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Delivery of gene-regulating agents: Internalization mechanisms and novel vectors

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ABSTRACT

The sequencing of the human genome was expected to generate a veritable explosion of therapeutics for regulation of gene function, either through modulation of gene expression or through the replacement of defect genes. However, nucleic acid-based pharmacological agents suffer from issues of low bioavailability and unfavorable pharmacokinetics, wherefore these prospects have not yet been realized.

One promising approach for regulation of gene function is a special type of antisense technology, referred to as splice correction. Aberrantly spliced mRNA is intimately associated with numerous serious illnesses, wherefore the ability to restore the correct splicing pattern is a highly attractive therapeutic approach. Another thriving oligonucleotide-based platform makes use of small (or short) interfering RNA (siRNA), double-stranded RNA sequences that efficiently silence expression of essentially any gene of interest. However, both platforms are limited by the inherent weaknesses of oligo- and polynucleotide-based agents, meaning that the development of efficient delivery vectors is a prerequisite for clinical translation. Short cationic peptide sequences, so called cell-penetrating peptides (CPPs), constitute an emerging category of delivery vehicles with the ability to convey various cargo molecules across the cell membrane, but numerous polymeric vectors (commonly referred to as ‘polyplexes’) are also under intense scrutiny for delivery of gene-regulating agents.

This thesis aims to delineate the internalization mechanisms of CPPs conjugated to a special type of splice-correcting oligonucleotide analogues (namely peptide nucleic acids (PNAs)), but it also presents a rationally modified CPP for delivery of splice-correcting oligonucleotides and plasmid DNA, as well as an entirely novel class of delivery vectors, so called polythiophenes, for siRNA delivery. Specifically, paper I examines the internalization routes of a number of CPP-PNA conjugates, papers II and III study the oligonucleotide and plasmid delivery efficacy, respectively, of the stearylated CPP transportan 10 (TP10), whereas paper IV examines the utility of a cationic polythiophene for siRNA delivery.

In conclusion, the research described herein provides novel data on internalization mechanisms of chemically distinct CPPs, as well as presents two novel agents for delivery of splice-correcting oligonucleotides, plasmid DNA, and siRNA, thereby adding additional tools to the toolbox for delivery of gene-regulating agents.