CRISPR-Based Allele-Specific Editing for the Treatment of Autosomal Dominant Retinitis Pigmentosa (ADRP)

Albena Kantardzhieva1, Andrea D’amico2, Daisy Lam1, Rossano Butcher2, Angelica Messana1, Akiko Noma1, Ryo Takeuchi1, Mariacarmela Allocca1, Mike Lukason1, Andrew Scharenberg1, Eric Pierce2, Qin Liu2 and Abraham Scaria1

1Casebia Therapeutics LLC, Cambridge, MA
2Department of Ophthalmology, Ocular Genomic Institute, Mass Eye and Ear, Harvard Medical School, Boston, MA

Retinitis pigmentosa is a group of inherited diseases causing retinal degeneration and can result from defects in more than 60 genes. Rhodopsin is a G-protein-coupled receptor, and a pigment found in rod photoreceptor cells. A point mutation in codon 23 of the rhodopsin (RHO) gene (g.129528801C>A) leads to the substitution of proline with histidine (P23H). Accumulation of the misfolded mutant rhodopsin in rod photoreceptor cells results in primary rods degradation and progressive visual loss. This mutation accounts for 10% of all advanced retinitis pigmentosa cases. Gene therapy has been challenging because the dominant effect of the mutant protein needs to be suppressed or disrupted. CRISPR/Cas9 technology allows for in vivo targeted gene disruption by introducing site-specific double strand breaks and could potentially offer treatment for diseases associated with dominant mutations in human RHO gene. Here, we have developed a method for specific inactivation of the human RHO-P23H mutant allele using Cas9 from Staphylococcus aureus (SaCas9) and a single guide RNA (sgRNA) that is highly selective for the P23H mutation to preserve the function of the wild type allele. To identify appropriate guides, a K562 cell line with a homozygous g.129528801C>A mutation (RHO-P23H cell line) was generated and used to examine the allele specificity of sgRNAs carrying spacers ranging from 18-24 nt in length. Multiple guides were identified that efficiently discriminated between the WT and the P23H alleles. No “off target” genetic modifications were detected for any of the tested guides using either directed (bio-informatic) or undirected approaches. We are currently testing the top Cas9/sgRNAs in vivo using both constitutive and self-inactivating AAV vector-based delivery systems in knock-in mouse models carrying the normal human RHO or mutant RHO-P23H genes. Taken together, our results suggest that specific inactivation of the P23H allele using CRISPR/Cas9 holds promise as a potential treatment for patients with ADRP due to the RHO-P23H mutation.