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Targeting of cell receptors and gene transfer efficiency: a balancing act

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Vectors conjugated with ligands recognized by cell surface receptors are of interest for cystic fibrosis gene therapy since these vectors would allow cell-specific targeting. However, an efficient and specific uptake may be abrogated by a subsequent intracellular trafficking leading to an inefficient gene transfer. This has been shown for polylysine substituted with mannose residues. While mannose-specific membrane lectins are predominantly expressed at the surface of airway cells and mannosylated complexes are the most efficiently incorporated glycosylated complexes in these cells, mannosylated complexes lead to a low gene transfer efficiency because of an inefficient exit from endosomal compartments, a high accumulation in lysosomes and an inefficient nuclear import. In contrast, the entry of low amounts of lactosylated complexes is balanced by more efficient intracellular trafficking, leading to an efficient gene transfer. This emphasizes that for a successful gene transfer, it is necessary to find the balance between efficient and specific uptake, and intracellular trafficking that overcomes the various cellular barriers and enables the plasmid to reach the nucleus.
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Cationic polymers are synthetic molecules that have been developed to introduce exogenous genes into cells to produce new proteins. For instance, poly-L-lysine which interacts with nucleic acids, is widely used to compact plasmid DNA containing a gene of interest and transfect cells with the resulting DNA/cationic polymer complexes. However, the main goal is to obtain cell-specific transfer which might be achieved by taking advantage of cell-surface receptors that mediate DNA uptake. Hence, bifunctional conjugates have been constructed in which the polylysine allows DNA binding and the ligand domain allows receptor-mediated delivery of the nucleic acid. Using this model, polylysine has been substituted with protein ligands, such as transferrin, to target airway cells through the transferrin receptor.[1] Sugar moieties commonly expressed on glycoproteins have also been used as ligands specific to receptors, called lectins. A large variety of cells express membrane lectins that selectively recognize glycoconjugates containing complex oligosaccharide structures.[2–4] Wu and coworkers achieved one of the first successes of *in vitro* and *in vivo* glycoconjugate-based transfection through the galactose-specific membrane lectin of hepatocytes by using DNA complexed with polylysine coupled to the asialoorosomucoid glycoprotein (for a review, see Ref. 5). The drawbacks with these strategies using proteins or glycoproteins are that the preparation and purification of such polypeptidic structures coupled to polylysine are difficult, their solubility may be very low and they may induce immune responses. One way of solving these problems may be to use cationic polymers substituted with small sugar units as ligands[3] (Figure 1). Plasmid DNA complexed with polylysine bearing lactosyl residues was found to efficiently transfect hepatocarcinoma (HepG2) cells and hepatocytes in primary culture, both of which express galactose-specific membrane lectin.[6–8] Similarly, plasmid DNA complexed with polylysine bearing mannosyl residues efficiently transfected human macrophages expressing a mannose/fucose-specific membrane lectin.[9]

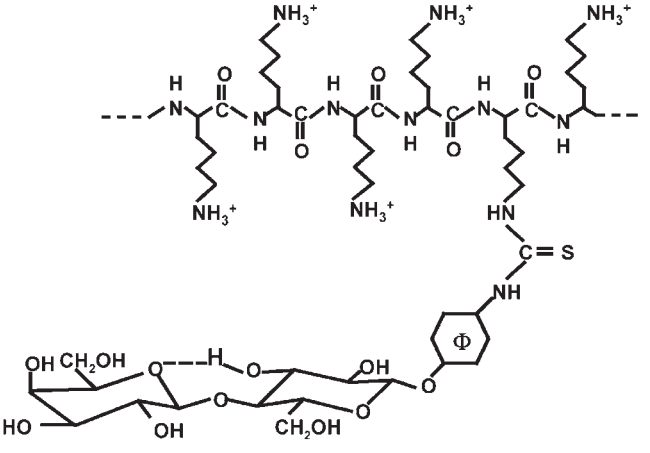

Figure 1 *Partial structure of lactosylated polylysine.*

Gene therapy could represent a potentially important advance in the treatment of cystic fibrosis (CF), which is the most common autosomal lethal recessive disorder in the Caucasian population. Presumably, the cells to target

