Title: Gene/mutation-independent gene therapy for inherited eye diseases

Abstract:
Despite the recent success of gene supplementation therapy for monogenic recessive diseases, therapeutic approaches to treat dominantly inherited diseases fall behind. Recently, we developed a new gene knock-in (KI) therapy that exploits AAV-Cas9-mediated homology-independent targeted integration (HITI) of the wild-type coding sequence (CDS) into the 5' untranslated region (UTR), more specifically immediately upstream of the Kozak sequence, of the disease gene. We tested this approach in the heterozygous $Rho^{P23H/wt}$ mice, which carry the most common dominant point mutation found in autosomal dominant Retinitis Pigmentosa (adRP) patients. We show that HITI-AAVs can mediate highly efficient gene insertion in mouse *Rho* 5'UTR in vivo. NGS results showed 43% alleles with successful 5'UTR *Rho* KI, 44% alleles with 5'UTR INDELs, and 13% unmodified alleles in the purified AAV-transduced rods. The $Rho^{P23H/wt}$ mice had significantly prolonged photoreceptor survival and visual function following the 5'UTR gene KI treatment. In summary, we developed a mutation-independent gene KI approach that targets 5'UTR of the disease gene and demonstrated its therapeutic potential to treat dominant diseases.