ANIMAL CELL TECHNOLOGY
Products of Today, Prospects for Tomorrow

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RECEPTOR-MEDIATED GENE DELIVERY INTO MAMMALIAN CELLS

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We have developed gene transfer systems which use the receptor-mediated endocytosis route to import DNA into mammalian cells. DNA gene constructs have been complexed with a polylysine-conjugated ligand (such as transferrin) for uptake via receptor-mediated endocytosis and polylysine-conjugated, endosome-disruption agents (such as replication-defective adenoviruses or peptides derived from the N-terminal sequence of influenza virus hemagglutinin) which allow cytoplasmic entry of the DNA. These complexes have been delivered to and expressed at very high level in a large proportion of target cells (up to 80% in primary fibroblasts, primary myoblasts or primary human melanoma cells).

Key words: DNA transfection; gene therapy; gene transfer; endocytosis; transferrin; polylysine; endosome disruption; adenovirus; influenza peptide

Introduction

Our work has been based on the idea of adopting cellular transport mechanisms for the uptake of nucleic acids into cells. In order to exploit the transferrin receptor mediated endocytosis, the iron transport protein transferrin that has been chemically conjugated to the DNA-binding polycation polylysine (1,2). Polylysine upon binding to the DNA also condenses it to a doughnut-like particle (3). The complexes have been found efficiently delivered into the endosomes of many types of cells. In some cell lines a high level gene expression has been found, in many cell types, however, low or no expression has been observed (4).

Results

It became clear that the DNA is accumulating in internal vesicles and subsequent steps that deliver the DNA to the nucleus work only inefficiently. Strategies had to be developed to prevent degradation of the DNA in lysosomal compartments and promote the transport to the nucleus. This has led to the development of an efficient gene transfer system (5) consisting of a transferrin-polylysine / DNA complex linked

to a replication-defective adenovirus (see **Figure**). After endocytosis of these combination complexes into endosomes the natural endosomal acidification process triggers a conformational change in adenoviral coat proteins which then destabilize the membranes; both the viral particle and the DNA are released to the cytoplasm.

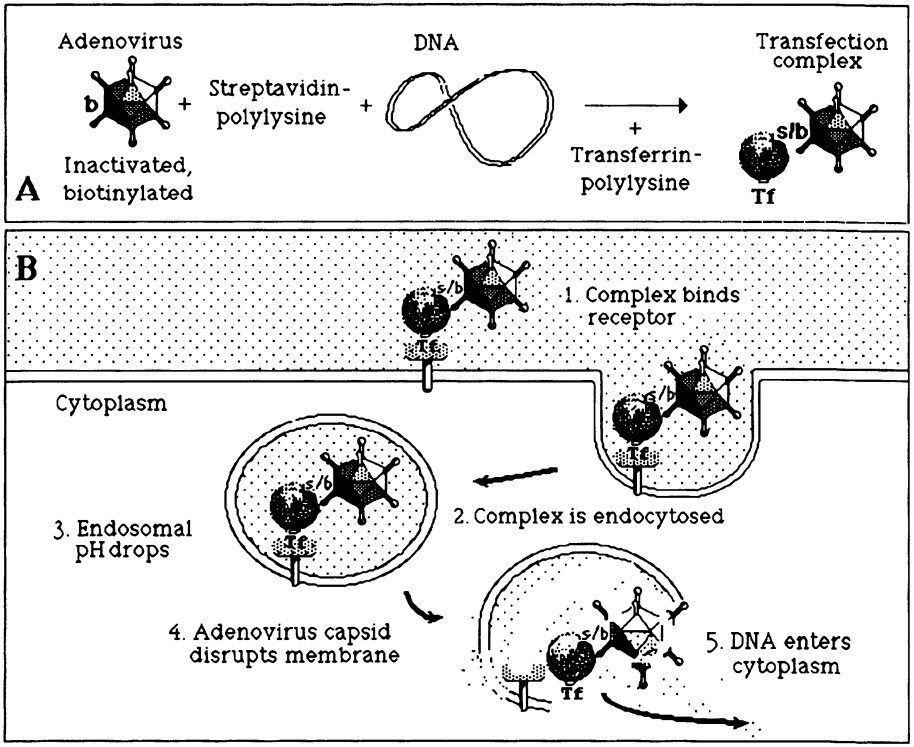

Figure: Formation (A) and uptake (B) of adenovirus-polylysine / transferrin-polylysine / DNA combination complexes into cells.