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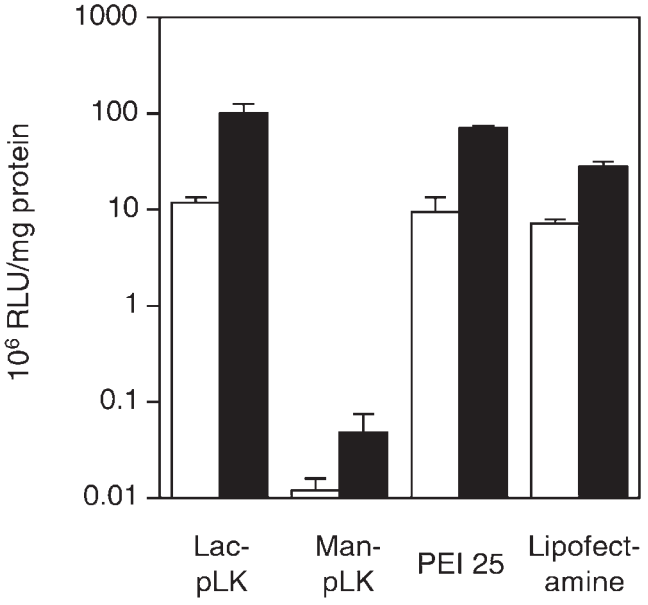

Figure 2 *Gene transfer with nonviral vectors into immortalized CF airway epithelial cells (ΣCFTE29o- cells; □) and CF airway gland serous cells (CF-KM4 cells; ■). Twenty-four hours after seeding, cells were incubated for 4 h in the presence of plasmid (pCMVLuc, 5 μg) complexed to lactosylated polylysine (Lac-pLK), mannosylated polylysine (Man-pLK), polyethylenimine 25 kDa (PEI 25) or Lipofectamine. Chloroquine (100 μM) was added to complexes made with polylysine. Forty-eight hours later, luciferase activity was measured by chemiluminescence.*

for efficient gene transfer in CF are the respiratory cells that are the predominant site of CFTR (cystic fibrosis transmembrane conductance regulator) expression and these were shown to be the airway surface ciliated cells and the airway gland serous cells.[10,11] Using various glycosylated polylysines as vectors, we have shown that some sugars enhanced the vector capability of polylysine and yielded high expression of the reporter gene luciferase in immortalized epithelial airway surface and airway gland serous cells, and in CF airway epithelial cells in primary culture.[12–14] Some of the most efficient glycosylated polylysines such as lactosylated or α-glucosylated polylysines, were at least as efficient as commercially available nonviral vectors like Lipofectamine and polyethylenimine[13,14] (Figure 2). Moreover, α-glucosylated polylysine allowed efficient *cftr* gene transfer and led to the expression of a normal CFTR protein on the membrane of CF cells.[14]

To better understand the mechanism of uptake of glycoplexes (plasmid/glycosylated polylysine complexes), we identified the membrane lectins expressed by immortalized normal and CF airway epithelial cells. A membrane lectin recognizing alpha-D-mannopyranosides was found on the surface of normal and CF airway surface epithelial cells and airway gland serous cells. In agreement with this finding, the most efficiently incorporated glycoplexes were those bearing alpha-D-mannose residues. However, glycoplexes made with mannosylated polylysine induced a very weak expression of the reporter gene in all the airway epithelial cells studied[13,14] (Figure 2). Therefore, the massive uptake of complexes by targeted cells does not mean that the complexes are able to efficiently transfer the gene. In the case of mannosylated complexes, inefficient intracellular trafficking probably occurs and abrogates the efficient mannose-specific uptake. In contrast, the entry of low amounts of lactosylated complex is presumably balanced by more efficient intracellular trafficking. Indeed, the low gene transfer efficiency obtained with mannosylated complexes as compared with lactosylated complexes was found to be due in part to their inefficient exit from the endosomal compartments and their high accumulation in lysosomes. In addition, nuclear import also appeared to be quite inefficient, since 95% of the intracellular mannosylated complexes were localized outside the nucleus[15] (Figure 3).

