce the number of endoscopic investigations in patients.

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FLOW CYTOMETRIC ANALYSIS OF DNA CONTENT AS A PROGNOSTIC INDICATOR IN SQUAMOUS CELL CARCINOMA OF THE URINARY BLADDER

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Flow cytometric DNA analysis was performed on 73 primary bladder squamous cell carcinomas treated at the Mayo Clinic between January 1970 and December 1975. Nuclei were extracted from paraffin-embedded archival material and isolated nuclei were stained with propidium iodide. Twenty-seven (37%) showed a DNA diploid or normal pattern; 17 (23%) exhibited a significant increase in the 4C peak (DNA Tetraploid) and 29 (40%) showed a distinct aneuploid peak. High grade (grades 3 and 4) and high stage (stages T_2-T_4) tumors had a significant higher incidence of abnormal (either tetraploid or aneuploid) DNA patterns than low grade (grades 1 and 2) and low stage (stages T_a/T_1/Tis) tumors ($p<0.005$). At 5 and 10 years after diagnosis, 67% of the patients with diploid tumor were free of disease, compared to 51% of those with tetraploid tumors and 12% and 8% respectively, of the patients with aneuploid tumors ($p<0.0005$). Furthermore, at 5 and 10 years after diagnosis, an estimated 18% of the patients with DNA diploid tumors will die of bladder cancer. In contrast, 53% of the patients with DNA tetraploid tumors and 83% and 86% of those with DNA aneuploid patterns will die of bladder squamous carcinoma by 5 and 10 years after treatment. ($p<0.0001$). Determination of nuclear DNA ploidy pattern by flow cytometry, provides important prognostic information for patients with squamous cell carcinoma of the bladder.

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INTERMEDIATE FILAMENT PROTEIN ANTIBODIES AND DNA ANALYSIS IN THE FLOW CYTOMETRY OF RENAL CELL CARCINOMAS.

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The clinical importance of the presence of an abnormal DNA stemline as a prognostic factor in renal cell carcinomas has been described before. Intermediate filament protein characterisation by immunohistochemical techniques have shown the characterisation of different subpopulatiuons in renal cell tumors. Intermediate filament protein antibodies and flowcytometric analysis, in combination with each other, has been used to detect different subpopulations in renal cell tumors.
The presence of an abnormal stemline could be detected in 19/44 patients in a single parameter analysis. The combination of intermediate filament protein antibodies and propidium iodide as a DNA staining showed the presence of an abnormal DNA stemline in 13/15 tumors instead of 9/15 tumors by single parameter flowcytometric analysis. Therefor we can conclude that the use of a two parameter flowcytometric analysis permits the detection of an abnormal DNA stemline and subpopulations in renal cell tumors. This might be usefull in the characterisation of the biological potentials of renal cell tumors and in the selection of patients for early additional therapy for surgery.

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LECTIN BINDING BY PROSTATE CANCER CELL LINES

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As part of our studies on the characteristics of cell lines derived from primary and metastatic prostate tumours the binding by the cells of lectins with different carbohydrate specificity is examined. After incubation of intact or neuraminidase-treated cells with fluorescein labelled lectins the binding is quantitated by analysis of fluorescence recovered by means of specific carbohydrates. So far the cell lines 1013L (from a primary tumour) and DU 145 (from a CNA metastatic lesion) have been examined for their binding of Concanavalin A (Con A), Lentil lectin (LCH), Wheat germ agglutinin (WGA), Crotalaria juncea agglutinin (CJA), Jacalin lectin (JFA), and Peanut agglutinin (PNA). The 1013L cells growing in suspension bind less galactose specific lectin (CJA) than the DU 145 cells which grow in monolayer. Both cell lines bind WGA with high affinity but while treatment with neuraminidase does not decrease the amount of WGA bound by 1013L, it considerably reduces the number of binding sites for this lectin in DU 145 cells. The differences in lectin binding between the two cell lines may reflect their different origin and perhaps also a difference in metastatic potential.

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PLASMINOGEN ACTIVATORS (PA) AND INHIBITORS ACTIVITY IN HUMAN BREAST AND PROSTATE CANCER CELL LINES : RELATIONSHIP TO HORMONAL DEPENDENCE.

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We showed recently (Pourreau-Schneider et al., J.N.C.I., 92 : 259 1989) that laminin increased cell associated urokinase type PA (UPA) in hormone insensitive breast cancer cell line MDA-MB 231. In estrogen sensitive breast cancer lines MCF7, 17β estradiol (E2) stimulated UPA secretion in a similar fashion on plastic, laminin, fibronectin or collagen. In addition, E2 acted in synergy with laminin in the production and release of tissue type PA. Growth of androgen-responsive prostate tumor cells might be controlled by androgens in a way comparable to the regulation of growth of mammary carcinoma cells by estrogens. The LNCaP cell line show continuous growth in vitro, produce prostate-specific acid phosphatase and contain androgen receptors : these cells respond to androgen with an increased growth rate and secretion of androgen-dependent proteins (Schummans et Al. : The prostate 12 : 55 - 1988).

Secretions of PA and their inhibitors using both solid phase immunoassays and zymographic analysis were measured in two prostatic cancer cell lines : hormonal sensitive LNCaP and hormonal insensitive DU 145. Data of their modulation either by androgens, antiandrogens and LHRH analogs may bring new possibilities in the manipulation of the malignant phenotype of prostatic tissues.

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ANALYSIS OF TRANSGLUTAMINASE ACTIVITIES IN PROSTATE CANCER CELLS: RELATIONSHIP WITH METASTATIC POTENTIAL.

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Transglutaminases (TGases) are enzymes catalyzing post-translational modification of a limited number of proteins through an acyl transfer reaction between peptide-bound glutamine residues and primary amine groups. The physiological role of cellular (or tissue type) TGase remains unclear, but there is increasing evidence for its involvement in growth regulation. Increasing tissue TGase activities are generally associated with decreasing rates of cell proliferation, e.g. during differentiation, ageing and contact inhibition. We have analyzed TGase activities in various human and rat prostate cancer cell lines and studied the relationship with metastatic potential. TGase activities were measured in cell homogenates by the incorporation of radiolabelled putrescine into N,N-dimethylated casein.
TGase activities were widely different in the various cell lines examined. Highest activity was observed in the PC-3 cell line (6.88 nmol/hr.million cells). A metastatic variant of the same cell line, PC-3LM, had a 40-fold lower activity (0.15nmol/hr.million cells). An inverse relationship between TGase activity and metastatic potential was observed also in the Dunning rat prostate cancer model.
These results suggest that TGase may serve as a marker for the metastatic ability of prostate cancer cells. The question whether loss of TGase activity is also a causative factor in the metastatic process cannot be answered as yet. One might speculate, however, that TGase may modulate metastatic behavior by modification of cell surface-associated proteins such as fibronectins and vitronectin, both known substrates for TGases. Reduced TGase may then result in a more negative cell surface and thus affect metastatic behavior.

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CIRCULATING OSTEOCALCIN (OC) AS A SENSITIVE PROBE FOR BONE REMODELLING PROCESSES IN PATIENTS WITH PROSTATIC CARCINOMA

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We have recently reported the application of serum OC values (normal range = 3.5-11.5 ng/ml) in patients with metastatic cancer of the prostate as a sensitive probe for both bone lesions improvement (elevated values) and worsening or stabilization (normal and/or subnormal concentrations) regardless of the therapy applied ($p < 0.01$). Blood OC level predicts the outcome of the therapy as early as 30 days and may help also in distinguishing a flare phenomenon from the early osseous progression. Further investigations revealed differences between subnormal mean OC level (<3.5 ng/ml) measured during disease stabilization and progression in patients treated with DES and Estracyt and normal OC values assessed in patients treated by orchiectomy (or LHRH analogs) plus either CPA or flutamide (5-8 ng/ml). Recorded subnormal OC values have not been reflective of a degree of bone matrix destruction. The cross-over from DES to CPA or a replacement of DES by placebo yields a substantial raise in OC level without changes in tumor metastatic activity. An increase in circulating cortisol concentrations (normal range = 80-180 ng/ml) was measured during administration of DES, Estracyt, and ethinyl estradiol (> 530 ng/ml) but neither in patients treated with antiandrogens, LHRH analogs, polyestradiol phosphate depot, and after orchiectomy (110-180 ng/ml), nor in a control group with BPH. Elevated serum cortisol values rarely parallel ACTH level and thus may be originated by either estrogen-induced changes in cortisol catabolic pathways or/and elevated blood CBG concentrations. Since glucocorticoids are confirmed as promotors of bone demineralization probably via a receptor-related mechanism, treatment with DES and Estracyt might provide an additional risk for osteopenia.

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THE METASTATIC POTENTIAL OF THREE HUMAN UROTHELIAL CELL LINES

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Three cell lines were obtained by the in vitro culture of three different human urothelial tumours; they were characterized either in vitro or in vivo, in order to study the metastatic phenotype of the tumour cells.
The three cell lines were the line TL40, obtained from a grade I not invasive papillary bladder cancer of a 55 years old male patient; the line SG65, derived from a grade IV invasive transitional bladder cancer of a 56 years old male patient; the line CG90, isolated from a grade III invasive papillary bladder cancer of a 72 years old male patient. The three cell lines were characterized in vitro according to the cell morphology, the growth kinetics, and the agar colony forming ability; the presence of surface tumour antigens was also investigated. The tumourigenicity and the metastatic ability of the three cell lines were studied in vivo by inoculating the cells into nude mice of both sexes at various ages and per various routes.
The biological behaviour of the CG90 cells was quite different from that of the TL40 and SG65 cell lines, which exhibited similar properties; the results so far obtained indicate that the metastatic potential of these tumour cells is not directly correlated to their growth abilities in vitro, i.e. to efficient growth kinetics and agar colony formation; it depends mainly on the antigenic profile of the cells and is influenced by the immunological status of the host: highly antigenic cells can produce metastases only in young immunologically immature nude mice.

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STUDIES OF TRACE ELEMENTS IN METASTASES AND PRIMARY TUMORS OF TESTIS

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Previous studies showed distinct differences in the concentration of several trace elements (Cd,Zn,Mn, Cr,Se,Pb) in cellular fractions, separated epithelium and stroma as well as in the unseparated tissues of primary prostatic carcinoma and metastases of the same patient.

In other investigations we found an increased con - tent of zinc in both the nuclear and mitochondrial fractions of tumour tissues compared with normal tissues of testicles.

In view of a possible interaction between the primary tumor and the metastases we decided to investigate the concentration of several trace elements in tissues, cellular fractions and separated epithelium and stroma of testicular tumor patients.

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METASTATIC POTENTIAL IN NSGCT STAGE I

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Following the protocol of clinical surveillance in non-seminomatous germ-cell tumors of the testis (NSGCT stage I a progression rate of 28% at Hannover Medical School was obtained after evaluation of 82 patients with a close follow-up between at least 2 years and 6 1/2 years.
Identification of risk factors with respect to progression on the level of the primary was the rationall of the study presented herein using a multi-parameter analysis including nuclear deoxyribonucleid acid (DNA) ploidy pattern, number of nuclei with hyperpentaploid (>5c) DNA content, morphometry and immunochemistry. Nuclear suspensions obtained from paraffin-embedded archival specimens (Hedley technique) were stained by the Feulgen-DNA method and examined employing the modular image analysis computer (MIAC-Autoplan, LEITZ, Wetzlar, FRG).
In 27 tumors studied so far (6 with progression, 21 without progression) the incidence of the >5c DNA cell population proved prognostic valuable indicator. 5/6 of the tumors with progression exhibited a >5c cell rate whereas only 4/21 of the tumors without progression did so.
Confirmation of these results at the entire material means the addition of a "tumor marker" which will permit more differentiated and adequate treatment strategies in NSGCT stage I.

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ANALYSIS OF RECESSIVE EVENTS INVOLVED IN MULTISTEP UROTHELIAL CARCINOGENESIS IN VITRO

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Several phenotypic stages can be defined during in vitro transformation of rat urothelium. We have used somatic cell hybridization to analyze the role of recessive events at different stages in this process.

A series of immortal and transformed bladder cell lines have been fused with each other, with immortal rodent fibroblasts and with a series of human transitional carcinoma cell lines and the phenotype of the resulting hybrids has been studied in vitro. Results of fusions of one immortal cell line (RM2) with fully transformed variants indicate that a suppressor function is lost in the transformed cells. Hybrids between transformed RM2 (suppressor$^-$) cells and four other immortal urothelial lines show no suppression of anchorage independence, indicating that these cells lack this suppressor function. However, some tumorigenic rat and human urothelial cells and a range of immortal mesenchymal cells do retain this function. In tumorigenic cell lines, the ability to suppress anchorage independence does not show a direct relationship with the phenotype of the parent cell since in at least one case a tumorigenic cell line which itself forms no colonies in agar lacks the suppressor function.

In a more extensive analysis using several anchorage independent urothelial cell lines and a test panel of rodent and human lines, urothelial lines have been assigned to several complementation groups based on their ability to suppress or be suppressed for anchorage independence. Our results indicate that cell lines established following the same carcinogen treatment regime do not all share the same genetic lesions. Our classification provides the basis for a rational approach to the selective cloning of a series of putative suppressor genes from urothelial cells using cDNA-mediated functional assays or retroviral insertional mutagenesis.

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Enhancement of chemically induced bladder carcinoma under immunosuppression by cyclosporin A

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The immunosurveillance theory describes that the loss of immunoexpression is one of the factors, being responsible for the development of cancer. Whether the selective immunosuppression of T cells by cyclosporine A (CsA) would enhance bladder tumors, induced by the known tumor initiator N-butyl-N (4hydroxybutyl) nitrosamin (BBN), should be investigated. 175 Wistar rats were randomized into 5 groups. I: only 0,05%BBN drinking solution over 8 weeks. II: identical to group I, in addition to 5mg/kg per day CsA for 11 weeks. III: identical to group I, in addition to 12,5mg/kg per day CsA for 11 weeks. Controls IV and V only CsA in doses of 5mg/kg/day (IV) and 12,5mg/kg/day(V) for 11 weeks. All animals were sacrificed at the end of 13th week.

