SYNTHETIC VIRUS-LIKE GENE DELIVERY SYSTEMS

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Introduction

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

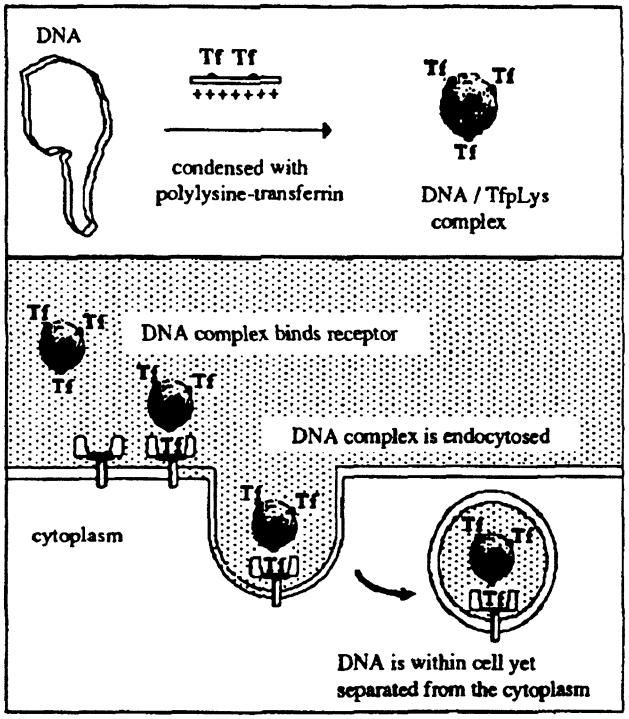

Figure 1 Gene transfer via the transferrin (Tf) receptor-mediated endocytosis pathway.

Viruses have acquired special mechanisms to release their genome from endosomes into the cytoplasm. In the case of membrane-free viruses (such as adenoviruses) the endosomal acidification process specifically activates viral coat protein domains that trigger disruption of the endosomal membrane. In the case of enveloped viruses (such as influenza virus) the viral membrane fuses with endosomal membranes. This has led us to the development of virus-like gene transfer complexes (4-7) consisting of DNA gene constructs complexed with a polylysine-conjugated cell-binding ligand (such as transferrin) and polylysine-conjugated, endosome-disruption agents (such as replication-defective adenoviruses or synthetic peptides derived from viral fusion sequences) which allow cytoplasmic entry of the DNA.

Transferrin-polylysine / DNA complexes linked to replication-defective and chemically (psoralen /UV) inactivated adenoviruses (6) 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). 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.

Results

One of our major goal has been the development of 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 or sequences in the VP-1 protein of rhinoviruses. At neutral pH these peptides 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. The peptides are able to disrupt liposomes, erythrocytes, or endosomal membranes triggered by the change to lower pH.

We have incorporated endosome-disruptive peptides into DNA complexes either via covalent conjugation to polylysine (6,7) or by ionically binding to DNA/polylysine complexes (see Fig.2). As a first step in assembling the complex, half of the negative charges of the plasmid DNA were saturated with transferrin-polylysine to provide a ligand for receptor-mediated endocytosis. The remaining nucleic acid charges were utilized for binding an excess of polylysine. To the resulting positively charged complex the negatively charged peptide was added to provide the endosome-disrupting activity. The complexes were used for transfection of cell lines such as murine BNL C1.2 hepatocytes, NIH 3T3 fibroblasts, B16 melanoma cells, or primary human melanoma cells. Reporter genes (luciferase, ß-galactosidase) or therapeutically more relevant genes (cytokines interleukin-2, GM-CSF; see next page) have been delivered. The (up to 10 000-fold) enhancement of gene expression mediated by the peptide strongly correlated with the capacity of peptides to lyse erythrocytes in an acidic environment.

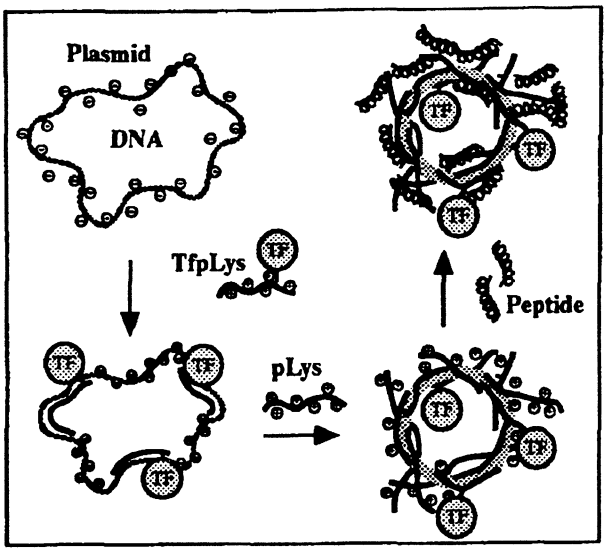

Figure 2 Synthetic virus-like gene transfer complexes. Complexes consisting of DNA, transferrin-polylysine conjugates (TfpLys) and membrane-disruptive peptides ionically (pLys, Peptide) bound to polylysine.

Conclusions

What are the future options? The delivery of genes to tumor cells is considered to be an exceptionally well-suited application of transferrin receptor-mediated gene delivery. Rapidly proliferating tumor cells can be transfected with highest efficiency, but also slowly-dividing or non-dividing cells are accessible for receptor-mediated gene transfer. One might consider using the system to deliver toxin genes directly into the tumor mass for elimination of these cells; however, the poor survival of our complexes in the blood stream coupled with the requirement to introduce the gene into most cells of the tumor limits this application.

We favor the concept of generating cytokine gene-modified melanoma vaccines (8). These are 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 also stimulates effector cells such as CTLs that are able to eliminate residual tumor cells in the patients' body. IL-2 expressing tumor cells should have the ability to directly activate a cellular immune response.

In mouse models using syngeneic melanoma cells (see ref. (8) and our unpublished results) the vaccination with gene-modified tumor cells prevented the development of tumor in the prophylactic setting (vaccination before challenge with unmodified tumor cells) or the therapeutic setting (vaccination of animals bearing small tumors). Transfection of human melanoma cultures (preferably with low passage numbers, ≤10 passages) by adenovirus dl1014 / transferrin-polylysine / DNA complexes resulted in high interleukin-2 expression levels between 1 000 and 190 000 units IL-2. Currently we are also evaluating peptide-transfection complexes as alternative to the use of adenovirus complexes. Murine B16 melanoma cells and primary human melanoma cells were transfected efficiently using the peptide method, resulting in high levels of the cytokines GM-CSF (up to 6 000 ng / 24 hrs / 10^6 B16 cells) or IL-2 (up to 12 000 units / 24 hrs / 10^6 human melanoma cells) secreted by the cells transfected with the corresponding genes. These results suggest that the synthetic virus-like DNA complexes may be useful for gene therapeutic application.

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Acknowledgements

We thank Claudia Denk, Helen Kirlappos, Margareta Kursa, Karl Mechtler, Medhya Saltik, Ursula Scheer and Alexandra Sinski for their excellent technical assistance. We are grateful to Dr. Gotthold Schaffner for providing the plasmid pGShIL-2tetr. We thank Dr. Susanne Schreiber and Dr. Georg Stingl for providing melanoma isolates.