Receptor-Mediated Small Interfering RNA Delivery in Sjögren's Syndrome

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Background:

Sjögren's syndrome (SjS) is characterized by xerophthalmia and xerostomia resulting from loss of secretory function due to immune cell infiltration in lacrimal and salivary glands. Current SjS therapeutic strategies employ secretagogues to induce secretion via muscarinic receptor stimulation. Based on our expertise on muscarinic type-3-receptor (M3R), we are utilizing ligands specific for MR to deliver siRNA into cells via receptor-mediated endocytosis, thereby altering epithelial cell responses to external cues such as pro-inflammatory or death signals while simultaneously stimulating secretion.

Methods:

MR agonist carbachol was conjugated with siRNA targeting caspase-3. To test siRNA efficacy after conjugation, HSG cells were transfected with conjugate. To test conjugate efficacy, conjugate was added to HSG cells in culture. Quantitative RT-PCR and immunofluorescence were used to detect caspase-3 gene and protein expression, respectively. Carbachol functionality was assessed using intracellular calcium release assays. A FAM-labeled DNA probe was designed to target the antisense strand of caspase-3 siRNA and in situ hybridization was utilized to detect conjugate entry into cells.

Results:

Transfection of conjugate into cells resulted in 80%-reduction in caspase-3 gene expression, confirming retained function of siRNA after conjugation. External conjugate treatment of HSG cells resulted in similar intracellular calcium release and induction of endocytosis as carbachol stimulation indicating that the carbachol portion of conjugate also retained function after conjugation. Using the FAM-labeled probe, conjugate uptake was visualized in HSG cells, indicating that the conjugate binds M3R and is taken into cells via receptor-mediated endocytosis as expected. In conjugate-treated HSG cells caspase-3 mRNA and protein expression was reduced 50% demonstrating the efficacy of our conjugate in siRNA delivery.

Conclusion:

Both siRNA and carbachol portions of the conjugate were shown to retain function after conjugation, and external treatment of HSG cells with conjugate shows promising results. Further in vitro/in vivo studies are needed to optimize conjugate entry and test efficacy of conjugate in the prevention of cytokine-induced apoptosis. This therapeutic strategy can easily be manipulated to target different genes of interest while maintaining cell-type specificity, and has many potential clinical applications in the treatment of SjS. Supported by NIH grant T32DE007200 and Sjögren's Syndrome Foundation Research Grant.