The percentage of exophytic ($p<0,004$) and infiltrative ($p<0,001$) bladder tumor expansion:

	I(%)	II(%)	III(%)	IV(%)	V(%)
exo.	4,2±0,4	8,0±0,9	11,6±1,4	0	0
inf.	0,7±0,08	2,5±0,3	3,3±0,4	0	0

A CsA dose dependent enhancement of bladder tumor expansion in II and III resulted.
Findings confirm that effective expression of immune response may be important in control of tumor development. An experimental model for immunosuppression is presented.

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N-NITROSO COMPOUNDS IN URINE OF RATS AND HUMANS WITH URETEROSIGMOIDOSTOMIES

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Ureterosigmoidostomies in both a rat model and in patients result in an increased incidence of colon tumours. Rectal urine of 26 patients and 46 female Wistar rats with ureterosigmoidostomies was analysed für nitrate, nitrite and N-nitrosamines and compared to 20 control volunteers as well as 10 unoperated rats. Bacterial investigations on feces-urine mixtures from both the rat model and ureterosigmoidostomy patients showed the presence of a complex nitrate-reducing bacterial flora. This bacterial flora actively reduced urinary nitrate to nitrite in humans and increased the endogenous formation of N-nitroso compounds significantly from 57,3±33,9 nmol/l in the control group to 97,9±66,5 nmol/l in the ureterosigmoidostomy group. However, no evidence of urinary nitrate reduction and increased nitrosamine formation in the rectosigmoid of rats was found.
The results support the N-nitrosamine theory of carcinogenesis of the colon following ureterosigmoidostomy in humans, but not in rats. As the rat model induces colon carcinomas, factors other than the increased endogenous formation of N-nitroso compounds in the rectosigmoid may contribute to the initiation of colon carcinomas following ureterosigmoidostomy.

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EXPRESSION OF PROSTATE-SPECIFIC ANTIGEN AND HUMAN GLANDULAR KALLIKREIN IN PROSTATE CELL LINES.

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Prostate-specific antigen (PA) is of high importance as a specific marker for prostatic cancer both in serological and in immunohistochemical assays. This prompted us to study in detail regulation of PA expression. Three PA cDNA clones, differing in splicing and in polyadenylation and the PA gene were isolated. Fragments of these PA cDNA clones were used for analysis of PA mRNA expression. In the LNCaP prostate tumor cell line PA expression could be stimulated by androgens. Pa expression is not detectable in androgen-independently growing prostate tumor cell lines.

PA is a member of a small family of kallikrein (-like) genes. This family also includes the pancreas/kidney kallikrein (hPK) and the human glandular kallikrein gene (hGK). The three genes have a similar organization and are clustered on chromosome 19. They also show a strong structural homology (60-80%). Interestingly, it was reported that hGK is expressed in the prostate (Chapdelaine et al. (1988) FEBS Lett. 236; 205-208). Using specific fragments of a recently isolated full length hGK cDNA clone we are currently investigating regulation of hGK expression.

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In vitro and in vivo antiproliferative effects of High Energy Shock Waves (HESW, Lithostar, Siemens) on Dunning P-AT-2 prostate cancer cells.

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Recently several in vitro and in vivo studies have reported antiproliferative effects of HESW on tumor cells. In our studies we evaluated the antiproliferative effect by clonogenic potential assays (double layer soft agar). In this way sensitivity to treatment with cytotoxic agents after HESW exposure was also investigated.
We used different approaches for in vitro HESW exposure. When a single cell suspension was exposed a dose dependent antiproliferative effect and an increase in Vinblastine cytotoxicity was found. However, when cells were exposed as a cell pellet there was no additional Vinblastine cytotoxicity. No effects were seen in a single cell suspension fixed by gelatine.
In vivo studies showed marginal effects on tumor growth after single HESW exposure. Vinblastine cytotoxicity was not enhanced significantly. However, when in vivo shocked cells were used an antiproliferative effect was evident in temporal growth curve analysis. These analyses also revealed an increased sensitivity for Vinblastin treatment.
Conclusions; The in vitro effects of HESW depend on the way in which cells are exposed to HESW. Single exposure on in vivo growing P-AT-2 tumor (doubling time; 2 days) has no direct growth inhibitory effect.

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THE EFFECT OF A METHOTREXATE-ANTI MEMBRANE ANTIBODY CONJUGATE AND ANTIBODY TARGETED LIPOSOMES ON THE HUMAN PROSTATIC TUMOUR LINE - PC3

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The treatment of prostatic tumours by chemotherapy is limited by the toxicity of the agents used which may be overcome by the targeting of such drugs to the tumour site. The effectiveness of such cytotoxic drug targeting in a prostate cancer model system was evaluated using a purified IgG fraction of rabbit polyclonal antiserum. IgG was linked either directly to methotrexate (MTX), via an active ester intermediate, or coupled to small unilamellar liposomes bearing MTX using the heterobifunctional coupling reagent SPDP and the two types of targeting systems compared both in vitro and in vivo. Coupling of the IgG to SPDP resulted in a 9% loss in binding, whilst linkage of the antibody to MTX resulted in a 70% retention of antibody activity and a small loss in the dihydrofolate reductase activity displayed by MTX. Comparison of the cytotoxicity of antibody-drug conjugates and antibody linked drug-bearing liposomes was achieved by measurement of (a) chromium release from pre-loaded PC3 cells and (b) the number of cells remaining in culture after treatment with targeting agents and controls. Both antibody bound liposomes and antibody-drug conjugates were as effective as free drug (at doses of 4μg/ml) in both experimental procedures. A study in vivo, using athymic nude mice bearing PC3 tumours, receiving 5 injections of drugs (1mg MTX per kg body weight) over a 14 day period showed significant reduction in tumour growth by both targeting systems when compared to free drug. Administration of antibody-drug conjugates, however resulted in a significantly greater tumour reduction than treatment with antibody-targeted liposomes and no significant difference was observed between the tumour growth of animals treated with 'targeted' or 'non-targeted' liposomes. Tissue distribution studies using [^{3}H]MTX revealed that the antibody-drug conjugates was preferentially accumulated within the tumour. Animals treated with 'targeted' and 'non-targeted' liposomes demonstrated a significantly higher tumour levels of [^{3}H]MTX than free drug, but accumulation in lung, liver, spleen and skin was significantly higher than both antibody-drug conjugates and free drug.

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IN VITRO GROWTH CHARACTERISTICS OF PROSTATE CARCINOMA. Josee J.König, Hans C.Romijn and Fritz H.Schröder, Department of Urology, Erasmus University, Rotterdam, The Netherlands.

We have studied the in vitro growth of 47 prostatic carcinoma specimens obtained at surgery. Epithelial cells were cultured from cell suspensions made by mechanical and enzymatic disaggregation. Outgrowths from 1mm3 tissue cubes (organ cultures) were also studied.
Short term (2-7 days) in vitro growth was evaluated in different culture media, especially designed for prostatic epithelium (PFMR4A and WAJC) and more generally applied (F10, F12, F12/MEM). Successfull growth in these media ranged from 90% in F12/MEM to only 40% in PFMR4A. While success of tissue culture was thus treatly determined by the medium choice, growth rate depended mainly on the addition and combination of fetal calf serum, growth factors and hormones. A negative effect on growth rate was observed for transferrin, while bovine pituitary extract and dihydrotestosterone showed no effect. Hydrocortison, epidermal growth factor, cholera toxin and insulin however all stimulated growth. The addition of 2% FCS proved optimal in combination with F12/MEM and stimulating growth factors.
Maintenance of long term (6 months) viability of tissue cubes giving rise to repeated vigorous outgrowth (up to 13 times) of pure epithelial colonies was possible in the same medium. Frequently mitoses could be observed in the middle of these colonies, so proliferation rather than shedding of cells takes place. Other characteristics of this medium are that growth of fibroblasts is absent, even in long term organ cultures and that cells can be passaged up to three times. This relatively easy formulated tissue culture medium is at present mainly applied in our laboratory for culture of malignant prostatic epithelium for cytogenetic analysis but can no doubt find wider application.

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THE ROLE OF THE GLUTATHIONE METABOLISM AND ITS MODULATION IN CHEMORESISTANT RENAL CELL CARCINOMAS

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High intrinsic chemoresistance contributes to the poor prognosis of patients with metastazised renal cell carcinoma (RCC). The glutathione metabolism, apart and independently from the P-170 glycoprotein efflux pump, seems to play an important role in the detoxification of chemotherapeutics in RCC.
We therefore determined the glutathione contents and the activities of related key-enzymes in 28 primary human RCCs and in matched normal renal tissue, evaluated the degree of chemoresistance in a tetrazolium based microculture assay (MTT), and studied the effect of glutathione depletion by Buthionine Sulfoximine (BSO), a specific inhibitor of the glutathione biosynthesis.
We found a significant increase in the glutathione metabolism in chemoresistant RCCs compared to sensitive cases ($p< 0,05$). The highest glutathione contents was detected in matched normal renal tissue, but without reaching significance. BSO led to a distinctive enhancement of cytotoxicity in RCCs depending on the chemotherapeutic used: Only minor potentiation of vinblastine effects, strong reversal of doxorubicin-, and a dramatic circumvention of platinum resistance.
Our results support the view that the glutathione metabolism has impact on the multidrug resistance of RCCs, and that its modulation may contribute to a more successful chemotherapy of these tumors.

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The Mechanism of Kidney Infarction after Ligation of the Left Renal Veins.

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To elucidate the efficiency of porto-renal drainage, we studied in our experimental animal-model on rabbits the effects for left renal vein ligation after right nephrectomy and suprarenal inferior cava ligation. The success of that radical operative procedure depends on the place of the ligation. In contrary to the right Kidney, which exhibits, as it is called by anatomists, a visceral type of venous drainage, the more parietal venous drainage of the left kidney by numerous porto-renal anastomosis can be used to examine the velocity of adaptation-mechanism. The aim of this study is to correlate the results from topographical histological staining with the postoperative renal function of the adaptation process after ligation of left renal vein. After sodium pentobarbital anesthesia, the left renal vein was ligated in 14 male rabbits after median laparotomy, right nephrectomy and ligation of the suprarenal inferior vena cava. In six cases we placed the ligation of the renal vein distal, in the rest proximal to a major vessel, which is called in rabbits "dorsolumbar vein". The development of a venous collateral circulation was demonstrated by aortorenal arteriography and venography. The postoperative renal function was controlled by evaluation of serum retention values, excretory urography, radioisotope renogram and sequence scintigraphy. After operation the rabbits showed only within a few hours after ligation of the renal vein of the left kidney a venous congestion of the kidney, that culminated in hemorrhagic infarction in those cases, in which the dorsolumbar vein was proximal to the ligature. In the control group only 1 out of 8 animals died as a result of pneumonia. In all our cases the development of the acute hemorrhagic infarction depended directly on the distance of the ligation to inferior vena cava and was responsible for the postoperative morbidity. According to our results, we assume that the ligation of the left renal vein after right nephrectomy and suprarenal inferior vena cava ligation is also tolerated in man as indicated by some reports from literature.

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Monitoring the Metabolic Changes in a human Testicular Non Hodgkin Lymphoma (NHL) Recurrence during Irradiation.

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We used 31 P Magnetic Resonance Spectroscopy (MRS) to follow up the metabolic changes of a NHL recurrence in a 76 year old patient before, during and after radiation treatment (total dose 56 Gy/28 fractions). The histological examination revealed a diffuse centroblastic high malignant NHL. The primary treatment was a left orchiectomy. Three weeks later the patient presented a recurrence and refused any other kind of therapy except irradiation. The 31 P MRS spectra of the tumor showed before starting irradiation, intensive PME (phosphomonoester) and PDE (phosphodiester) signals that overlapped the Pi (inorganic phosphate) signal. After 25 Gy irradiation the decrease of the PME and PDE signals was observed. The Pi peak appeared, and revealed an intratumoral pH of 7.08 very close to that in the normal opposite testis (7.06). At the end of the irradiation, 56 Gy, the tumor dissappeared and a complet remission was achieved.
The intense PME signal (before starting the irradiation) as a sign of high phospholipid synthesis, correlate well with the large number of mitotic figures present in the primary tumor as well as with the short tumor doubling time revealed by the fast growing of this recurrence.
The intratumoral pH of 7.08 (after 25 Gy) very close to the normal value is a sign of a well oxigenated tumor and has a good prognostic value, i. e. a good response to irradiation. The PME peak decrease could be a good pathological marker of the steady-state response to irradiation.
All the above mentioned in vivo metabolic changes are in good accordance with the clinically achieved complete response and were as well confirmed by the MRI follow up.

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STABILITY AND HETEROGENEITY IN UROLOGICAL TUMORS: RESULTS OF TRANSPLANTATION OF 131 DIFFERENT UROLOGICAL TUMORS INTO NMRI NU/NU MICE.

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Xenotransplantation of human renal-, bladder, testicular- and prostatic cancer into NMRI nu/nu mice was done to define tumor heterogeneity and stability and to evaluate the sensitivity to chemo- and immunotherapy.
Different urological tumors from 131 patients were transplanted into nude mice to evaluate growth characteristics, identity of primary and xenotransplanted tissue during long-term follow-up, stability, correlation of tumor growth of the xenotransplanted tissue and the follow-up of the corresponding patients and the individual identity of preclinical and clinical therapeutic results.
The acceptance rates of the different tumors ranged between 87% for RCC and 12% for testicular tumors. The results of transplantation of 81 different RCC showed a large variety in respect to tumor doubling time, DNA-analysis, hormonal and biochemical activity and sensitivity to chemo- and immunotherapy. However each tumorline in itself was remarkably stable in up to 100 subpassages. In contrary to RCC, transplanted bladder and prostatic cancer showed an intraindividual instability concerning these parmeters. Of special interest was the observation that chemotherapy-sensitivity changed within the same tumor during subpassaging.
From our data we conclude that human RCC are of a great interindividual heterogeniety especially in respect to tumor growth, tumor biology and sensitivity to chemotherapy and that bladder and prostatic tumors show a remarkable intraindividual heterogeneity. It therefore has to be claimed to treat every patient with RCC with an individual strategy depending on "his" tumor biology, whereas treatment of prostatic and bladder carcinoma requires for example the simultaneous application of different treatment modalities to take into account the intraindividually varying tumor clones.

