TARGETING GENE THERAPY TO PROSTATE CANCER

Oliver Cassesse1, Mariam Andrawiss3, Michel Perricaudet1 and Philippe Berhov1

1Département de Recherche en Urologie, Hôpital Saint Louis, 75475 Paris Cedex 10, France. Laboratory of Génétique des Virus Oncogènes, URA 1301 CNRS, Institut Gustave-Roussy, 91805 Villejuif, France.

No prostate cancer treatment has proven to be as efficient as soon as the tumor becomes invasive. It is therefore of some importance to design a new therapeutic approach using cellular and molecular biology tools. A major breakthrough may come from the developments 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 lines (Int. J. Oncol. 6: 333-343, 1995), human-rodent xenografts and non human primates. By using recombinant adenovirus expressing the gene reporter β-galactosidase (rAd-β-gal) and the suicide gene thymidine kinase (Ad-HSV-tk) we have tested in vitro the efficiency of transfection as well as the toxicity of the adenoviral 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 some staining. This epithelial selectivity to rAd was further confirmed on the immortalised human prostatic cancer cell lines PNT2 and PNT1A, known to be extremely insensitive to transfection. Confirmation of this selectivity has been carried out in human prostatic cancer xenograft on nude mice while 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.

GENERATION OF ANTI-IDIOYPE MONOCLONAL ANTIBODIES RELATED TO PSA

Philippe Berthou1, Olivier Cassesse1, Mariam Andrawiss3, Michel Perricaudet1 and Philippe Berhov1

As a consequence of the idiotype network theory it has been suggested that interaction of an antibody anti-idiotypic antibodies must be able to substitute for the nominal antigen. Therefore, the development of monoclonal anti-idiotypic antibodies (Ab2) bearing the internal image of a tumor-associated antigen (TAA) is of great interest.

Prostate specific antigen (PSA) has proven to be as 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 maker 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 advances 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 MAbs IgG1-kappa(Ab2) was crosslinked to Keyhole Limpet Hemocyanin and used as immunogen. BALBIc mice were immunized with Ab1-kappa, which specifically reacts with PSA and their splenocytes were fused with SP2/Ø mouse myeloma cells. In 3 fusions, 6 anti-idiotypic antibodies specifically reacting with anti-PSA MAbs were isolated from more than 3000 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAbs binding pocket and not for the C2 (outside pocket) of IgG. Furthermore, these Ab2 were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAbs IgG. All Ab2 showed clear binding to anti-PSA MAbs in nolis, whereas their reactivity was abolished in reductant conditions, indicating that Ab2 recognize a conformational epitope compromised of hexas and light chain, and not a structural epitope outside anti-PSA MAbs binding site. It is also likely that these Ab2 have 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 MAbs. For the generation of anti-idiotypic antibodies (Ab3) resembling Ab1 (Ab1), NK-92 cells were immunized with Ab3 and selected. Ab1 and Ab3 were tested for their binding to PSA and their ability to induce antibodies competitive to PSA. Ab1 induced Ab2 reactivity against PSA but not with antigen-negative cell lysis, indicating Ab1 induction.

In summary, we have isolated 2 anti-idiotypic 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 MAbs binding pocket.

P 18

POLYAMINE METABOLISM AND PROGRAMMED CELL DEATH IN PROSTATIC CANCER CELLS


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 pathway needed for this programmed cell death is retained. Recent evidence indicates that 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 some staining. This epithelial selectivity to rAd was further confirmed on the immortalised human prostatic cancer cell lines PNT2 and PNT1A, known to be extremely insensitive to transfection. Confirmation of this selectivity has been carried out in human prostatic cancer xenograft on nude mice while 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

GENERATION OF ANTI-IDIOYPE MONOCLONAL ANTIBODIES RELATED TO PSA

Philippe Berthou1, Olivier Cassesse1, Mariam Andrawiss3, Michel Perricaudet1 and Philippe Berhov1

As a consequence of the idiotype network theory it has been suggested that interaction of an antibody anti-idiotypic antibodies must be able to substitute for the nominal antigen. Therefore, the development of monoclonal anti-idiotypic antibodies (Ab2) bearing the internal image of a tumor-associated antigen (TAA) is of great interest.

Prostate specific antigen (PSA) has proven to be as 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 maker 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 advances 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 MAbs IgG1-kappa(Ab2) was crosslinked to Keyhole Limpet Hemocyanin and used as immunogen. BALBIc mice were immunized with Ab1-kappa, which specifically reacts with PSA and their splenocytes were fused with SP2/Ø mouse myeloma cells. In 3 fusions, 6 anti-idiotypic antibodies specifically reacting with anti-PSA MAbs were isolated from more than 3000 hybridomas. Of these, two blocked binding of Ab1 to PSA, indicating that specificity for the anti-PSA MAbs binding pocket and not for the C2 (outside pocket) of IgG. Furthermore, these Ab2 were tested in Western blots to determine the reactivity with reduced and non-reduced anti-PSA MAbs IgG. All Ab2 showed clear binding to anti-PSA MAbs in nolis, whereas their reactivity was abolished in reductant conditions, indicating that Ab2 recognize a conformational epitope compromised of hexas and light chain, and not a structural epitope outside anti-PSA MAbs binding site. It is also likely that these Ab2 have 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 MAbs. For the generation of anti-idiotypic antibodies (Ab3) resembling Ab1 (Ab1), NK-92 cells were immunized with Ab3 and selected. Ab1 and Ab3 were tested for their binding to PSA and their ability to induce antibodies competitive to PSA. Ab1 induced Ab2 reactivity against PSA but not with antigen-negative cell lysis, indicating Ab1 induction.

In summary, we have isolated 2 anti-idiotypic 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 MAbs binding pocket.