119 Cell targeting for CF gene therapy: Identification of a new specific cell ligand and selection of infectious papillomavirus mutants

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Papillomavirus VLPs have the potential to deliver genes into numerous cell lines. However, HPV pseudovirions do not or transduce poorly human airway epithelial cells. The aim of this study was to redirect the tropism of HPV VLP vectors to human airway cells. In a preliminary study, three peptides, described as airway cell ligands, were inserted into the L1 major capsid protein of HPV-16. Cell internalization of these chimeric VLPs compared to wild-type VLPs into 16HBe14O- cells was increased, being dependent on the peptide use and the position of the insertion within L1. However, no gene transfer with the corresponding pseudovirions was detected in airway cells. To increase gene transfer, the minor capsid protein L2 was inserted within the chimeric VLPs. However still no gene transfer was detected in airway cells. This lack of gene transfer could be due either to lack of trafficking by an infectious pathway in airway cells or to the low level of infectious pseudovirions generated.

To overcome these difficulties, we searched for other ligands using a phage display system. A new peptide, LSPIRMR, that binds specifically to IB3–1 and S9 cells was identified. In addition, to improved the understanding of the entry pathway of VLPs into cells, the airway epithelial cell responses to transduction with HPV pseudovirions is currently being investigated by suppression subtractive hybridization (SSH). As there is a possibility that HPV pseudovirions are non-infectious in airway epithelial cells, we are also producing auto-replicative pseudovirions to select chimeric particles that infect airway cells. As a first step, we have obtained autoreplicative pseudovirions in 293 FT cells.

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120 Click chemistry: a promising approach to improve targeting ability of non-viral pulmonary gene transfer

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Background: Non-viral pulmonary gene transfer for the treatment of acquired or hereditary lung diseases including cystic fibrosis, requires the development of safe and highly efficient synthetic vectors. Recently we discovered a novel class of synthetic gene delivery systems, triblock and tetrafunctionnalized block copolymers, that promoted high gene expression in the lung. Moreover we demonstrated that partial galactosylation of these block copolymers dramatically increased their gene expression efficiency in the lung.

Aim: To further improve the airway epithelial cells targeting through galactose receptor recognition, we decided to synthesize full-galactosylated block copolymers using "click" chemistry. The synthetic pathway relies on the copper-catalysed Huisgen 1,3-dipolar cycloaddition between azide-terminated polymers and the galactose moiety that contains an alkyne group.

Results: The azide-terminated block copolymers were obtained quantitatively in a two steps procedure involving tosylation of hydroxyl groups and nucleophilic substitution with sodium azide. The alkyne containing galactose was synthesized from peracetylated galactopyranoside by reaction with butynol followed by sodium methoxide mediated deprotection. The optimized "click" reaction was performed in aqueous media at 55ºC for 2 days in the presence of a copper catalyst and led to a quantitative galactose functionalization of the extremities of block copolymers.

Conclusion The "click" chemistry strategy allowed the quantitative functionalization of triblock and tetrafunctionnalized block copolymers. These full-galactosylated block copolymers may improve transgene expression in the lung in terms of efficiency and safety compared to existing non-viral vectors.

121 Impact of polymers on lipid cationic mediated gene transfer

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Background: Amphiphilic block copolymers have recently been developed and displayed very efficient in vivo transfection activity in various tissues. However their mechanism remains unknown and better understanding of this mechanism represents a major goal for the development of this new class of vectors.

Methods: We observed that lutrol, an amphiphilic block copolymer does not allow to transfect in vitro cells, suggesting that environment (in vitro or in vivo) is strongly involved in their mechanism. However electron microscopic examination of cells transfected with DNA formulated with Lutrol indicated that DNA molecules were internalized. The present study aimed at understanding this discrepancy between in vitro and in vivo transfection behaviour of block copolymers.

Results: We observed that cell incubation with amphiphilic block copolymers prior to cationic lipid mediated transfection improves the reporter gene expression. Among the various steps going from cell internalization to transcription that could be improved by the block copolymer we found that the DNA molecules internalization step is probably the most important barrier on which the block copolymer played.

Conclusion: Our results support that amphiphilic block copolymers improve transfection efficiency by allowing a better crossing of the cell membrane barrier both in vitro or in vivo. However even if the mode of entry of DNA in the presence of block copolymer in cells is probably the same, the transfection efficiency is very different. It is generally accepted that cells cultured in vitro mostly internalize DNA by endocytosis requiring cationic lipid to escape the endosomes. DNA molecules do not probably follow this pathway of internalization in vivo and thus do not required cationic lipids.

122 Synthesis and transfection properties of a series of lipidic neamine derivatives

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To develop novel bio-inspired non-viral vectors for lung gene therapy of cystic fibrosis, we synthesized a series of cationic lipids with a neamine headgroup which incorporates rings I and II of the natural antibiotic aminoglycoside neomycin B. Indeed, we reasoned that neamine might constitute a straightforward and versatile building block for synthesizing a variety of lipophilic aminoglycosides and modulating their characteristics such as size, topology, lipophilicity, number of charges and charge density. Neamine derivatives bearing long dialkyl chains, one or two neamine headgroups and five to ten protonatable amine functions were prepared through the selective alkylation of the 4′- or the 5-hydroxyl function in ring I and ring II of neamine, respectively. The transfection activity of the twelve derivatives synthesized was investigated in in vitro gene transfection experiments using several mammalian cell lines, including human pulmonary cells (A549 and 16HBE). The results allowed to unveil interesting structure-activity relationships and to identify a formulation incorporating a small neamine derivative as a highly efficient gene delivery system.

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