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THE SIGNIFICANCE OF TUMOR HISTOLOGY AS A PROGNOSTIC PARAMETER FOR PATIENTS WITH SUPERFICIAL BLADDER CANCER TREATED WITH INTRAVESICAL BACILLUS CALMETTE-GUERIN

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Between March 1983 and March 1988, 62 patients were treated for superficial bladder transitional cell carcinoma (TCC) ($Ta/T_1/Tis$) with intravesical Pasteur strain BCG. The protocol consisted of 6 weekly instillations followed by 10 monthly instillations. The results of BCG therapy were compared beetween two groups of patients: group I - patients having TCC with favorable histology (gr. I-II/Ta,T_1) and group 11 with unfavorable histology (gr.III-IV/T_1 or CIS). 19 of 21 group I patients and 21 of 41 group II patients were thiotepa failures. The 62 patients were followed from 6 to 64 months with a mean follow-up of 25 months. The mean follow-up in both groups was similar (23 vs 26 months). The relationship of local recurrence according to tumor histology is given in the table:

	TUMOR HISTOLOGY					
	Total		Group I (favorable)		Group II (unfavorable)	
	No	(%)	No	(%)	No	(%)
Recurrence	22	(35)	3	(14)	19	(46)*
No recurrence	40	(65)	18	(86)	22	(54)
Total	62	(100)	21	(100)	41	(100)

*p<0.03

84% of patients having tumors with favorable histology and 54% of those with unfavorable histology were free of disease at 3 years after BCG therapy (p<0.03). As predicted, our study indicates that intravesical BCG appears to be significantly more effective in patients with favorable histology.

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URINARY SECRETION OF CYTOKINES AFTER INTRAVESICAL BCG IMMUNOTHERAPY

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The mode of action of intravesical Bacillus Calmette-Guérin for prophylaxis of recurrent superficial urothelial bladder carcinoma is largely unknown,although evidence suggests that immunological mechanisms play a major role in its therapeutic effect. In an attempt to further clarify this question, urine probes of patients receiving intravesical BCG were analyzed for the presence of interleukin-1, interleukin-2 and tumor-necrosis-factor before therapy and in intervalls of 2 hours after the 6th instillation of BCG (Pasteur strain), using sandwich-ELISA and biological assays.
The results show high urinary titers of IL-1, IL-2 and TNF in the urine 2 - 8 hours after instillation, returning to baseline values within 24 hours. Particularly high titers were identified for TNF with individual maxima of >6000 U/2h in bio-assay, corresponding to >40 ng/2h in ELISA. Biological activity could be inhibited specifically by an anti-TNF-α antibody, suggesting the presence of TNF-λ. These results suggest a strong inflammatory activation of the local immune system of the bladder, the cytokines themselves being possibly responsible for the recurrence free state after BCG immunotherapy.

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Effects of Keyhole Limpet Hemocyanin on normal transitional epithelium and chemically induced bladder carcinoma

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Keyhole Limpet hemocyanin (KLH) is a potent immunostimulator. To investigate its effects on normal urothelium forty two Wistar rats were randomized in KLH nonsensitized and KLH sensitized groups, treated by intravesical instillation (0,25ml) of different KLH doses (KLH 0,1mg/ml - 50mg/ml). The experiments were repeated with rabbits, to exclude species specific effects. After being sacrificed, no group demonstrates any histological change of bladder mucosa, lamina propria or musle. The proliferation rates measured by BrDu labeling index were identical in each group, thus followed that intravesical instillation of KLH in normal bladder does not induce morphological reaction like cellular mucosal infiltration or granuloma. In a second series the effects of KLH on chemically induced bladder carcinoma, using a known carcinogen N - butyl - N (4hydroxybutyl) nitrosamin (BBN) were investigated. Forty Wistar rats were treated with KLH 12,5mg i.ves. + 0,5mg s.c./2x/week over a period of 8 weeks after being sensitized with 1x1mg KLH s.c., additionally to 0,05% carcinogen BBN feeding over 8 weeks. Compared to the controls (n=40), KLH treatment resulted in a statistically significant reduction ($p<0,02$) of exophytic (9,8+2,7% vs. 14,2+2,8%) and infltrative (2,7+1,6% vs. 4,6+1,3%) bladder tumor growth. The experimental findings confirm the concept of KLH immunotherapy in superficial bladder cancer.

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TOXICITY OF SULPHONATED ALUMINIUM PHTALOCYANINE (ALSPc) PHOTOSENSITISED LIPOSOMES DIRECTED TO BLADDER CANCER CELLS BY MONOCLONAL ANTIBODY G4 IN VITRO

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There is a need for drugs independent of cell cycle whilst maintaining some specificty to the tumor cells. Antibody targetting of liposomes might be specific of tumor cells and active against cells in the resting phase but their toxicity is limited by the rate of internalisation. ALSPc encapsulated liposomes do not require internalisation since cytotoxic action is to be primarily by generation of free radicals on exposure to light of specific wavelenghts. A partially purified fraction of hydrophilic ALSPc was encapsulated in liposomes that has been limited to monoclonal antibody G4.

G4 is a murine monoclonal IgM which has been able to detect transitional cell carcinoma (TTC) in human bladders by intravesical injection (Urol. Res., 1986, 14 : 145). The phototoxic effects of these liposomes were determined on two human bladder carcinoma cell lines ; one bearing an antigenic determinant recognized by G4 (647 V), the other without any reactivity with G4 (T 24). The phototoxic effect was determined using a tetrazolium based colorimetric assay (MTT). Antibody dependant cytotoxicity was observed with 647 V and not with T 24 depending upon exposure to activating red light. This selective photolysis of carcinoma cells in vitro suggests alternative route for endovesical treatment of bladder cancer.

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INTERFERON ALFA-2b FOR IN-VITRO-SENSITIVITY TESTING OF HUMAN RENAL CELL CARCINOMA (RCC)

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A 27% complete and partial remission rate has been reported for patients with lung metastases of renal cell carcinoma (RCC) treated with daily intramuscular injections of interferon alfa-2 (36×10^6 U)(Fossa et al., Cancer 57,1986). But up to now the discussion about the optimal therapeutic interferon dosage (low, intermediate or high dose) is still controversial. Therefore we investigated 38 different human RCC's in vitro treated with increasing doses of interferon alfa 2b. Cell response under treatment was detected by simultaneous measurement of tumor-cell kill rates (TCKR) and proliferation rates (PR).
After tumor nephrectomy a macroscopicly homogeneous tumor area lacking fibrosis, necrosis and haemorrhage was excised for in vitro cell preparation. In earlier investigations the in vitro preparation procedure used in this assay has been proved to obtain pure tumor cell cultures employing cytogenetic (Kovacs et al.,Int. J.Cancer,1987) and immunocytochemical methods (de Riese et al., Invest.Urol.3,1989). After 3-7 days of in vitro growing the first cell passage was done: The cells were transfered to 24-well-multiplates for in-vitro sensitivity testing with therapeutic agents as recently described to obtain TCKR and reduction of PR for each tumor simultaneously (de Riese et al., Invest.Urol.3, 1989). Under interferon treatment based on in vivo dose of 30×10^6 U per patient 9 (23.6%) different RCC's showed in vitro either a TCKR of more than 50% or a reduction of PR of more than 50%, none of the investigated tumors showed a response in both criteria. A strong correlation was observed between TCKR, reduction of PR and increasing interferon dosages.
Transferring these data to in vivo conditions individual treatment of metastatic renal cell carcinoma with the highest tolerable interferon dose seems recommendable using the drug's cytostatic effect only. Administering these high doses of interferon alfa-2 the patients' immune system will be competely suppressed.

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ANTIPROLIFERATIVE EFFICACY OF GAMMA-INTERFERON AND TUMOR NECROSIS FACTOR ALPHA IN A RAT RENAL CELL TUMOR MODEL SYSTEM; ESTABLISHMENT OF A RESISTENT TUMOR LINE

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In a syngeneic rat renal cell tumor model system we have investigated the antiproliferative efficacy of rec. rat-gamma-interferon (IFN) and rec. human tumor necrosis factor alpha (TNF), as single agent or in combination drug tests. Also the role of tumor volume at start of therapy was investigated. The tumor is transplanted subcutaneously (s.c.) by implanting tumorpieces of 50 mg. The BRMs are administered peritumorally, IFN 3 times and TNF 5 times per week. Treatment starting two days after tumor implantation with 8,000 or 80,000 Units IFN per rat (spec. act. 7E6 U/mg protein) has a dose-dependent inhibiting effect on tumor growth. 1 or 10 µg TNF per rat (spec. act. 6E7 U/mg protein) has not a significant effect on tumor growth , 100 µg TNF, however, results in a significant growth inhibiting effect. Different combinations of IFN and TNF have additive or synergistic antiproliferative effects. The combination of 80,000 Units IFN and 100 µg TNF completely inhibits tumor growth without any obvious toxic effects on the rats.

When treatment is started at a tumor volume of 0.2-0.5 cc, IFN or TNF alone have no effect on tumor growth. The combination 80,000 Units IFN and 100 µg TNF, however, stabilizes tumor volume even when treatment was started at a tumor volume of 2-5 cc.

60 days after start of therapy in the group treated with 80.000 IFN and 100 µg TNF in one rat a tumor started to grow. After reimplantation therapy with the most effective combination of IFN and TNF could not inhibit tumor growth.

Conclusions: IFN and TNF have, when combined, synergistic antiproliferative effects against renal cell carcinoma. Cell biological and biochemical experiments on the resistent tumor line can give us insight in the mode of action of the Biological Response Modifiers.

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NEW TREATMENT MODALITIES FOR METASTATIC RENAL CELL CARCINOMA: TUMOR NECROSIS FACTOR IN COMBINATION WITH OTHER CYTOKINES AND CHEMOTHERAPY

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To answer the question whether the efficacy of monotherapy with Tumor Necrosis Factor (TNF) in metastatic renal cell carcinoma (RCC) might be increased by combining it with other cytokines or chemotherapeutic agents, we studied the effect of both mono- and combination therapy on transplantable human RCC in the nude mouse model.

Four well characterized human RCC established in NMRI nu/nu mice were treated either with TNF (0,75 mg/kg i.p. 5x/week), Interferon-α-2a (IFN-α-2a), Interferon-γ (IFN-γ), Interleukin-2 (IL-2), Mitomycin C, cis-Platinum or Epirubicin alone. A second set of animals received TNF in combination with one of the other cytokines or chemotherapeutic drugs. Response to therapy was evaluated by tumor volume measurements, histology, immunohistochemistry, flow cytometry and BRDU-labeling and compared to an untreated group.

In monotherapy, only IFN-α-2a, IFN-γ and IL-2 produced objective tumor responses. Although TNF alone did not cause a significant tumor growth depression, combined therapy with TNF and IFN-α-2a was superior to any other combination regimen and lead to an objective tumor response in 80% of the animals with 60% complete remissions. Antitumoral activity was shown to be as well cytotoxic as antiproliferative by immunohistochemistry and flow cytometry and resulted in a rapid decrease of tumor cells.

In conclusion, the combination of TNF and IFN-α-2a was highly effective in the treatment of human RCC in the nude mouse model while TNF monotherapy and the combination of TNF and chemotherapy were unsatisfactory. Preliminary results of a clinical phase I/II trial being underway in our department seem to confirm the efficacy of TNF-IFN-α-2a combination therapy in patients with metastatic renal cell carcinoma.

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INTERFERON-ALPHA, -GAMMA AND TUMOR NECROSIS FACTOR AND THEIR EFFECTS ON THE GROWTH POTENTIAL AND CLASS I, II ANTIGEN EXPRESSION OF RENAL TUMOR XENOGRAFTS.

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The direct in vivo antiproliferative effect of alpha- and gamma-Interferon (IFN) as well as Tumor Necrosis Factor (TNF) has been studied on renal tumor xenografts. Antiproliferative effects of single agents and combinations at different concentrations have been evaluated. BalbC nu/nu mice were used and the drugs were administered subcutaneously peritumoral (S.C.), intraperitoneally (I.P.) or intraveneously (I.V.) three to five times a week. Treatment started 24-48 hours after S.C. implantation of 2.10E6 tumor cells or when tumor volume reached 65 cubic mm.

Treatment started after measurable tumor growth was not effective in reducing tumor growth. I.V. treatment with an alpha-IFN/TNF combination even resulted in a slight stimulation of tumor growth. Efficacy of alpha- and gamma-IFN showed to have an optimum dose of 150 IU/g for S.C. treatment. TNF showed less activity and antiproliferative effects appeared to be dose dependent. Treatment with this drug was most effective with 30,000 IU/g applied five times a week. Combinations of both alpha- and gamma-IFN with TNF showed to have synergistic antiproliferative efficacy against the Renal Cell Carcinoma (RCC) xenografts. S.C. administration of 150 IU IFN/g three times a week combined with 30,000 IU TNF/g five times a week during six weeks completely inhibited tumor growth of most tumor lines. No tumor growth could be detected six months after completion of therapy.

Class I and Class II antigen expression tested on frozen sections using mABs W6.32 and B8.11.2 in a DAB peroxidase assay showed that alpha-IFN and TNF can augment HLA Class I expression of RCC xenografts. HLA Class II expression was induced or augmented by gamma-IFN and TNF.

Because induction or augmentation of Class I and Class II antigens was found in the BRM sensitive RCC xenografts we conclude that HLA Class I and Class II expression may be related to enhanced sensitivity for immunotherapy.