One common intracellular limiting step encountered

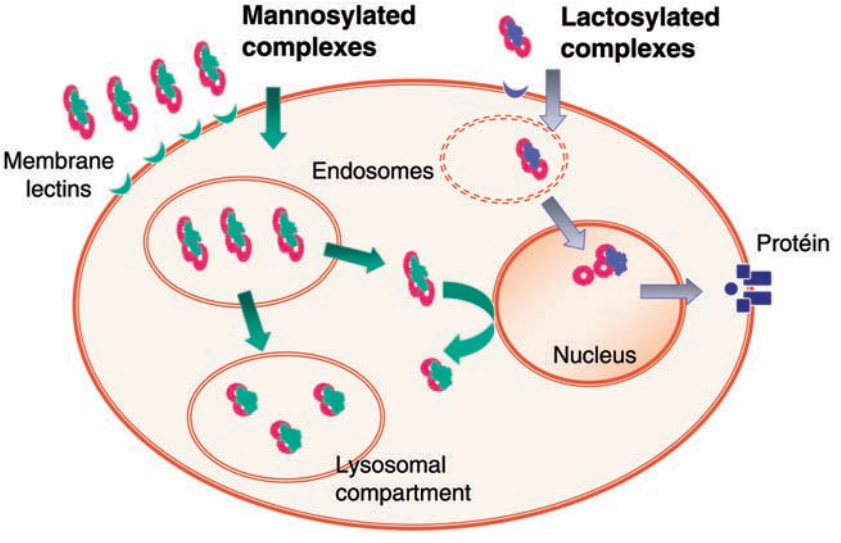

Figure 3 *Schematic drawing of mannosylated complexes and lactosylated complex delivery pathways in airway epithelial cells. While mannose-specific membrane lectins are predominantly expressed at the cell surface and mannosylated complexes are the most efficiently incorporated glycosylated complexes, mannosylated complexes lead to a low gene transfer efficiency as compared with lactosylated complexes because of an inefficient exit from endosomal compartments, high accumulation in lysosomes and inefficient nuclear import.*

when using polylysine as vector is poor endosomal exit. Hence, all the experiments need to be conducted in the presence of membrane disrupting agents, such as chloroquine or fusogenic peptides, which increase the transmembrane passage of the plasmid DNA into the cytosol and/or limit lysosomal enzyme degradation of endocytosed material.[16,17] Unfortunately, these membrane disruptive agents are not devoid of toxicity, thereby making *in vivo* experiments difficult to design. To circumvent this drawback, we have constructed a polylysine partially substituted with histidyl residues, which become cationic upon protonation of the imidazole groups at slightly acidic pH. This protonated polymer induces the leakage of plasmid/histidylated polylysine complexes from acidic vesicles and hence favors plasmid release into the cytosol.[18] This histidylated polylysine was shown to efficiently transfect various cell types, including immortalized normal and CF airway surface and airway gland serous cells.[18,19] Histidylated polylysine complexes are likely to be incorporated into cells through non-specific uptake. So far, attempts to enhance the specificity and the level of gene expression by adding sugar on histidylated polylysine have failed; while cellular uptake of glycoplexes prepared with such glycosylated polylysine derivatives was efficient, the transferred gene was poorly expressed (unpublished data).

These results emphasize that for successful gene transfer using synthetic vectors, the goal is not necessarily to obtain substantial cellular uptake of the complexes, but rather to find the balance between efficient and specific uptake, and intracellular trafficking that overcomes the various cellular barriers and enables the plasmid to reach the nucleus. With this objective, we recently developed glycosylated polyethylenimines that appear to be efficient for gene transfer into airway epithelial cells, as they undergo a receptor-mediated uptake and retain the desired endosomal swelling property of the sugar-free polymer.[20]

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