“Let There Be Light!” rAAV Mediated Delivery of shRNA in a Canine Model of Autosomal-dominant Retinitis pigmentosa

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Autosomal-dominant Retinitis Pigmentosa (adRP) is caused by diverse gain-of-function mutations in rhodopsin (RHO). The purpose of this study is to aid in the development of a mutation-independent gene therapy. The strategy consists of using RNAi to degrade the endogenous target transcript while sparing an introduced resistant mRNA (hardened) with an altered sequence. Development of a single therapeutic reagent to treat all RHO mutations will maximize utility, reduce cost and reach a larger affected population. Methodology consisted of characterizing the ability of 3 specially designed shRNAs to suppress the expression of Wild-Type and 2 Mutant RHOs through flow cytometry, fluorescence microscopy, and quantitative real-time PCR. Development of the gene therapy vector through the cloning of the hardened RHO into the AAV integrated shRNA plasmids followed. Results reflected that shRNA was able to suppress the expression of Wild-Type and Mutant RHOs. Suppression efficiency ranged: 33% to 99%. Formation of colonies was achieved in some trials during cloning of the hardened RHO gene into the rAAV2 integrated shRNA plasmids; however, litigation through diagnostic restriction digestion and sequence analysis showed absence of “hardened” RHO. The data clearly indicates that the shRNAs had a statistically significant effect in degrading the endogenous target transcript for all RHOs. This data suggests the efficacy and utility of the allele-independent gene therapy strategy In Vitro. AAV development requires two ligation events on either side of insert (low probability but achievable). The series of combination RNA suppression and replacement AAV vectors may also be useful for the treatment of adRP In Vivo.

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