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Immune status in renal cell carcinoma

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Different protocols of immunotherapy of metastatic renal cell carcinoma have been studied recently, success rates, however, were very heterogenous. The purpose of this study was to assess the pretherapeutic immunological status of patients with renal cell carcinoma and the course of several immune parameters during application of the xenogeneic biological response modifier keyhole limpet hemocyanine (KLH). Determined were differential blood counts, lymphocyte subpopulations, β-2-microglobulin (β-2-m), tumor necrosis factor (TNF), neopterin, immunoglobulins, fibronectin and ferritin prior to any therapyin 14 patients with metastatic as well as in 15 patients with non-metastatic renal cell carcinoma and 15 healthy volunteers. Furthermore these parameters were recorded during applications of KLH (Immucothel R, biosyn, Stuttgart; 1 mg i.m. in 4 weekly-intervals) in 10 patients with metastatic and in 5 patients with non-metastatic disease prior to the KLH-injections and one day and one week thereafter. The pretherapeutic immunological status of patients with metastatic disease was characterized by reduced T4- and T8-cell counts and significantly reduced B-cell counts. Significantly increased were granulocyte counts, β-2-m, Neopterin and TNF. In patients without metastases, only β-2-m and Neopterin were increased significantly. During KLH-application, in patients with metastases, there was a decline of lymphocyte subsets and the T4/T8-ratio, which correlated with progress of disease. Humoral immune parameters showed no changes compared to pretherapeutic values. In patients without metastases, cellular immune parameters showed stable values during KLH-application and neopterin, β-2-m and TNF increased considerably. These findings indicate immunosuppression in patients with progression of disease which may hinder the immunostimulating effects of biological response modifiers during immunotherapy.

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THE CORRELATION BETWEEN THE PLOIDY OF RENAL CELL CARCINOMAS AND THE REACTION OF PATIENTS TO THE ACTIVE SPECIFIC IMMUNO-THERAPY WITH MODIFIED AUTOLOGOUS TUMOUR CELLS

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Patients with aneuploid tumours have shorter survival times than those with diploid tumours. On the other hand the active specific immunotherapy with NDV modified autologous tumour cells may possibly influence the course of the neoplastic disease(1). The purpose of this study was to examine if the ploidy of renal cell carcinomas is related to the delayedtype hypersensitivity (DTH) reactivity of the immunized patients.

Following resection of a renal cell carcinoma active specific immunotherapy (ASI) with Newcastle disease virus (NDV) modified autologous tumour cells was performed in 38 patients in a clinical phase I study. The DNA ploidy of the tumour suspensions was determined by flow cytometry with a mixture of propidium iodide (10 ug/ml) and 4'-6-di-amino-2-phenyl-indole (DAPI 2 ug/ml). The DTH reaction and the peripheral blood lymphocyte subsets of the vaccinated patients were sequentially assessed within the first postoperative year and related to the DNA ploidy of the carcinomas.

Results: In all patients with aneuploid tumours the serum levels of OKT 8 positive cells increased markedly following the vaccination. The DTH scores in these patients, unlike in those with euploid tumours, showed a decreasing tendency.

Conclusion: The patients with aneuploid tumours seem to show during the ASI an increase of suppressor cell levels.

Lit.:
Schirrmacher et al, Cancer Metastasis 1989 (in press)

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Effects of Keyhole Limpet Hemocyanine (KLH) and low doses of Cyclophosphamide on immune parameters in patients with metastatic renal cell carcinoma.

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Immunotherapy with biological response modifiers (interferons, interleukin 2) has gained increasing importance in the treatment of metastatic renal cell carcinoma. Assessing immune parameters in these patients, however, we found lack of consistent immune response to the xenogeneic immunostimulant KLH and indications for immunosuppression. Immunosuppression may hinder the potention antitumoral effects of immunotherapeutic agents in tumor patients, thus being responsible for the lack of therapeutic success noted in many studies. Immunosuppression mediated by T-suppressor-cells can be overcome by application of low doses of cyclophosphamide, either via a reduction of T-suppressor-cell number or via reduction of T-suppressor-cell function. We tested the effect of combination of low doses of cyclophosphamide and KLH (300 mg Cy/m² i.v., 3 days later 1 mg KLH (Immucothel R, biosyn, Stuttgart), in 4-weekly-intervals) on immune parameters in 8 patients with metastatic renal cell carcinoma. Recorded were differential blood counts, lymphocyte subpopulations, neopterin, β-2-microglobulin, tumor necrosis factor (TNF), immunoglobulins, fibronectin and ferritin before and one day and one week after the KLH-application. In cellular immune parameters, reductions in the T4/T8-ratio were noticed less frequent than in a previous study, in which KLH had been applied without cyclophosphamide. In humoral immune parameters, increases in β-2-microglobulin, TNF, IgG and IgM were more frequent when KLH was combined with cyclophosphamide, indicating that the humoral immune response to KLH can be augmented by low doses of cyclophosphamide in patients with metastatic renal cell carcinoma.

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A DOUBLE-BLIND RANDOMIZED CONTROLLED STUDY OF THE EFFECT OF FLUTAMIDE ON BENIGN PROSTATIC HYPERTROPHY: CLINICAL EFFICACY.

Nelson N Stone and Arnon Krongrad NY NY, Gerald W Chodak and Paul S Ray Chicago Ill, Joseph A Smith Salt Lake Ut, Peter T Scardino Houston Tx, Robert B Smith La Calif, Om P Khanna Phil Pa, David F Paulson Durham NC, B Kasimis East Orange NJ, and Robert J Spiegel, Rudolph O Neri Kenilworth NJ. (Presentation to be made by Dr. Stone).

A multicenter randomized double-blind study was undertaken to evaluate the efficacy of the antiandrogen flutamide in treating patients with urinary obstruction from BPH. Patients were randomized to either placebo or flutamide (750 mg/d) for a treatment period of up to 6 months. Response was determined by prostate volume (PV), maximum uroflow rates (U) and urinary symptom scores (USS).

Of the 84 patients enrolled in the study 58 patients have completed a minimum of 3 months treatment. Thirty patients in the placebo group (PG) had a median age of 65 years (50-88) and 28 in the flutamide group (FG) 66 (55-81). The initial and 3 month PV's for PG were 51 cm^3 and 53 cm^3. The median decrease in prostate volume for the FG was 18% by 3 months and 41% by 6 months ($p<.01$).

Uroflow was measured prior to and after bladder filling by catheter. Patients on flutamide had a median improvement in uroflow of 35% and 43%, respectively. In contrast, patients on placebo noted a 18% decrease in uroflow.

Urinary symptoms improved by 50% in both groups. Sixty-nine per cent of the FG and 40% of the PG reported adverse experiences. There were no severe cases of gynecomastia or impotence in patients on flutamide.

Serum testosterone (T), estradiol (E) and prostate specific antigen (PSA) were obtained in 17 patients (8-F, 9-P). No changes were noted in patients on placebo. T increased by 79.7% by 6 months while PV decreased by 38.3% and PSA by 65.8%. No changes were noted in E. All values returned to baseline 6 weeks off flutamide.

Flutamide is a safe and effective treatment for BPH. Minor complaints of breast pain or gynecomastia did not influence study participation. Maximum effect may require up to 6 months or longer of treatment.

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IF ADVANCED PROSTATIC CANCER IN PATIENTS OLDER THEN 75 YEARS IS A TRULY INDICATION FOR A HORMONAL TREATMENT ?.

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Forty three patients aged 76 to 85 years (mean 79.1) with advanced prostatic cancer (stage C or D) were treated by oral administration of diethylstliboestrol diphosphate (Honvan) combined with bromocriptine (Parlodel). The treatment was commenced only in patients with clinical symptomps and/or elevated serum marker (acid & prostatic acid phosphatases) levels and was stopped in patients with clinical & enzymatic normalization - delayed & intermittent therapy. The lowest therapeutical doses of drugs were used. Of patients 14 (32.5%) had distant metastases at the first presentation.

During the mean observation time of 65 months 16 (37.2%) patients have died but only 7 due to prostatic cancer progression.Twenty seven (62.8%) patients are still alive and 18 of them show no symptomps of neoplastic disease.

The results when compared with the group of 101 patients with stage C or D of prostatic cancer, younger then 75 years gave us the following conclusions:

-in older group the beginning of the treatment could be postponed by mean of 15 months,
-the periods of cessation of the treatment were longer in older group by mean of 12 months,
-the older patients required lower doses of drugs,
-eleven (25.0%) patients of older group did not required any treatment showing no progression of cancer
-the death rate due to progression of prostatic cancer was higher in younger group.

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PARENTERAL POLYESTRADIOL PHOSPHATE (PEP) AS SINGLE DRUG THERAPY OF PROSTATIC CANCER

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49 patients with cytologically and/or histologically confirmed prostatic cancer were treated with parenteral PEP at initial dosages of 160, 240 or 320 mg every fourth week for 6 months. After that maintenance doses varying from 80 to 240 mg/4th week were used. The patients were followed up to 42 months.

During the initial 6-month period, castration values of testosterone (T) were reached with the 240 and 320 mg doses, but not with the 160 mg dose. Maintenance dosages at 80 and 160 mg were insufficient for reaching castration levels of T, even in patients treated with 240 or 320 mg initial doses.

All patients were clinically followed by SPCG criteria. Mean observation time was 23 months (6-42 months). 11/49 patients were classified as non-responders. Only 3 cases of minor cardiovascular complications were observed.

Our opinion is that parenterally given oestrogen, in an adequate dosage, is an alternative with few side effects compared to combined perorally/parenterally given oestrogen in hormonal treatment of prostatic cancer.

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SUBJECTIVE AND BIOCHEMICAL RESPONSE IN HORMONE RESISTANT PROSTATIC CANCER DURING TREATMENT WITH FLUTAMIDE

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Pain relief represents the principal goal of treatment of hormone resistant prostatic cancer.

26 patients (pts) with progressive and symptomatic prostatic cancer in spite of castration, entered an ongoing phase II study with the aim to evaluate the response rate of Flutamide (250 mg x 3 per os daily) (median age: 69 years; performance status: 0/1:14; 2/3: 12; median serum PSA: 285 U/l).

Subjective response was assessed after at least 6 weeks treatment. The use of analgesics and of concomitant radiotherapy was taken into account. Treatment with Flutamide was discontinued in pts with progression and/or in case of intolerable toxicity. The concomitant changes of serum PSA were also reported (remission: >50% decrease; progression: >50% increase of the pre-treatment value). If possible, "objective" response was also assessed based on the EORTC criteria. At present 21 pts are evaluable for response.

Subjective response at 6 weeks was as follows: remission: 1 pt; no change: 13 pts; progression: 7 pts (4 of them "early" progressions). Subjective response at 12 weeks: Remission: 1 pt; no change: 3 pts; progression: 10 pts.

Subjective progression was significantly correlated with a >50% increase of serum PSA.

3 pts experienced nausea/vomiting which necessitated dose modification or discontinuation of the drug.

Conclusion: Flutamide (250 mg x 3 per os daily) is ineffective in hormone resistant prostatic cancer as concerns subjective response. Serum PSA might be useful as a marker in hormone resistant prostatic cancer, where other "objective" tumour markers often are lacking.

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ENDOCRINE TREATMENT OF EXPERIMENTAL PROSTATIC CANCER

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The effects of chronic treatment with various endocrine treatment modalities on tumor growth, body and organ weights were investigated in male Copenhagen rats bearing transplanted Dunning R3327 (PAP) prostate tumors. After transplantation of a piece of tumor, the rats were treated in 2 experiments during 4-11 weeks by surgical castration, with buserelin implants, cyproterone acetate (CPA, 3 and 10 mg s.c.), the aromatase inhibitors aminoglutethimide (2 x 2 mg) and 4-OH-androstenedione (2 x 2 mg) p.o., the antiprogestin mifeprisone (2.5 mg RU 486), the somatostatin analogue Sandostatin (2 x 3 µg) both as single treatment and in combination with surgical castration, buserelin implants or CPA. When compared to controls, the aromatase inhibitors and the antiprogestin did not cause tumor growth inhibitory effects. Surgical castration was the most effective way of treatment (52-53% growth inhibition) compared to buserelin implants (38%) and CPA (29-33%). Treatment with Sandostatin caused a slight but not significant inhibitory effect as single treatment (12%) and in combination with buserelin implants (45%), but no additional anti-tumor effects were observed in combination with surgical castration (50%) or CPA (30-32%). Body weight decreased by treatment with buserelin implants, CPA and Sandostatin. Mean pituitary weight increased after surgical castration and decreased after treatment with the LHRH and somatostatin analogues or mifepristone. Testes weight decreased strongly after treatment with buserelin implants or CPA. Adrenal weight decreased after treatment with CPA or buserelin implants. Prostate weight decreased after treatment with surgical and medical castration, CPA or mifepristone. Pancreatic weight showed only a slight increase after Sandostatin treatment and a decrease after antiprogestin treatment. In conclusion: surgical castration, buserelin implants and CPA caused significant growth inhibitory effects in the Dunning rat prostate cancer model. The somatostatin analogue Sandostatin showed only minor, not significant growth inhibitory effects as single treatment and in combination with buserelin implants. Two aromatase inhibitors and an antiprogestin were not effective. This study is supported by the Dutch Cancer Society (KWF).

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BASAL AND ACTH-STIMULATED ADRENAL STEROIDS IN PROSTATIC CANCER PATIENTS DURING TREATMENT WITH LH-RH ANALOGUE AND FLUTAMIDE

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Basal levels and ACTH-induced increments (Δ-values) of serum dehydroepiandrosterone (DHA) and its sulfate (DHAS), 4-androstene-3,17-dione (A-4) and cortisol and serum aspargine aminotransferase (ASAT) were measured in prostatic cancer patients before and after 6 months of treatment with LH-RH-analogue, with flutamide and with LH-RH-analogue + flutamide respectively. The results are given in the table as per cent of pretreatment values:

	LH-RH analogue	Flutamide	LH-RH-analogue + flutamide
N	9	11	8
DHAS	94%	54% ***	44% ***
DHA	121%	50% *	48% **
Δ DHA	103%	81%	99%
A-4	62% **	96%	42% ***
Δ A-4	81%	94%	97%
Cortisol	105%	94%	93%
Δ Cortisol	88% *	87% ***	99%
ASAT	136%	172%	196%

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

While the pronounced decrease in basal levels of adrenal androgens is in accordance with previous findings of Labries group (1) we could not confirm their results concerning a selective inhibitory action of flutamide analogues upon adrenal androgen response to ACTH stimulation. The effects of flutamide on adrenal androgens may be mediated via liver interaction (2).

