Gene delivery of nucleic acid therapy targeting *MAPT* for the treatment of Alzheimer's disease

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Background:

For Alzheimer's disease (AD) pathology, the abnormal accumulation of phosphorylated tau protein is a key hallmark of AD. Tau protein is encoded by the *MAPT* gene. Nucleic acid therapeutics targeting *MAPT* therefore may represent a promising approach to AD treatment. In this study, an *MAPT*-targeted antisense oligonucleotide (ASO) was synthesized to downregulate the expression of pT181. Having set up an AD cell model with total tau and phosphorylated Thr181 overexpressed in the glyceraldehyde (GA)-induced SH-SY5Y cells, which were subsequently differentiated into neuronal cells. Then transfection efficacy of ASO was evaluated and a liposomal delivery system was developed to penetrate the BBB. To enhance BBB penetration, the ASO-loaded liposomes were further modified with transferrin (TF). Ongoing work is focusing on optimization of the ASO delivery system, by evaluating it in the 3D-chip BBB models, to improve therapeutic delivery to the brain.

Methods:

SH-SY5Y cells were treated with GA to induce abnormal tau phosphorylation. Phosphorylation levels of Tau protein on Thr181, Ser199 and Ser396 were quantified using enzyme-linked immunosorbent assay (ELISA). Confocal imaging was utilized to compare the neurite length between undifferentiated SH-SY5Y cells and neuronal cells differentiated following Shipley's protocol. ASO-loaded liposomes were prepared with or without transferrin (TF) conjugation via EDC/NHS reaction. Liposomes were characterized by dynamic light scattering (DLS) and fluorescence plate reader. Then the permeability to BBB was assessed through confocal image and quantified by ImageJ analysis.

Results:

The MTT assay revealed the viability of cells was significantly reduced from 0.7 mM GA induction. GA treatment increased the intracellular at Thr181, Ser199 and Ser396 tau expression. A neuron-like model was established via differentiation of SH-SY5Y cells with retinoic acid (RA) treatment. Confocal imaging and Image analysis demonstrated that GA treatment reduced axon length, which was restored following ASO administration. According to the DLS measurement, the particle size and zeta-potential of the liposomes remained stable within 7 days of preparation. The ASO encapsulation efficiency reached 49.5%. The grafting rate of TF was 41%. TF-functionalized liposomes demonstrated enhanced permeation across BBB *in vitro* compared to non-functionalized liposomes.

Conclusions:

GA-treated SH-SY5Y cells successfully model tau hyperphosphorylation characteristic of AD. The *MAPT*-target ASO demonstrates neuroprotective effects in the neuronal cell model. TF-modified liposomes enhance ASO penetration across the BBB. Ongoing work focuses on optimizing the ASO delivery system for BBB penetration and evaluating its efficacy using advanced BBB models and analytical methods.