115* New targeting method for nucleic acid transfer into the lung

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Background: Nucleic acid transfer into the lung is a promising approach to treat various acquired or hereditary lung diseases. Synthetic cationic vectors were currently used for gene transfer to the lung but their high instability and toxicity limited their efficacy in vivo. Recently, we discovered a novel class of gene delivery systems to the lung: nonionic block copolymers.
Aims: Our aim is to improve nucleic acid transfer efficiency of this novel class of vectors. Recently, it was shown that galactose residues promoted the entry of cationic vectors into human airway epithelial cells which mostly involved a receptor-mediated endocytosis. Thus, galactose molecules will be covalently linked to the extremities of block copolymers to specifically target airway epithelial cells through galactose receptor recognition.
Methods: Galactosyl residues were grafted on block copolymers by chemical synthesis. A CAT (chloramphenicol acetyl-transferase) reporter plasmid was formulated with galactosylated block copolymers and injected in the lung of mice by intratracheal instillation. After 2 days, reporter gene expression was evaluated in lungs.
Results: A 30 fold increase of CAT expression is observed with galactosylated block copolymers when compared to ungalactosylated block copolymers. Histochemical and histopathological analysis will be performed to better understand the enhancement of transfection efficacy.
Conclusions: This new targeting method could improve transgene expression in terms of efficiency and safety. Thus, we conclude that galactosylated block copolymers could specifically deliver nucleic acid molecules to the airways of cystic fibrosis mice. Thus novel perspective for knockdown of specific gene in lung by RNAi could be envisaged.
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116* Variations on hydrophobic domains in Lipophosphoramidates results in lung transfection's improvement and lower toxicity

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The design of safe and efficient vectors for gene delivery is a current challenge. Viral-based systems are for now, one of the most efficient methods to deliver genes into the cells. However, immunogenic and oncogenic complications are two of the main disadvantages of this transfection approach. As an alternative, synthetic carriers have been developed, notably based on cationic lipids.
Our objective was to evaluate the in vivo transfection capacity of three cationic carriers that (KLN47, EP8e, BSV-18) differ by the nature of their hydrophobic domain, respectively oleyl, linoleyl and phytanyl chains. Formulated in NaCl 0.9%, they are associated with 50 μg of pDNA encoding Luciferase. Then, lipoplexes are injected into the tail vein of mice and Luc expression was analysed up to 72 h by in vivo bioluminometer (NightOwl II, Berthold). We first observed that Luc expression was exclusively located into the lungs. Secondly, the highest transgene expression was obtained 24 h after transfection and progressively decreased. Thirdly, EP8e and BSV-18 were much more efficient than KLN47. Moreover, contrary to KLN47, both new vectors did not induce any mice death and their lower toxicity was confirmed by ALAT/ASAT measurements. The structure-activity relationship is crucial for a gain of effectiveness and inocuity.
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117* Histidinylated linear PEIs as new DNA carriers for *in vitro* and *in vivo* gene transfer with low toxicity

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Development of non-toxic and efficient gene delivery systems is among the most challenging requirement for gene therapy. Synthetic carriers have been developed for their non-immunogenicity, as well as for their easiness of handling. Lipids and polymers have so far demonstrated their effectiveness and linear PEI (lPEI) was found to offer opportunity to treat cystic fibrosis. Herein, we designed lPEI derivatives bearing histidinyl moieties to increase the buffering effect of lPEI in order to facilitate the endosome escape of the polyplexes. First, grafting of the amino acid was conducted through the Mickael addition of the N-acryloyl-L-histidine on the secondary amine of the polymer. Grafting was set between 4 and 50% and this was verified through ^{1}H and ^{13}C NMR. *In vitro* transfections with various cell lines, including human pulmonary cells (A549 and 16HBE), indicated that, when mixed with a luciferase encoding plasmid, His-lPEI characterized with a histidine content of 25 mol % was able to reach the highest luciferase activities. Compared to unmodified lPEI, this new polymer was at least as efficient while the toxicity level was remarkably very reduced. *In vivo* gene delivery experiments in mice, via either systemic or intranasal administration routes, demonstrated the effectiveness of this His-lPEI to transfect the lungs and confirmed its low toxicity. Thus, histidinylated lPEI derivatives may constitute promising weakly toxic non-viral vectors for lung gene therapy of cystic fibrosis.
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118* The Adenovirus chimeric vector Ad5F35/GFP-CFTR efficiently restores the CFTR deficiency in human airway epithelia via apical surface transduction

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Background: *CFTR*, the gene mutated in cystic fibrosis (CF), was the first human gene to be delivered to patients using Adenovirus serotype 5 (Ad5) vector and these clinical trials have revealed several limitations of Ad5 vectors. In this study, the gene delivery of GFP-CFTR by a chimeric Ad5Fi35 vector to human airway epithelial and tracheal cells was analyzed for transduction efficiency, correction of chloride channel activity, GFP-CFTR cellular localization and vector toxicity.
Methods: Transduction efficiency by Ad vectors was assayed by flow cytometry, cellular trafficking of GFP-CFTR by confocal fluorescence microscopy, CFTR function by a radiotracer flux method and by measure of transepithelial ion transport.
Results: High doses of Ad5 vector was detrimental to the endogenous CFTR function in normal cells transduced by Ad5GFP, and to exogenous CFTR delivered by Ad5GFP-CFTR in CFTR(–) cells. This inhibition of CFTR function was associated with the viral capsid proteins involved in the RGD-integrin cell entry pathway used by the vector.
A chimeric vector, Ad5Fi35GFP-CFTR which uses a RGD-independent cell entry pathway, showed minimum effect on CFTR function even at high doses. More importantly, Ad5Fi35-GFP-CFTR transduced reconstituted airway epithelia from CF patients via the apical surface and restored the CFTR function at relatively low vector doses.
Conclusion: Ad5Fi35 represents a potential vector for efficient gene delivery to the airway epithelium.
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