1) Belanger et al: J Clin Endocrinol Metab 59:422,1984
2) Fukushima et al: J Clin Endocrinol Metab 47:788,1978

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INTERFERON AND HORMONE-SENSITIVITY IN PROSTATIC CANCER CELLS
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Cultured PC-3 cells, derived from a human prostatic adenocarcinoma metastatic to bone, contain, in our experience, androgen receptors (AR) but are insensitive to testosterone. Hydroxyflutamide, an anti-androgen that competes with androgens for binding to cellular receptor, does not affect PC-3 cell proliferation, $_{-10}$(like testosterone), at concentrations ranging from 10^{-10} M to 10^{-6} M, after 3 and 6 days of treatment.
As we recently showed that natural ß-Interferon (ß-IFN) is able to modify steroid hormone receptor content in breast and endometrial cancer cells and to promote the antiproliferative action of antiestrogen and progestins, we investigated,in the present work, the effect of ß-IFN on AR level in PC-3 cells.
AR were evaluated by a whole cell assay, after 3 days of treatment with ß-IFN at concentrations ranging from 10 IU/ml to 1000 IU/ml. Low doses of ß-IFN were ineffective, while 100 IU/ml determined an AR increase of approximately 100% with respect to control. The effect of 1000 IU/ml on the AR enhancement was impressive (the increase reached about 500% with respect to control). Work is in progress to evaluate if receptor modulation that we obtained can restore hormone sensitivity in our model.

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TRANSPLANTATION OF HUMAN NORMAL PROSTATE OR BPH TISSUE INTO NMRI NU/NU MICE: RELIABILITY OF AN EXPERIMENTAL MODEL

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We tried to answer the question whether transplantation of human normal prostatic and BPH tissue into NMRI nu/nu mice can be a reliable model for studies of the induction of BPH, its biological behaviour and therapy experiments.

Human normal prostatic and BPH tissue was transplanted subcutaneously on both sides of the thoracic wall of male, orchiectomized mice. Standardized hormone substitution was performed by addition of silastic implants containing 5-alpha-dihydrotestosterone (DHT) and/or estradiol (E_2).
As a first attempt for the evaluation of therapeutic effects mice additionally were treated with two plant extracts (contained in Prostagutt N^R and Cernilton N^R).
Tumor volume was measured weekly. After two months the tissue was processed for histological examination and PSA staining.

BPH could be induced in *normal* human prostate tissue by simultaneous stimulation with DHT and E_2.

Two months after transplantation of BPH, vital tissue was found histologically in all cases. Under hormonal stimulation tumor volume increased significantly ($p<0.08$) and typical histological alterations were observed.
The application of the therapeutic agents led to a significant growth inhibition ($p<0,08$). However no histological alterations occured after treatment with these drugs.

From our data we conclude that transplantation of human normal prostatic tissue or benign hyperplastic tissue into nude mice is a promising model for the investigation of the induction of the disease, its biological characteristics and possible therapeutic concepts.

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DO STEROID ANDROGENS IN EXPRESSED PROSTATIC FLUID REFLECT THE PATHOLOGY OF THE PROSTATE GLAND?

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The ratio of the steroid androgens testosterone (T) and 5 alpha-dihydrotestosterone (DHT), when measured in prostatic cancer tissue, is greater than that measured in benign prostatic hyperplasia (BPH) and normal tissue (Habib,1979)
If these changes in androgens are reflected in the expressed prostatic secretion (EPS) then analysis of this fluid might provide a valuable clue to prostatic pathology.

0.1 to 0.8 ml of EPS was collected from each of 20 men (mean age 73.1 years) by transrectal prostatic massage immediately prior to transurethral resection for symptomatic BPH. Prostatic tissue was collected in each case and part reserved for biochemical analysis. EPS was also collected from a second group of 16 younger patients (mean age 46.6 years) presenting to the outpatient clinic with a variety of complaints but who clinically had neither BPH or prostate cancer. Androgen concentrations were measured by radioimmunoassay after extraction from lyophilised EPS and from prostate tissue.

Results show that both T and DHT can be measured quantitatively in EPS from these patients, and that in patients with BPH the T/DHT ratio is >1, similar to that measured in tissue. In the younger patients a greater variation in EPS androgen concentrations was seen, reflecting the heterogeneous nature of this group. Difficulties in expression of absolute androgen levels arise from variations in volume and concentration of EPS but the steroid ratios were independent of these variables.
The application of the technique to patients with prostate cancer will be discussed.

Habib et.al. Br.J.Cancer (1979)39,700

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IMMUNOCYTOCHEMICAL LOCALIZATION OF ESTROGEN RECEPTORS (ER) IN NORMAL AND DISEASED HUMAN PROSTATES Harald Schulze and Siegfried Claus, Herne, FRG (Presentation to be made by Dr. Schulze)

Using the monoclonal ER antibody H222 with the ABC (avidin-biotinylated peroxidase complex) method we investigated the histological localization of ER in normal and diseased human prostates by immunocytochemistry.
Prostate tissue was obtained from 3 organ donors (15 to 37 years old; group I), from 14 prostates removed by radical prostatectomy (n=10) or cystoprostatectomy (n=4) of patients with no or mild obstructive symptoms (52 to 74 years old, mean age 64 years; group II) and from 11 prostates with benign hyperplasia (BPH) causing severe obstructive symptoms removed by suprapubic prostatectomy with obstructing tissue weight of > 60 gm (mean weight 82 gm) (60 to 83 years old, mean age 73 years; group III). The prostates were opened anteriorly and longitudinal or sagittal sections through the verumontanum were obtained.
In all prostates examined (n=28) intense specific ER immunocytochemical staining was found in nuclei of the transitional epithelium of prostatic urethra and periurethral prostatic ducts.
In the periurethral stroma (submucosa) less intense immunoreactive staining was found in prostates of all 3 groups. In the prostatic stroma of non-obstructive prostates (grps. I and II) specific ER staining was found in stromal nuclei, with the highest concentration of specifically stained nuclei in non-cancer stroma in prostates of group II. In contrast, stroma of obstructive BPH (group III) contained no specifically stained nuclei.
In prostatic glandular epithelium specific staining was seen only in a small percentage of acini in the non-obstructive prostates (grps. I and II), mostly in coincidence with a basal cell hyperplasia.
Our results indicate that the stromal growth of human BPH causing obstructive symptoms may not be mediated via ER. (Supported by DFG Schu 616/1-2. H222 was provided by Abbott Labs.)

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3β-HYDROXY-Δ^5-STEROID DEHYDROGENASE ACTIVITY IN BENIGN AND MALIGNANT HUMAN PROSTATIC TISSUE.

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3β-Hydroxy-Δ^5-steroid dehydrogenase (EC 1.1.1.145, Isomerase) converts the important adrenal androgen DHEA to androstenedione (Ae), and is a key enzyme for the formation of "active" androgens from "inactive" precursors.

We have established a sensitive method for quantifying this enzyme in human prostatic tissue: prostatic tissue homogenates were incubated with [^{3}H]-DHEA (0.5 µM) for 60 min, steroids were extracted with ether, separated by HPLC, and quantified by LSC.

The main results were, (1) a low enzyme activity was detected in benign (Mean ± SEM [n], nmol/h/mg DNA, 0.04 ± 0.01 [8]) as well as in malignant (0.03 ± 0.01 [12]) tissue of previously untreated patients, (2) in 11 metastases from 4 patients the activities were found in the ranges of the primary tumors except one metastasis with an about threefold activity. (3) From the latter patient four further metastases were obtained at a later time point, when the patient had relapsed from endocrine treatment. They revealed an in part exessively increased isomerase activity (up to 4.21 nmol/h/mg DNA). In these tissues the isomerase activity was also reflected by the endogenous Ae concentration.

It is concluded that individual tumors convert DHEA at a high rate to Ae, and subsequently to testosterone and DHT. Therefore, in these patients castration alone may be insufficient to inhibit androgen-stimulated cancer cell proliferation.

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Influence of in vivo treatment with the aromatase inhibitor 1-Methyl-ADD on androgen metabolism in human prostate

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For development of benign prostatic hyperplasia (BPH) not only an intraprostatic excess of DHT is discussed but also an increased ratio of estrogens to androgens. Thus, inhibition of peripheral aromatization of androgens to estrogens by aromatase inhibitors was created as a new conservative therapeutic concept for BPH. In this context, however, it is completely unknown whether such an aromatase inhibitor may also influence intraprostatic androgen metabolism, which, in turn, might lead to an altered DHT concentration within the prostate.

Therefore, we measured kinetic parameters of the DHT forming enzyme 5α-reductase and the DHT removing enzymes 3α-HSOR red and 3ß-HSOR red in epithelium and stroma of normal (n=10) and hyperplastic (n=16) prostates as well as of hyperplastic prostates from 10 patients treated for 7 days with the aromatase inhibitor 1-Methyl-ADD (5 or 10 mg/kg) before suprapubic prostatectomy. From kinetic parameters, a so called DHT enrichment index was calculated. $P < 0,05$ was considered significant.

The main results were: (1) Concerning normal prostate, the mean DHT enrichment index (± SEM) in epithelium (2,6± 1,2) was lower than in stroma (4,4 ± 0,5). (2) Concerning BPH, again the epithelial index (2,3 ± 0,3) was lower than the stromal one (3,1 ± 0,3). The difference in stroma between normal prostate and BPH was significant. (3) After treatment of BPH patients with the aromatase inhibitor, again the mean DHT enrichment index in epithelium (7,3 ± 1,8) was lower than in stroma (8,3 ± 1,2). These values were significantly higher as compared to those of untreated donors.

In conclusion: In BPH lower DHT enrichment indices are present than in normal prostate. This finding does not support the hypothesis of DHT accumulation as an etiological factor for BPH. Treatment of BPH with 1-Methyl-ADD leads to an intraprostatic metabolic shift to DHT, which even exceeds the relatively high DHT shift in untreated normal prostates.

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GENOMIC ORGANIZATION AND STRUCTURE OF THE NORMAL AND ABERRANT HUMAN ANDROGEN RECEPTOR

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The cloning of cDNAs encoding the human androgen receptor (hAR) has revealed this protein to be composed of 917 amino acids with a calculated Mw of 99 kDA. Structurally, the hAR consists of a large N-terminal domain, containing an acidic region and several homopolymeric amino acid stretches, followed by a DNA-binding domain and a ligand-binding domain. Such a structural organization is common to all steroid hormone receptors.

The complete protein coding region of the hAR gene was found to be divided over eight exons. The total length of the gene exceeds 90 kb. The sequence encoding the N-terminal region is present in one exon. The two putative DNA-binding fingers are encoded separately by two small exons. The information for the hormone-binding domain is split over five exons. Positions of introns are identical to those reported for the chicken progesterone receptor and the human oestrogen receptor genes. Analysis of genomic DNA using various specific probes revealed that the human androgen receptor is encoded by a single-copy gene which is localized on the X-chromosome.

Southern blot analysis of genomic DNA was used to investigate the gross structure of the androgen receptor gene of 13 patients with reported androgen insensitivity (AIS). Results showed identical hybridizing DNA-restriction fragments in normal subjects and in 13 patients with AIS. From these data it was concluded that large deletions in the human androgen receptor gene are not the main cause of androgen insensitivity. Recently in one AIS patient a point mutation in the exon/intron boundary of exon 4 was identified by sequencing of the PCR (polymerase chain reaction)-amplified androgen receptor exon. The consequences of this mutation for the functioning of the androgen receptor is presently under investigation.

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DELIMITATION OF AN EUKARYOTIC ANDROGEN RESPONSE ELEMENT

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Although androgens are assumed to be active through imperfect palindromes based on the TGTTCT motif which is the right arm of the glucocorticoid/mineralocorticoid/progesterone/androgen response element of the mouse mammary tumour virus (MMTV), no satisfactory definition of an androgen response element (ARE) has been determined for an eukaryotic androgen-responsive gene. By means of DNA-cellulose competition analysis and mobility shift assays, and comparison with a Dra I-Pst I fragment of the MMTV long terminal repeat (LTR), a 500bp fragment of the rat ventral prostate PBP C3(1) gene intron 1 was shown to have high affinity for partially-purified androgen-receptor complexes. Ladder retardation defined the putative ARE region to the 3' 250bp portion of this fragment. Based on these data, the oligonucleotides ARE 1 (GCACTTCAGTACTAGGGTTTCTGGTTATGTTGTTTTGTGA TGCAAATGTTAGAGCC), ARE 2 (TATAGGATGTTTGAACATAGTACGTGATGTTCTCAAGA TAGTAATGAAAT) and ARE 3 (AGAGTTAAGAGAGAACAACTTGGCTAACATTCGAGCTGT GATATTTATAG) and their complementary strands were synthesised, annealed, and assessed in gel retardation assays using GAATTGGTCGACTTGGCTTCAATCCAACCCGGGAAGCTT (NF1 binding site consensus sequence) and a perfect GRE palindromic element as competitors. Sequences were also synthesised with overlapping ends for orientation-specific ligation into the plasmids pBLCAT2 and pBLCAT3 (B. Luckow & G. Schutz. Nucleic Acids Res. 15, 5490, 1987). After transfection by electroporation into CAPE 1 prostate epithelial cells ARE capability was verified.

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PROCESSING OF THE ANDROGEN RECEPTOR IN HUMAN LNCaP CELLS

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The human LNCaP (Lymph Node Carcinoma of the Prostate) cell line is responsive to androgens with respect to growth and specific protein synthesis. This cell line was used to study the role of the androgen receptor in the biochemical mechanism of action of androgens. For that purpose androgen receptors were in situ photo-labeled with the synthetic androgen ^{3}H methyltrienolone (R1881) and analysed on SDS-PAGE.
In addition to the 110 kD androgen receptor, a 43 kD protein was specifically labeled with ^{3}H-R1881. This protein has no affinity for other steroid hormones and is structurally unrelated to the human androgen receptor as was demonstrated by limited proteolysis with chymotrypsin.
The androgen receptor in LNCaP cells is a rapidly turning over protein with a half life of 2.5 hours. 1 hour after incubation of hormone depleted LNCaP cells with ^{3}H-R1881, the androgen receptor undergoes an upshift in molecular weight on SDS-PAGE, indicating a covalent modification of the receptor molecule. For the progesterone receptor, a similar upshift in molecular weight on SDS-PAGE reflects a phosphorylation step. Studies are in progress to determine if androgen receptor processing also involves a hormone induced phosphorylation.