The use of replication-defective and chemically (psoralen /UV) inactivated adenovirus (6) avoids most of the potential hazards associated with viral vectors. The delivered gene is carried on the exterior of the adenovirus, being therefore far less restricted in size or sequence of the DNA to be delivered. Transfer of 48 kb DNA molecules, and high level expression of the full length (8 kb) human factor VIII cDNA in primary fibroblasts, myoblasts and myotubes has been demonstrated.

A potential application of the gene transfer system may be the development of a cytokine gene-modified melanoma vaccine. This is supposed for application in patients with minimum residual disease where most of the tumor mass has been removed by surgery. A tumor cell culture has to be established, transfected with a immunstimulatory agent such as a cytokine gene, irradiated with 100 Gy in order to block replication. Such a cytokine-gene modified irradiated vaccine when injected s.c. into the patient is supposed to induce a systemic immune response against residual tumor cells that otherwise lead to metastasis formation.

Cytokines are important actors in the activation of an immune response. Interleukin 2 (IL-2) is produced by stimulated T-helper cells that have seen e.g. tumor-antigen presented by cells such as macrophages or dendritic cells. IL-2 stimulates effector cells such as NK cells or CTLs that are able to circulate in the patients body and to eliminate residual tumor cells. IL-2 expressing tumor cells should have the ability to directly activate these effector cells.

We have started first animal experiments in the M3 melanoma model. Murine M3 melanoma cells transfected with cytokine gene constructs produce high levels of cytokines (24,000 units IL-2 / 10^6 cells/24 hrs; 1200 ng IFN-γ/10^6 cells/24 hrs). Transplantation of $1x10^5$ IL-2 transfected M3 cells into syngeneic DBA/2 mice showed loss of tumorigenicity (0/6 animals), whereas 6/6 animals treated with $1x10^5$ untransfected M3 cells developed tumors. In order to test a biological efficacy of the cancer vaccine, animals were immunized with irradiated, IL-2 transfected M3 cells. The vaccination resulted in a systemic protection in all animals against subsequent challenge with tumorigenic doses of unmodified M3 cells (7).

Conclusions

What are the future options? On the one hand we want to further develop the cancer vaccine into a stage suitable for a clinical trial, on the other hand we are developing completely synthetic versions of the gene transfer system. We have replaced the whole virus by small synthetic peptides similar to sequences occurring in the hemagglutinin of influenza virus. At neutral pH this peptide have an inactive, rather unordered structure, whereas at acidic, endosomal pH they adopt the active structure of an amphipathic helix that can interact with the lipid membrane and destabilize it. We have incorporated such endosome-disruptive peptides into complexes containing DNA, transferrin and polylysine. The peptides within the complex are able to disrupt liposomes (8) or endosomal membranes (C.P. and E.W., unpublished results) triggered by the change to lower pH and substantially (up to 10,000-fold) augment the gene transfer (8,9).

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PAPER OF WAGNER

Spier: While recognising that the introduction of exogenous genes into animal cells is important have you examined the moral issues involved in the use of such techniques for the enhancement of human functions rather than the alleviation of disease ?

Wagner: The concept of our work is that it is not the permanent transfer of genes into cells. We chose the applications of the tumour vaccines because once it has worked there will not be any gentically modified cells remaining in the patient. The gene transfers we use lead to episomal DNA and not integrated DNA.