Efficient Non-viral Gene Therapy for Stargardt's Disease with pH-Sensitive Multifunctional Lipid ECO Plasmid DNA Nanoparticles

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Purpose

Monogenic retinal dystrophies can be treated with gene replacement therapy (GRT) due to easy localized delivery into retina. Here, we developed a nanoparticle-based GRT using pH-sensitive multifunctional lipid ECO and therapeutic ABCA4 plasmids to treat Stargardt's disease (STGD), which requires delivery of large *ABCA4* gene.

Methods

Therapeutic plasmids expressing ABCA4 induced by rod photoreceptor-specific RHO promoter and non-specific CMV promoter were designed. Subretinal treatments were performed using ECO/pRHO-ABCA4 and ECO/pCMV-ABCA4 nanoparticles to *abca4*$^{-/-}$mice (STGD model). Treatment efficacy was evaluated by analysis of A2E accumulation in the RPE after 6 months. ABCA4 expression was evaluated by qRT-PCR and IHC at 7 days and 8 months after a single injection.

Results

GRT using nanoparticles reduced A2E accumulation at least for 6 months. Demonstrated by HPLC (Figure 1A) and quantitative A2E levels (Figure 1B) of treated *abca4*$^{-/-}$ mice, an averaged 35% reduction in A2E levels was observed for ECO/pRHO-ABCA4 and a 15% reduction for ECO/CMV-ABCA4. ABCA4 mRNA (Figure 1C) were measured by PCR in mice treated with both nanoparticles. Expression driven by RHO promoter was 10-fold higher in treated eye than control at 8 months, but the expression driven by CMV was slightly but not significantly higher than control. IHC staining for ABCA4 protein (Figure 1D) revealed significant protein expression for ECO/pRHO-ABCA4 treated group and no significant difference between

ECO/pCMV-ABCA4 group and control. The expression driven by RHO demonstrated excellent tissue specificity in the retinal outer segments.