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CHARACTERIZATION OF ANTIBODIES AGAINST THE HUMAN ANDROGEN RECEPTOR.

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Two approaches were followed for preparation of rabbit antibodies against the human androgen receptor (AR). First, an AR cDNA fragment encoding part of the N-terminal domain of the receptor was cloned behind the beta-gal gene and expressed in E. coli. The partially purified and renaturated fusion protein was used for immunization. The rabbit antiserum obtained was able to immunoprecipitate selectively radioactive labelled androgen receptors (nuclear receptor, 100kD form) obtained from human prostate LNCaP tumor cells and from calf uterine tissue. No other steroid receptors were precipitated by the antiserum.
In the second approach the contribution of different structural elements of the AR to immunogenicity were analyzed with prediction programs and peptides of 15 to 25 aminoacid residues synthesized. Conjugates of the peptides were injected into rabbits. Polyclonal antibodies against two of these peptides (SP60 and SP61) selectively precipitated the 3H-R1881 labeled AR isolated from nuclei of LNCaP tumor cells. These antibodies against different parts of the N-terminal region of the AR also precipitated the photo-affinity labeled 100 kD androgen receptor, as demonstrated by SDS-electrophoresis and recognized a 100 kD band after Western blotting of LNCaP proteins. In sucrose gradients the anti-SP60 and SP61 antibodies shifted the sedimentation constant from 4.5 to 7 S, indicating complex formation of the receptor with the antibodies. Both antibody preparations specifically stained nuclei of epithelial cells in frozen sections of benign prostate hypertrophy tissue. (See abstract Th.H.v.d.Kwast et al.)
In conclusion: the N-terminal part of the human androgen receptor contains an immununogenic region, which can be used for selective generation of antibodies against this receptor.

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IMMUNOHISTOCHEMICAL DETECTION OF THE HUMAN ANDROGEN RECEPTOR IN PROSTATIC TISSUES.

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The majority of prostatic carcinomas is initially androgen-dependent for their growth, but during progression of the disease most of the tumors eventually become androgen-independent. The mechanisms leading to androgen-independency are unknown. The expression of androgen receptor (AR) by prostatic tumor cells indicates the responsiveness of a given tumor to hormonal treatment or castration. Immunohistochemical detection of AR in prostatic tissues might provide an easy method on small biopsy samples or cytological smears to get an insight into tumor heterogeneity for AR and to evaluate androgen dependence at diagnosis and during follow-up of prostatic cancer.
Rabbit antibodies against synthetic peptides were generated with specificity for unique epitopes of the N-terminal part of the human AR (see abstract of E. Mulder et al.). Cryostat sections of prostatic tissues were fixed in formalin and subsequently dehydrated in chilled methanol and acetone. After washing in PBS tissues were incubated overnight with anti-AR antibody and an indirect conjugated peroxidase method was employed for visualization. AR-specific antibodies selectively stained the nuclei of the secretory epithelial cells lining hyperplastic prostatic glands. Basal cells remained unstained. Rather faint staining of nuclei of stromal cells was observed. The results indicate that these antibodies are of potential use for the evaluation of the expression of AR in prostatic cancer.
Immunohistochemical analysis of prostatic tumors for expression of AR is currently under investigation.

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RELEVANCE OF LOW ANDROGEN LEVELS AND ADRENAL ANDROGENS IN THE GROWTH OF TRANSPLANTABLE HUMAN PROSTATIC CARCINOMAS.

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To investigate the necessity of total androgen ablation therapy in prostate cancer patients, the androgen dependent human prostatic tumor lines PC-82 and PC-EW were used as models. Plasma testosterone (T) levels of castrated nude mice bearing exponentially growing tumors were hormonally manipulated by implanting Silastic capsules filled with cholesterol mixed with varying proportions of T. These implants resulted in plasma-T levels of 0.2-20.0 nmol/l. The PC-82 tumor regressed when circulating T levels were below 0.3 nmol/l (0 % T-implant; n=9) corresponding with an intratumor dihydrotestosterone (DHT) level of 1.4 ± 0.8 pmol/g tissue. A stable tumor size was obtained at plasma-T levels of 0.8 ± 0.8 nmol/l (5 % T-implant) with intratumor DHT concentrations of 3 - 4 pmol/g tissue, which can be appointed as the threshold level for growth response. From these results it is concluded that total androgen suppression is not necessary to prevent growth of the human prostate cancer model PC-82.

Preliminary results from the PC-EW tumor model showed that, compared to the PC-82 tumor, regression after androgen withdrawal was more pronounced and that stimulatory levels of intratumor DHT in the PC-EW were lower.

In addition, the effects of the adrenal androgens dehydroepiandrosterone (DHEA) and androstenedione (A) on the growth of the PC-82 tumor were studied. Substitution of tumor bearing castrated nude mice with Silastic implants containing A and DHEA resulted in peripheral concentrations of 9.2 ± 3.9 and 13.5 ± 2.9 nmol/l, respectively. Growth of the tumor in DHEA implanted mice was not stimulated. Substitution with A led to a peripheral T level of 10.7 ± 2.8 nmol/l, and an intratumor DHT level of 21.3 ± 1.6 pmol/g tissue, causing an increase in tumor burden. It is concluded that levels of adrenal androgens, which lead to plasma-T concentrations exceeding the above-mentioned threshold level for growth stimulation, can play a stimulatory role in growth activation of the human prostatic carcinoma PC-82.
This study was supported by the Dutch Cancer Society through grant IKR 87.8.

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TISSUE SPECIFIC EFFECTS OF PROGESTERONE ON STEROID RECEPTORS IN THE FEMALE RABBIT UROGENITAL TRACT

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High affinity progesterone receptors (K_D = 1-2 nM) could be demonstrated in both cytoplasmic and nuclear fractions prepared from estrogenized rabbit urethra, bladder and vagina. Progeterone binding sites in the bladder had a broader hormonal specificity than those in the urethra or vagina. Since progesterone is known to cause a marked reduction in its own receptors in the uterus, the effect of progesterone administration on progesterone and oestrogen receptors in the uterus, vagina and urethra of rabbits was studied. After 24h of progesterone treatment the concentration of cytosolic progesterone receptors decreased to about 25% of the control value in the uterus, whereas no significant change in receptor concentration was observed in the vagina or the urethra. The concentration of the nuclear progesterone receptor did not change in any of the three tissues studied. The apparent dissociation constant (K_d) of nuclear progesterone receptor increased after progesterone treatment in all three tissues. Although the K_d of the cytosolic progesterone receptor also increased in all tissues, the difference was significant for only the vagina and urethra. The concentration of cytosolic estrogen receptors in the uterus decreased significantly ($p < 0.001$) after progesterone treatment whereas the K_d value increased slightly ($p< 0.05$). In vagina or the urethra, there was no change in either estrogen receptor concentration or K_d values after progesterone treatment. These data clearly show that the reduction by progesterone of progesterone and estrogen receptor concentrations occurs only in the uterus and not in the vagina or the urethra. The reduction in nuclear progesterone receptor affinity after progesterone treatment which was observed in all three tissues of the urogenital tract may have a biological significance.

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INHIBITION OF AROMATASE ACTIVITY BY STEROIDAL AND NONSTEROIDAL COMPOUNDS IN CULTURED HUMAN GENITAL SKIN FIBROBLASTS

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Although it is well documented that the extratesticular "peripheral" aromatization is the major source of estrogen formation in men and recent observations indicate that estrogens might play an essential role with respect to the onset and maintenance of benign prostatic hyperplasia (BPH), no data are available on the effect of potential inhibitors of aromatase in peripheral tissues.
Since fibroblasts cultured from human skin biopsies contain the aromatase-enzyme, we studied the ability of various steroidal and nonsteroidal compounds to inhibit aromatase activity in homogenates of genital skin fibroblasts. Cell homogenates were incubated under standardized conditions with 50 nM ^3H - androstenedione both in the presence or absence of increasing concentrations (10^{-9} - 10^{-5}M) of the compounds to be tested.
Among the steroidal agents tested, 1-methyl-androsta-1,4-diene-3,17_dione could be identified as a very potent inhibitor. This steroid is of particular interest since it will be now used in clinical studies to treat disorders in which estrogens are thought to play a pathogenetic role such as BPH or mammary carcinoma.

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ARE HORMONE RECEPTORS IN RENAL CELL CARCINOMA OF ANY THERAPEUTIC VALUE ?

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Controversies still exist about the value of hormonal treatment in renal cell carcinoma (RCC) and data about steroid receptor contents in RCC are contradictive. The aim of our study was therefore to look for androgen, progestin and estrogen binding in human RCC with accepted methods and, if positive receptors were found, to stimulate or block these receptors in the animal model to evaluate the true biological potential of these findings.
Specimens of 21 RCC were analysed for the presence of androgen, progestin and estrogen receptors by agar gel electrophoresis and a dextran-coated charcoal assay. Receptor-positive tumors were transplanted into NMRI nu/nu mice and subpassaged.
An androgen receptor could be demonstrated in 3 tumors at concentrations of 7 to 19 fmol/mg protein. Progestin and estrogen receptors were not detectable in any of the tumors. Two of the three androgen-positive tumors could be successfully established and subpassaged in the nude mouse model. Tumor growth in these two cases could be enhanced by treatment with 5-alpha-dihydrotestosteroneacetate while therapy with cyproteroneacetate or estrogens or with castration of male mice lead to a significant tumor remission with characteristic histological changes. One corresponding patient with metastatic disease was treated therefore with cyproteroneacetate and acheived a partial tumor response.
In conclusion, our results demonstrate in contrary to those of other authors that only androgen receptors are detectable in a subset of RCC. In the nude mouse model androgen-receptor-positive tumors respond to antiandrogen therapy. Therefore antiandrogen treatment might be effective in a subset of androgen receptor positive patients with metastatic RCC. In contrary, no rational could be found for treatment of metastatic RCC with medroxyprogestinacetate, while therapy with androgens in RCC might even lead to a progression of the disease.

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Androgen- and EGF-Receptors in carcinoma of the prostate

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In the surgical specimens of 15 patients treated by radical prostatectomy the androgen and in some cases the epidermial growth factor (EGF)-receptors were determined.
Comparing tumour stages localized primaries exhibited a higher androgen-receptor content than tumours penetrating the capsule ($pT_{1/2} \hat{=} 65.2$ vs. $pT_{3/4} \hat{=} 37.4$).
No difference could be found regarding the tumour volume (volume $>$ 10 ccm $\hat{=}$ 54.8, volume $<$ 10 $\hat{=}$ 52.4), PSA values (PSA $>$ 20 $\hat{=}$ 46.0, PSA $<$ 20 $\hat{=}$ 52.2) and grading of the tumour ($G_{1/2} \hat{=} 50.6$, $G_3 \hat{=} 46.8$).
In the same way no difference could be found between benign prostate hyperplasia and carcinoma determined in the same specimen.
The median EGF-receptor values are 23.3.
Follow up is to short to correlate these values to disease free survival.

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PROGNOSTIC FACTORS IN PROSTATIC CANCER PATIENTS

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In a continuing study of various endocrine and other factors that might have prognostic value in prostatic cancer patients, we have recently compared the expression of certain antigens in fixed and frozen tissue. These include c-myc protein, c-fos protein, EGF receptor and TGF-β receptor expression and also prostate specific antigen (PSA), prostatic acid phosphatase (PAP) and Ki_{67} expression. Immunocytochemical procedures have been used on samples from the whole populations (85) but comparisons with m RNA expression using Northern Blot analysis has been undertaken with a proportion of the samples. EGF receptor expression as assessed by using 3 different monoclonal antibodies appears to be located in a variety of epithelial and mesenchymal elements within the tumour sample and should be considered in relation to assays using lignand binding. To date the Ki_{67} antigen expression relates to grade and patient survival. PAP expression was heterogeneous in many samples and correlated with grade whereas PSA although varied in intensity did not correlate with histopathological grade.

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INVOLVEMENT OF EPIDERMAL GROWTH FACTOR AND ITS RECEPTOR IN IMPLANTATION AND GROWTH OF HUMAN PROSTATIC CARCINOMA CELL LINES, TRANSPLANTABLE INTO NUDE MICE.

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Heterotransplantation of human prostatic carcinoma tissue into athymic nude mice has proven to be a very significant approach for studying the properties and growth requirements of human prostatic cancer tissue in vivo. The epidermal growth factor receptor (EGFr) concentration was estimated in membrane preparates of 6 transplantable human prostatic cancer cell lines in vivo, using scatchard plot analysis. The EGFr was detectable in all (androgen dependent and independent) cell lines with concentrations that varied from 6-60 fmol/mg protein. Preliminary experiments showed that the concentration of EGFr in the hormone dependent PC-82 tumor might be regulated by androgens.
The hormone independent tumor PC-135, which contained around 30 fmol/mg protein EGFr (Kd: 0.63 nM), was used as model for studying the the possible role of EGF in transplantation and growth of human prostate cancer tissue in nude mice. PC-135 tumor tissue was grafted in male mice and female mice (having reduced circulating EGF levels) and in mice that were sialoadenectomized (removal of the submandibular glands), which also results in diminished circulating levels of EGF. The take rates of PC-135 tumor tissue at 40 days after transplantation in female (50%) and in sialoadenectomized male mice (58%) were significantly retarded compared to the acceptance of tissue in male nude mice (92%). It appeared that tumors once developed did not differ with regard to their respective growth rates. Experiments with EGF substitution in transplanted mice are in progress, as are EGFr estimations in tumor tissues of the EGF manipulation experiments. The present results indicate that EGF is involved in the take rather than in the growth rate of the PC-135 human prostatic carcinoma tissue in nude mice.

