The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/20881

Please be advised that this information was generated on 2018-10-19 and may be subject to change.
P 17
TARGETING GENE THERAPY TO PROSTATE CANCER
(Enrique Casasola1, Martim Andrawiss1, Michel Perricaudet1 and Philippe Berthon1)
1Département de Recherche en Urologie, Hôpital Saint Louis, 75475 Paris Cedex 10, France, Laboratoire de Génétique des Virus Oncogènes, URA 1301 CNRS, Institut Gustave Roussy, 94805 Villejuif Cedex, France.

No prostate cancer treatment has proven to be as efficient as soon as the tumor becomes invasive. It is thus 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 the development of a human gene therapy, (i) the transduction by viral vectors or transfection by DNA-mediated systems of appropriate target cells, (ii) the 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 Urol Oncol 3: 333-343, 1995), human-rodent xenografts and non human primates. By using recombinant adenovirus expressing the gene reporter β-galactosidase (β-gal), and the suicide gene thymidine kinase (HSV-Tk) we have tested in vitro the efficiency of transfection as well as the toxicity of the β-gal/ganciclovir treatment. In primary cultures of human prostatic cancer epithelial and fibroblastic cells, we observed a transduction of the reporter gene in 100% of the epithelial cells with 100 virus particles per cell, while the fibroblastic cells displayed scattered staining. This epithelial selectivity to β-gal was further confirmed on the immortalized human prostatic 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 β-gal 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 19

As a consequence of the idiotype network theory it has been suggested that intensive immunisation with anti-idiotypic antibodies must be able to substitute for the normal antigen. Therefore, the development of monoclonal anti-idiotypic antibodies (Abs) 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. However biological advices 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-KH , 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 3000 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAb binding pocket and not for the C2 (outside pocket) of IgG. Furthermore, these Abs were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAb IgG. All Abs showed clear binding to anti-PSA MAb in nodules. Western blots with sera from patients with carcinoma showed no reactivity with these Abs, i.e., whether they are able to induce antibodies capable to compete with the parental anti-PSA MAb IgG. For the generation of anti-idiotype antibodies (Ab3) resembling Ab1 (Ab1-I), NZW rabbits were immunized with Ab1-I and as a control Ab2. From a serum pool of 12 rabbits, Ab2 showed reactivity with PSA but not with antigen-negative control cell lysates, indicating Ab1-I induction.

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

P 18
POLYAMINE METABOLISM AND PROGRAMMED CELL DEATH IN PROSTATIC CANCER CELLS
R. Schipper, A. Otten, R. Rutten, W. Langs, J. Schalken, A. Verhohfstad 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 cells can be triggered (by calcium ionophores) to undergo apoptosis, i.e., the tumor becomes invasive. It is thus of some importance to design a 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 the development of a 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. Urol. Oncol. 6: 333-343, 1995), human-rodent xenografts and non-human primates. By using recombinant adenovirus expressing the gene reporter β-galactosidase (β-gal, rAd-RSV-βgal) and the suicide gene thymidine kinase (HSV-Tk) we have tested in vitro the efficiency of transfection as well as the toxicity of the β-gal/ganciclovir treatment. In primary cultures of human prostatic cancer epithelial and fibroblastic cells, we observed a transduction of the reporter gene in 100% of the epithelial cells with 100 virus particles per cell, while the fibroblastic cells displayed scattered staining. This epithelial selectivity to β-gal was further confirmed on the immortalized human prostatic 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 β-gal 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 20
PROSTATE TUMOR GROWTH ENHANCEMENT AND MODULATION OF NATURAL KILLER CELL ACTIVITY BY TRANSFORMING GROWTH FACTOR BETA 1 (TGF β1). Hans E. Contrator and Evelyn R. Barrack, Baltimore, MD. (Presented by Dr. Contrator)

Engineered TGF- β1 overexpression in prostate cancer cells (Dunning subline R3327 MATLyLu) causes significantly larger tumors than parental MATLyLu (MLL) tumors. Importantly, TGF- β1 over-producing (TGF-OP) and control tumors both have the same doubling time. Flow cytometry revealed that both tumor types also had the same percentage of cells in S-phase. Therefore TGF-β1 overexpression affects tumor size without affecting the rate of proliferation or growth. However, in T-cell deficient animals like nude mice or nude rats no size differences between TGF-OP and MLL tumors after inoculation of 5x105 tumor cells was seen, from which we assumed that an inhibition of T lymphocytes in TGF-OP tumors is responsible for the smaller tumor size. The same effect of eliminating size differences between TGF-OP and MLL tumors was observed in Copenhagen rats which Natural Killer (NK)-cell activity had been either stimulated by daily intraperitoneal injection of 1.2 mg Poly I:C, a potent stimulator of interferon production, or inhibited by injection of 20μg anti-ASGM-antibody (Wako, Richmond VA) i.p. 3 days before cell inoculation and at additional intervals (days 2,10,14,17) afterwards, implying a role of NK-cells in prostate tumor growth modulation by TGF-β1 (Contrator / Breul / Barrack, J.Urol. 151 :366A, 1994). Note that NK-cell activity in nude mice and in nude rats is no size differences between TGF-OP and MLL tumors after inoculation of 5x105 tumor cells was seen, from which we assumed that an inhibition of T lymphocytes in TGF-OP tumors is responsible for the smaller tumor size. The same effect of eliminating size differences between TGF-OP and MLL tumors was observed in Copenhagen rats which Natural Killer (NK)-cell activity had been either stimulated by daily intraperitoneal injection of 1.2 mg Poly I:C, a potent stimulator of interferon production, or inhibited by injection of 20μg anti-ASGM-antibody (Wako, Richmond VA) i.p. 3 days before cell inoculation and at additional intervals (days 2,10,14,17) afterwards, implying a role of NK-cells in prostate tumor growth modulation by TGF-β1 (Contrator / Breul / Barrack, J.Urol. 151:366A, 1994). These data represented a paradox because both T- and NK-cells seemed responsible for tumor growth enhancement in TGF-OP tumors. Assuming an influence of inoculated cell number on prostate tumor growth in nude mice, tumoricidal inocula with increasing numbers of TGF-OP or MLL tumor cells were examined for their ability to form tumors. Interestingly, after inoculation of 2x105, 1x106 or 5x106 tumor cells no difference in tumor volume was observed between MLL and TGF-OP tumors (2x105: 6.69±0.83 mm3, 7.22±0.48, 1x106: 5.91±0.7, 7.21±0.89, 5x106: 4.84±0.81, 7.28±0.85 ) (mean ±SD). In contrast a significant difference between TGF-OP and MLL tumor volumes in nude mice was observed after inoculation of 5x106 tumor cells (5x106: 1.89±0.30 mm3, 3.42±0.35 (p<0.005)). The growth promoting effect of TGF-β1 is also apparent in nude mice but only in low number cell inocula. Therefore effects of TGF-β1 on Tumor growth in vivo are not responsible for the observed growth promotion in TGF-OP tumors. Thus, it appears that in rats TGF-β1 overexpression may enhance tumor growth at least in part by inhibiting NK-cell function and thus enabling more tumor cells to survive immune surveillance and produce larger tumors. (Supported by CA15924 and DFG GO 183/1)