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

These stages are involved in development of human gene therapy, (i) the transduction by viral vectors or transfection by DNA-mediated systems of appropriate target cells, (ii) enhancement of prostate-specific gene expression, and (iii) the feasibility assessment of different effector genes to direct "suicide" or proliferation control of the targeted prostate cancer cells. To test these alternatives, we have standardised in vitro and in vivo models: human prostatic epithelial and fibroblastic cells (Int. J. Oncol. 6: 333-343, 1995), human-rat xenografts and non human primates. By using recombinant adenovirus expressing the gene reporter β-galactosidase (rAd-RSV-βgal) and the suicide gene thymidine kinase (Ad- HSV-tk), we have tested in vitro the efficiency of transduction as well as the toxicity of the β-galactosidase treatment. In primary cultures of human prostatic cancer epithelial and fibroblastic cells, we observed transduction of the reporter gene in 100% of the epithelial cells with 100 virus particles per cell, while the fibroblastic cells displayed sparse staining. This epithelial selectivity to RAd was further confirmed on the immortalised human prostatic cancer cells lines PNT2 and PNT1A, known to be extremely insensitive to transfection. Confirmation of this selectivity has been carried out in human prostate cancer xenograft on nude mice while tk toxicity was assayed in vitro and in vivo.

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

**P 18**

**POLYAMINE METABOLISM AND PROGRAMMED CELL DEATH IN PROSTATE CANCER CELLS**

R. Schipper, A. Otten, R. Rutten, W. Lang, J. Schalken, A. Verhoefstad Pathology, Urology, University Hospital Nijmegen, P.O. Box 9101, 6500 HB Nijmegen

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

**P 19**

**GENERATION OF ANTI-IDIOYPE MONOCLONAL ANTIBODIES RELATED TO PSA**

U. Umero, H. Kitagawa, H. Oono, S. Hirao, Y. and Okiyama E.


As a consequence of the idiotype network theory it has been suggested that high molecular weight anti-idiotypic antibodies must be able to stabilize for the normal antigen. Therefore, the development of monoclonal anti-idotype antibodies (Abα) bearing the internal image of a tumor-associated antigen (TAA) is of great interest.

Prostate specific antigen (PSA) has proven to be useful as an indicator for disease progression and response to treatment in prostate cancer (PCA). Since tumors derived from other organs do not show PSA expression, PSA has been successfully used as a marker in PCA patients. However because of PSA production from normal prostatic cells, a number of false positive has been observed in patients with BPH. Moreover, recent investigations revealed that several molecular forms, i.e., free-PSA, complexed-PSA, exist in the serum and the seminal fluid.

Biological activities of these have not been clarified, i.e., the regulation of PSA activity. These characteristics make this antigen a good candidate for anti-idiotypic approach.

For generation of Ab2, anti-PSA mouse MAb IgG1-kappa(Ab1) was cross-linked to Keyhole Limpet Hemocyanin and used as immunogen. BALB/c mice were immunized with Ab1-k-HA, which specifically reacts with PSA and their splenocytes were fused with SP2/0 myeloma cells. In 3 fusions, 6 anti-idiotypic antibodies specifically reacting with anti-PSA MAb were isolated from more than 300 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAb binding pocket and for the C2 (outside pocket) of IgG1. Furthermore, these Ab2 were tested in Western blots to detect the reactivity with reduced and non-reduced anti-PSA MAb IgGs. All Ab2 showed clear binding to anti-PSA MAb in nodal, leukemic, and breast cancer cells, but not in dermal fibroblasts, indicating that Ab2 recognize a conformational epitope compromised of heavy and light chain, and not a stuctural epitope outside anti-PSA MAb binding site. It has also initiated the functional characterization of these Ab2, i.e., whether they are able to induce antibodies capable to compete with the parental anti-PSA MAb. For the generation of anti-idiotypic antibodies (Ab3) resembling Ab1 (Ab1-1), NZW rabbits were immunized with MAb2. Sera from rabbits immunized with Abα showed reactivity with PSA but not with negative-control cell lysates, indicating Ab1 induction. In summary, we isolated 2 idiotype MAbs that appear to bear the internal image of PSA. Such Ab2 can be tettled for PSA enzymatic activities and therapeutic potential. We are currently performing cross-blocking RIA to determine whether these Ab2 recognize different epitopes in anti-PSA MAb binding pocket.