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STRUCTURE AND EXPRESSION OF C-MYC PROTO-ONCOGENE AND GENE FOR ANDROGEN RECEPTOR IN HUMAN BLADDER AND PROSTATIC CANCERS

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Recurrence and invasion of superficial bladder carcinoma is scarcely predictable in individual patients and several efforts have focused on finding new indices of invasive tendency. Recently,the recombinant DNA techniques, which allow to dissect the genome of cancer cells and to compare it to the genome of a normal cell, provide new tools to identify possible DNA alterations specifically associated to neoplastic cells and to tumor progression. Evidence exists that DNA hypomethylation is frequently associated with neoplasia. However, a significant correlation between extent of DNA hypomethylation and stage and grade of the disease has never been found. We have studied the methylation at the CCGG sequences in the 3' end of the c-myc proto-oncogene in bladder cancers. Semples from 30 patients, 7 normals and 23 neoplastic bladders at different stage and grade were considered. The overall c-myc methylation levels were significantly reduced in carcinomas. Moreover, 5 CCGG sites showed different methylation degree in the various neoplastic samples.A significant correlation between disease invasivness and methylation level at specific sites was found. However, in each group of patients with the same hystological grade, heterogeneity has been observed. Evaluation of the patients at 24 month follow-up showed a possible correlation between degree of methylation and recurrence of the disease, not in the advanced, but only in the early phases of the disease. This may be relevant for the therapeutic care decision. The recent availability of the molecular DNA probes for the androgen receptor gene in our lab has allowed to perform similar studies in prostatic cancers. In addition, levels androgen receptor RNA will be evaluated both in hyperplastic and neoplastic prostatic samples. The procedures employed in the last studies and relative preliminary results will be presented.

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EPIDERMAL GROWTH FACTOR RECEPTORS ARE PRESENT ON BOTH NORMAL AND CANCEROUS HUMAN TESTICULAR TISSUE.

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Epidermal growth factor receptors (EGF-Rs) are known to be present on many types of tissue. Although EGF-Rs have been found in rat leydig cells no one has so far established their presence in human testicular tissue.

The results we have obtained using a radioligand exchange assay with ^{125}I as our radiolabel, indicate that EGF-Rs are present on normal tissue with a single high affinity binding site (Kd = 3.27 + 0.59 x 10^{-10}M). The receptor concentration ranged from 0.07-0.21 x 10^{-12} moles/mg of protein. Competition studies with other peptide like substances have also confirmed the specificity which EGF has for its receptor.

Immunohistochemical studies using the streptavidin-biotin technique clearly show that the EGF-Rs are located in the interstitial tissue and not within the seminiferous tubules. The molecular weight of the receptor was also obtained by crosslinking studies yielding a value of 125 kDa which is thought to represent a proteolysed form of the binding site.

With respect to cancerous tissue, EGF-R's were not detected in seminoma tissue but were found on teratoma tissue at a decreased number. Immunohistochemistry was also performed to confirm the previous biochemical results. In the case of the teratoma tissue the EGF-Rs were located in specific areas of the tumour at a much higher density to that observed in the normal human testicular tissue.

From this study we have therefore established that EGF-Rs are expressed in some cancerous testicular tissues. With respect to their presence in normal testicular tissue we are presently investigating the role which EGF may play in steroidogenesis.

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OPPOSITE MODULATION OF GENE EXPRESSION OF TGF-α AND EFG IN RENAL CARCINOMAS: CORRELATION WITH TUMOR PROGRESSION

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Polypeptide growth factors and their receptors play an important role in tumor formation and progression. Epidermal growth factor (EFG) and its embryonic counterpart transforming growth factor-(TGF)-α are involved in the regulation of proliferation and differentiation of epithelial cells. Since prepro EGF mRNA is expressed in murine kidneys and since the cDNA for TGF-α was originally isolated from a renal carcinoma cell line, we have decided to study human renal carcinomas for the expression of mRNA coding for TGF-α and EGF, resp. as well as for the EGF/TGF-α receptor using the Northern blotting technique. Tumor tissue was obtained by nephrectomy from more than 40 patients and frozen in liquid nitrogen until use. All renal carcinomas were found to express a specific 4.8 kb TGF-α mRNA. For comparison, adjacent normal tissue from the same kidney was tested. Although the TGF-α gene was expressed in some of the normal renal tissue samples, the degree of expression was significantly higher in tranformed regions of the kidney. Correlation with histological analysis of the tumor sample showed that quantitative differences in TGF-α expression correspond with tumor progression and differentiation, undifferentiated carcinoms. When the same tissue specimens were assayed for the expression of EGF- and EGF-receptor mRNA, the 5 kb-EGF-mRNA was found in all normal tissues but could not be identified in any of the renal carcinomas. EGF-receptor expression was increased in the majority of the renal carcinomas We speculate that the down-regulation of EGF-expression with the concominant up-regulation of TGF-α expression reflexts the switch from an adult to an embryonic growth factor for growth regulatory processes. Supported by W. Sander & Boehringer Ingelheim Fond.

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EPIDERMAL GROWTH FACTOR RECEPTOR IN ADENOCARCINOMA OF THE KIDNEY

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Reports on epidermal growth factor(EGF)involvement in malignant growth of different cancers indicate an important role of this peptide in development of normal and malignant tissue. As little is known about the factors influencing the growth of adenocarcinomas of the kidney and as reports on the response of hypernephromas to steroid therapy are somewhat controversial, we initiated studies to lay ground for possible new pathways of diagnosis and treatment of this carcinoma. No tumor markers are currently available to detect the adenocarcinoma of the kidney at an early stage.

Reports on amplified oncogenes such as N-myc becoming indicators of the stage of tumor growth in neuroblastoma and as well as an oncogene amplification in lung tumors indicate the importance of oncogenes and theirproducts as potential tumor markers.

As we show here a significantly higher expression of EGF-receptor in adenomatous carcinoma tissue in the kidney compared with the surrounding presumably normal tissue we suggest that the EGF receptor may be a useful tool in determining the stage of the tumor. Another finding was the low level of EGF-receptor found in urothelial carcinoma which had invaded the surrounding tissue. Serum EGF was tested as an indicator of progress of tumor growth. There was no difference between pre- and post-surgical serum EGF-levels. Furthermore we studied Tumor Necrosis Factor levels and tried to find a correlation to the increased EGF-receptor levels. No such correlation could be found. It is known that steroid dependent tumors such as breast cancer contain EGF-receptors in an inverse correlation to steroid-receptor levels. We found no such correlation between EGF- and androgen-receptor concentration.

Further work will be required to establish the value of the EGF-receptor as a diagnostic tool in determining tumor stage in a less subjective manner than that provided by current methodology.

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DETECTION OF ACIDIC FIBROBLAST GROWTH FACTOR A FGF IN URINE OF PATIENTS WITH TRANSITIONAL CELL CARCINOMA (TCC) OF THE BLADDER AS A POTENTIAL TUMOR MARKER

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Several lines of investigations indicate a potential role of fibroblast growth factor related gene producte in the biological behavior of urothelial tumors. Heparin binding mitogenic activity has been found in human and murine TTC. Oncogenes related to FGF gene family have been isolated from human bladder tumor cell lines. An enzyme immunoassay (EIA) specific for Acidic Fibroblast growth factor has been developped (Anal. Bioch. 1988, 173, 328). Aliquots from 24 hours urine collection were assayed on 351 patients for detectable levels of aFGF. The EIA was positive in 7% of 56 controls ; 13,5% of 111 patients with benign prostatic hypertrophy ; 23,3% of 60 patients with metastatic carcinoma of the prostate ; 19% of 42 patients with history of TCC and no detectable tumor ; 0% of 6 TA TTC ; 38% of 21 T1 TTC ; 16% of 12 patients with non metastatic renal cell carcinoma (RCC) and 66% of 6 M + RCC. Statistical nalysis of these date indiates that detection of FGF related immuno-reactivity in urine of patients with TCC may be useful for the non invasive follow up this disease.

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HER-2, (ERB-B2, NEU, MAC117) AMPLIFICATION AND EXPRESSION IN TRANSITIONAL CELL CARCINOMA.

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The oncogene HER-2 is a presumed growth factor receptor first isolated in ethylnitrosurea induced glioblastomas in rats. Amplification of HER-2 has been reported in several tumour types and proposed as a good prognostic indicator in breast carcinoma equivalent to lymph node status.

Over a two year period 80 cases of transitional cell carcinoma (GI 24%, GII 47%, GIII 25%, C.I.S. 4%) have been analysed, 25 on two or more occasions. DNA from patients' lymphocytes and 'normal' urothelium and 22 other intravesical pathologies (cystitis, catheter trauma, schistosomiasis) have been used as controls. All gels for Southern analysis were ethidium stained to assess loading. They were subsequently reprobed for p53 a single copy gene on the same chromosome (but opposite telomere) to assess loading further and exclude aneuploidy. Autoradiographs were scanned with an L.K.B. laser densitometer linked to an I.B.M.-A.T. computer but this rarely conferred any advantage over naked eye comparison.

Amplification was found in 1/20 grade I, 4/38 grade II, 5/20 grade III tumours and one case of carcinoma in situ. The highest level of amplification was found in a grade I tumour. Amplification developed in 2 cases during the course of the study. DNA was also extracted from cells voided in urine in 5 cases prior to B.C.G. treatment for carcinoma in situ. One of these showed amplification.

Levels of mRNA were measured by Northern analysis (35 cases) and these showed correlation with gene amplification. One case showed a doubling of transcript level as its clinical behaviour and grading deteriorated. Sampling ceased in 1988 and the one year clinical follow-up is awaited with interest.

This data suggests that HER-2 amplification may be a useful specific prognostic marker in bladder cancer.

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EPIDERMAL GROWTH FACTOR (EGF) - RECEPTOR IN UROTHELIAL CARCINOMAS

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Growth factors and their receptors have recently attracted much interest in oncologic research for their role in normal and neoplastic growth. Receptors of Epidermal Growth Factor (EGF) could be detected in various human tumours by immuno-staining methods. The human urothelial tissue is exposed extensively to the EGF of the urine. On the other hand an increase of proliferation in the cultured urothelium after EGF stimulation is well known. The relation between the invasive potency of the tumours and the detectability of EGF-receptor in urothelial neoplasias is therefore of particular importance.

We examined the tissues of 25 urothelial carcinomas with the monoclonal antibody (mAb) EGFR1, directed against the EGF-receptor (EGFR). The detectability of the EGFR decreased from the G1 through G2 to G3-tumours.
In the papillary carcinomas more EGF-receptor was found than in the invasive carcinomas. Increasing areas consisting of EGFR negative cells were observed in grade 3 tumours; in several cases there was even no EGFR-reactivity in the whole neoplastic material. Similarly, the invasive carcinomas showed less EGFR positive areas than papillary tumours.

Conclusion: The EGF-receptor seems to undergo certain modification in the course of dedifferentiation of urothelias carcinomas. Some additional mediators are possibly also required for growth and invasive capability of urothelial neoplasias.

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PRODUCTION OF TRANSFORMING GROWTH FACTORS AND RESPONSE TO EXOGENOUS GROWTH FACTORS BY THE PROSTATIC CARCINOMA CELL LINE DU 145.

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The secretion of growth factors by some transformed cells is thought to enable these cells to proliferate in low serum concentrations as well as reducing the dependency upon exogenous growth factors. Using an androgen independent prostatic carcinoma cell line (DU 145), we characterised the production of molecules with transforming growth factor (TGF)-like activity. The expression of EGF receptors and the response to exogenous EGF were also examined.

Serum-free medium conditioned by these cells showed TGFα activity, using a radioimmunoassay for TGF, as well as competing activity in EGF radioreceptor assays. Concentrated conditioned medium was further purified by reverse-phase high performance liquid chromatography (rHPLC). We found one peak of TGFα -like activity, as well as several other peaks which were immunologically distinct from TGF, but demonstrated competitive activity in EGF radioreceptor assays. Measurement of EGF receptors by competition and saturation analysis revealed high levels of receptors ($2.5 \times 10^5 + 1 \times 10^5$) with one high affinity binding site (1nm + 0.5). In contrast to other carcinoma cell lines, exogenous EGF had no significant effect on ^{3}H-thymidine incorporation.

There remains the possibility that this carcinoma cell line has by producing its own growth factors, little or no need for exogenous growth factors. The levels of factors produced may be auto-stimulatory, although this remains to be determined.

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Life span of stromal and epithelial prostatic cells

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The development of benign prostatic hyperplasia progresses over decades. Since tumor growth is characterized by an increasing cell proliferation rate and/or a decreasing cell death rate, we were interested in evaluating the mean life span of prostatic cells, which is an indirect parameter for the mean cell death rate in a tissue. For this purpose, we measured the activity of superoxide dismutase (SOD) which is known (a) to correlate with life span of cells and (b) to decrease with advancing age of cells. For evaluation of life span the SOD values in prostatic stroma and epithelium were compared with those of the postmitotic skeletal muscle.
SOD activities were determined by inhibition of autoxidation of pyrogallol (4,7 mM). The enzyme activity was calculated from the difference in the increase of absorbance at 420 nm with and without adding stromal or epithelial homogenates of human normal and hyperplastic prostates as well as homogenates of human skeletal muscle from donors aged 20 to 86 years.
The main results were: (1) In skeletal muscle, the mean SOD-values (4,0 mU/mg protein; 5,0 mU/µg DNA) were significantly (p at least < 0,005) higher than in prostatic stroma (2,1 mU/mg protein; 1,7 mU/µg DNA) and epithelium (1,4 mU/mg protein; 0,2 mU/µg DNA). (2) In skeletal muscle as well as in prostatic stroma and epithelium,a significant (p at least < 0,005) age related decrease of SOD activity was found. The slope of the age related regression lines was highest in skeletal muscle and lowest in epithelium. (3) The initial SOD activity, i.e. the activity in tissue homogenates of rather young donors was highest in skeletal muscle and lowest in epithelium.
The comparison of SOD values between skeletal muscle and prostatic stroma and epithelium led to the conclusion that the average life span of stromal cells is longer than 30 years, of epithelial cells longer than 2 years. Thus, development of benign prostatic hyperplasia is characterized by a rather low cell death rate, at least in the stroma.

