Peptide dendrimer as siRNA transfection reagent

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RNA interference (RNAi) allows effective and specific silencing as described by Tuschl and co-workers in their proof-of-principle experiment demonstrating that synthetic double stranded small interfering RNA (siRNA) could achieve sequence-specific gene knockdown in a mammalian cell line by promoting the degradation of complementary mRNA via RNA induced silencing complex (RISC).[1] Potential therapeutic applications of RNAi are crucially dependent on the delivery of siRNA into the cytosol to avoid that this step becomes a bottleneck. Naked or chemically modified siRNA delivery is of limited application and therefore nanoparticles encapsulating siRNA molecules have been investigated as a more general method to bring siRNA into cells.

We have previously explored a collection of peptide dendrimers for the transfection of plasmid DNA and siRNA and found efficient reagents that obeyed distinct structure-activity relationships. Of crucial importance were the distribution of cationic charges across the three dendrimer generations for DNA, the two outer generation only for siRNA and in both cases the use of DOTMA/DOPE as helper lipids.[2,3,4]

We are now exploring peptide dendrimers as delivery agents for siRNA in the absence of the helper lipids. In this project, a library of 100 peptide dendrimers was prepared by solid phase peptide synthesis and their gene silencing ability investigated. The biological experiments included treatment of HeLa, CHO, HEK-293, PC-3, HT-1080, SH-SY5Y and CACO-2 cells by the new transfection agents and siRNA targeting GAPDH (siGAPDH) or scrambled (siNC) in the absence and presence of serum. The knockdown efficiency was measured by monitoring enzyme activity of GAPDH and quantification of GAPDH mRNA level. The parameters necessary for efficient gene silencing have been discovered and optimized to lead to some only amino acid and some lipid-containing dendrimers.

Additionally, we discovered that diastereomers and enantiomers of these lead compounds influence and ultimately allowed a higher transfection efficiency. In order to understand the underlying principle, these potent compounds were then coupled to fluorophores that maintain the overall knockdown efficiency and therefore allow studies on the internalization process and intracellular localization of siRNA and dendrimer by flow cytometry and confocal microscopy.

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