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ABERRANT EXPRESSION OF RAF AND ABL IN RAT PROSTATIC CANCER CELLS

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An important molecular approach to study the complex processes implicated in prostatic carcinogenesis, is to determine steady state mRNA levels of potentially relevant genes. One important category are the proto-oncogenes. Previous studies on involvement of oncogenes in prostatic carcinogenesis, using primary human prostatic tumors, have shown that ras and myc might be related to the progression of this disease. We tested this hypothesis in the Dunning R-3327 rat prostatic cancer model system that consists of a series of sublines that represent the different stages of tumor progression in prostatic cancer. We were unable to show a correlation between the expression of the ras and myc proto-oncogenes and the state of progression of the disease. Expression levels and patterns of other proto-oncogenes, however, appeared to be more useful as marker for malignancy and/or progression of rat prostatic cancer. The raf proto-oncogene appeared to be overexpressed in anaplastic lines. Furthermore, an aberrant or raf related transcript of 2.0 kb was found in all Dunning tumors, whereas this specific transcript was not detectable in the normal prostate. Also, Northern blot analysis using a 5' v-abl probe revealed an aberrant 2.1 kb abl homologous transcript which was not detected in the normal prostate. The nature of these aberrant transcripts is not elucidated yet, nevertheless they can be useful markers to study carcinogenesis.

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EXPRESSION OF HIGH MOLECULAR WEIGHT FORMS OF bFGF IN RAT TUMOR CELLS.* Heinz-W. Goebel, Gerhard Aumüller and Ulrich Rausch, Dept of Anatomy and Cell Biology, Robert-Koch-Str.6, 3550 Marburg, FRG

INDRODUCTION: It has been suggested that factors other than sex hormones play an important role in normal and abnormal growth of prostatic cells. The physiologic function of these growth factors is only partially understood. In this study, both the transplantable Dunning R3327 rat tumor model system and the dorsolateral rat prostate (DLRP) were used as a tool to define the pre-sence and identity of rat prostatic growth factors (RPGF).

METHODS: Frozen Dunning tumor (AT3) and DRLP were homogenized and the protein fraction between 1,9M and 3,8M $(NH_4)_2SO_4$ precipitation was collected. Samples were dialyzed and purified with affinity chromatografy (anti-bovine bFGF IgG). The bound protein was eluted with 2M NaCl, separated with SDS-PAGE and blotted onto nitrocellulose sheets. After blocking the unspecific binding sites, immunodetection of bFGF-like proteins was performed using a polyclonal rabbit antibody raised against purified bovine pituitary bFGF(1).

RESULTS: Western blot analysis of bFGF enriched extracts of the AT3 Dunning tumor and DLRP tissue showed besides the previously reported 18-19kD RPGFs, additional staining of 24kD, 30kD, and 46kD immunoreactive proteins. The 46kD protein is the predominantly band, containing 90% of the immunoreactive material in both tissues.

DISCUSSION: In addition to described 18-19kD RPGFs and other closely related heparin-binding growth factors (HBGF) we found high molecular forms of bFGF-like growth factors in both normal and transformed rat prostatic tissue. These large forms exert trophic activities as recently shown with bovine tissue preparations(1). It is not clear yet, whether these high molecular forms are precursor molecules, nor the low molecular forms truncated growth factors either.

1. Grothe C, Zachmann K, Böhlen P, Unsicker K, Westermann R 1989 High molecular weight forms of basic fibroblast growth factor recognized by a new anti-bFGF antibody. FEBS-Lett (in press)

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REGULATION OF GROWTH OF LNCaP HUMAN PROSTATE TUMOR CELLS BY GROWTH FACTORS AND STEROID HORMONES.

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The mitogenic activity of several growth factors on androgen responsive LNCaP human prostate tumor cells was studied in 6 day cultures. Basic FGF (20 ng/ml) -but not acid FGF (up to 50 ng/ml)- stimulated cell proliferation twofold (increase in DNA content per culture versus control cultures). A twofold stimulation was also observed at 1 ng EGF/ml or 10 ng TGF-alpha/ml. Interestingly, TGF-beta (0.02 ng/ml), which did not affect cell proliferation when added alone to the culture medium, inhibited the EGF and TGF-alpha induced growth. Addition of PDGF, insulin or IGF I, did not affect cell proliferation compared to controls.

The synthetic androgen R1881 (0.1 nM) stimulated cell proliferation three- to fourfold. TGF-beta did not affect androgen stimulated growth. However, EGF acted synergistically with androgen on LNCaP cells to induce growth (sevenfold). This synergistic effect of androgen and EGF was inhibited by TGF-beta. Androgens increase the number of EGF receptors from 11500 to 28500 sites/cell. LNCaP cells are thus sensitive to EGF (TGF-alpha) and TGF-beta decreases the growth response to EGF. Androgens increase the growth response to EGF, probably due to up-regulation of EGF receptor levels.

Progestagen and estrogen receptors are not detectable in LNCaP cells. However, progesterone (1 nM) and estradiol (10 nM) can stimulate LNCaP cell growth and EGF receptor levels. These steroids compete with androgen for binding to the androgen receptor and their affinity for the androgen receptor parallels the effects on cell growth. Finally, antiandrogens do not inhibit androgen responsive growth of LNCaP cells. Both cyproterone acetate (100 nM) and anandron (100 nM, RU 23908) have striking growth stimulatory effects and increase acid phosphatase secretion.

We conclude that LNCaP cells contain a modified androgen receptor system both with respect to steroid specificity and anti-androgen sensitivity. One of the mechanisms involved in the hormonal stimulation of the cells is an increase in the number of EGF receptors.

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INTERMODULATION OF GROWTH FACTOR AND STEROID HORMONE ACTIONS IN NORMAL AND NEOPLASTIC PROSTATIC CELL LINES.

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Interrelationships that undoubtedly exist in the activities of temporal factors (hormones and growth factors) transiently or continuously expressed and responded to in prostatic tissues are at present poorly defined and are clearly highly complex. It is also becoming apparent that interdependencies between these classes of growth regulators may differ in normal growth processes from those involved in tumour growth with the inference that such alterations may be directly involved in oncogenisis. Using a series of cell lines derived from human prostatic tumours (LnCap., Du145, PC3) and from normal (CAPE 1) and neoplastic (CPA 1) canine tissues, we have examined growth responses to androgens, glucocorticoids, EGF, TGFs α and β, insulin and IGFs I and II in both serum supplemented and serum free medium. In addition we have evaluated growth factor receptor expression in the presence of these agents measured by both ligand binding (whole cell and membrane) assays, immunocytochemistry and by hybridisation analyses of ^{m}RNA expression using cDNA probes applied to Northern blots. In serum free medium the effects of these growth modulators were well defined with EGF, TGF$^{\alpha}$, insulin and IGF_I being significantly mitogenic in all the cell lines examined. These effects were either abolished or very significantly attenuated by the presence of serum. Androgens and glucocorticoids had no effect on EGF receptor expression in the androgen sensitive cell lines (CAPE 1 and LnCap.) grown in serum free medium and did not augment growth responses to EGF or TGFα. Our results suggest complex pathways for androgenic regulation of EGF receptor concentrations in androgen dependent prostatic cell populations involving a number of interacting factors, including the potential presence of autocrine ligands and serum factors.

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POLYAMINE REQUIREMENT FOR CANCER CELL PROLIFERATION.

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Polyamines, either provided by the novo synthesis from ornithine or by uptake from the environment via an active polyamine transport system, play an essential role in cell proliferation. Numerous in vitro studies have demonstrated that growth is generally arrested in the presence of polyamine biosynthesis inhibitors such as a-difluoromethylornithine (DFMO). We have studied the ability of various polyamines and derivatives to restore growth under conditions where the novo synthesis was blocked by DFMO. These experiments were performed using PC-93 prostate cancer cells, depleted of polyamines by exposure to 1 mM DFMO for 3 days. Such treatment resulted in complete suppression of endogenous putrescine (Pu), substantial reduction of spermidine (Sd) levels and slight elevation of spermine (Sm). Cell growth upon culturing in the presence of different polyamines was quantitated by means of a colorimetric test (MTT-assay). Growth rates were fully restored to normal by Sd, Sm, N1-acetyl-Sm, N1-acetyl-Sd, Pu and N8-acetyl-Sd (Sd being the most effective compound), but not by acetyl-Pu. Analysis of intracellular polyamines showed that re-establishment of growth was associated always with (largely) restored Sd levels. Sm and acetylated polyamines were apparently converted to Sd via polyamine interconversion pathways, involving acetylases (SAT) and polyamine oxidase (PAO). Subsequent experiments showed that blocking polyamine interconversion by a PAO-inhibitor prevented restoration of growth and of endogenous Sd levels by acetyl-ated compounds. These results demonstrate the critical involvement of Sd in cell proliferation and the importance of polyamine metabolism in regulation of intracellular Sd levels. Effective application of polyamine synthesis inhibitors in vivo should therefore include the elimination of polyamine uptake and/or interconversion

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REVERSIBLE ANTIPROLIFERATIVE EFFECTS OF SURAMIN ON ANDROGEN RESPONSIVE CELLS IN CULTURE.

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Suramin, a polyanionic compound used in the treatment of trypanosomiasis, probably acts through inhibitory effects on growth factor mediated cell proliferation. The effect of suramin on proliferation of LNCaP cells (human prostate tumor) and DDT-1 cells (hamster ductus deferens tumor) was studied. Both tumor cells contain androgen receptors and show androgen responsive growth. Addition of 1 to 10 nM testosterone (T) maximally stimulated proliferation of DDT-1 cells (3- to 7-fold). DDT-1 cells secrete immunoreactive PDGF. Addition of 1 to 10 ng PDGF/ml or 0.1 to 10 ng bFGF/ml to the culture medium stimulated DDT-1 cell proliferation to the same extent as the androgen. Addition of T to the medium containing 10 ng PDGF/ml or 0.1 to 10 ng bFGF/ml did not further increase cell proliferation, illustrating that these growth factors can replace the requirement for T. Suramin (100 uM to 1 mM) inhibited both basal and T stimulated cell growth (6 to 7 day cultures), suggesting that the growth is mediated by secretion of growth factors that act in an autocrine way. Suramin (100 uM) completely inhibited the ability of PDGF and partially of bFGF to stimulate cell growth. Lower concentrations of suramin stimulated both cell growth and detachment of cells.

Addition of 1 nM dihydrotestosterone or 0.1 nM R1881 (a synthetic androgen) to LNCaP cells stimulated cell growth 3- to 4-fold (6 day cultures). A 2-fold stimulation was observed with 1 ng EGF/ml. Cells were cultured in the presence or absence of 0.1 nM R1881 or 1 ng EGF/ml, with or without suramin (10 uM to 1 mM). 1 mM suramin completely blocked the androgen and growth factor induced growth. At 100 uM suramin half maximally inhibited growth stimulatory effects of EGF. Suramin decreased 125I-EGF binding to LNCaP cells. This indicates that suramin either competes with EGF for binding to the EGF receptor or binds EGF and so prevents interaction of EGF with its receptor. The antiproliferative effect of suramin on both DDT-1 and LNCaP cells was reversible. Cells cultured in the presence of suramin for 6 days were androgen and growth factor responsive following addition of fresh medium.

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PARTIAL CHARACTERIZATION OF PROSTATIC EPITHELIUM INHIBITING FACTOR.

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Stromal cells from several different tissues are known to produce both mitogenic and growth inhibiting factors. In previous papers we reported the modulation of the clonal growth of prostatic carcinoma cell lines by prostatic fibroblasts in vitro. The putative inhibiting factor was shown to be present in conditioned medium (CM) of such fibroblasts and referred to as Prostatic Epithelium Inhibiting Factor (PEIF).

The present study aimed at further characterization and purification of this factor. PEIF-activity accumulated in the medium covering confluent monolayers of prostatic fibroblasts but not of skin fibroblasts. This production was obligatory dependent on the presence of bovine serum albumin (or fetal calf serum) in the medium. The effect of PEIF on prostate-cancer cells was tested by means of the MIT-test using the PC-3 cell line plated at a density of 2,000 cells/well. By the use of such tests a dose-dependent inhibition of PC-3 cell growth was demonstrated. The factor appeared to be heat-labile: heating for 5 minutes at temperatures over 70°C resulted in a gradual decrease in activity; at 100°C all inhibition was lost. By performing ammonium sulfate precipitation according to Dixon, the pellet formed between 40 and 50% saturation level contained nearly all PEIF-activity. At this point 70% of the total amount of protein present in the CM stayed in solution. SDS-PAGE on 15% gels under reducing conditions showed a clearly defined band at approximately Mr 10,000 in addition to a large amount of serum albumin. This 10 kDa band was not present in all other fractions obtained by stepwise increasing the saturation level. This molecular size is quite similar to that of other growth modulating factors.

At this stage we have gathered proof that PEIF has a small molecular weight of approximately 10 kDa. Because it is heat-labile too it is very likely to be distinct from known growth inhibitors such as TGFβ. Further efforts will be made to disclose the identity of PEIF as well as to unravel the intriguing role serum albumins seem to play in its production.

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USE OF POLYMERASE CHAIN REACTION FOR THE ANALYSIS OF ONCOGENE AND ANDROGEN RECEPTOR EXPRESSION IN CULTURED CELLS, PROSTATE AND BLADDER SPECIMENS.

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The Polymerase chain reaction (PCR) is a powerful method in the analysis of DNA or RNA sequences from minute amounts of starting material. Using two specific oligonucleotide primers the sequence between them is amplified exponentially by the thermostable DNA Polymerase Taq I. The amplified DNA sequence can subsequently be analysed by restriction endonucleases and hybridization techniques.

To investigate the role of androgenreceptor and oncogenes in development and progression of prostate carcinoma and bladder cancer we established amplification procedures for androgen-receptor, fos and Ha-ras specific sequences starting from RNA of cultured cells and used these techniques to measure expression of androgenreceptor and c-fos in prostate biopsies and expression of Ha-ras in bladder biopsies and bladder cells obtained by bladder washings.