Splice Modulation Therapy in Inherited Retinal Diseases

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Background: Antisense oligonucleotides (AONs) are small DNA/RNA molecules able to modulate pre-mRNA splicing, including the correction of aberrant splicing processes due to genetic mutations. Therapeutically, AONs are employed for a variety of inherited diseases, including Duchenne muscular dystrophy, spinal muscular atrophy, metabolic disorders etc. In this talk, I will mainly focus on the use of AONs for inherited retinal diseases (IRD), a group of disorders that is highly heterogeneous, both at the genetic and clinical level. Our proof-of-concept work on the correction of a recurrent splicing defect underlying *CEP290*-associated Leber congenital amaurosis (LCA), one of the most severe subtypes of IRD, has led to the initiation of a clinical trial, with positive interim results, demonstrating safety and in some subjects efficacy upon intra-ocular AON delivery. We expanded the use of AONs for the treatment of other subtypes of IRD, including Stargardt disease which is caused by *ABCA4* mutations.

Methods: Recently, we established a panel of *ABCA4* midigenes together encompassing the complete *ABCA4* gene, with which the effect of any *ABCA4* variant on pre-mRNA splicing can readily be assessed *in vitro*. Following site-directed mutagenesis, wild-type or mutant midigenes were transfected into HEK293T cells to identify variants affecting *ABCA4* pre-mRNA splicing. For variants resulting in aberrant *ABCA4* splicing, AONs were designed, and co-transfected with the midigenes to assess their efficacy to correct splicing defects. For several variants, patient-derived fibroblasts and/or iPSC-derived photoreceptor progenitor cells were treated with the AONs, and rescue efficacy was determined at the RNA level.

Results: In total, we identified more than a dozen different *ABCA4* variants that affect pre-mRNA splicing. The majority of variants resides in introns, and leads to the insertion of pseudoexons that are predicted to disrupt the reading frame and thus loss of ABCA4 protein function. Another, recurrent, *ABCA4* variant (c.768G>T) led to an exon extension of 35 nucleotides(nt), thereby also disrupting the reading frame. Whereas some variants showed splicing defects in all cell types tested, others showed a more retina-specific splicing defect. For all variants that resulted in pseudoexon insertion, this process could be corrected by one or more AONs specifically targeting the corresponding pseudoexon. Intriguingly, also the 35-nt extension caused by the common c.768G>T variant could be rescued completely by blocking the alternative splice donor site in intron 6, forcing the spliceosome to use the original splice donor site of exon 6.

Conclusion: AONs appear to be an effective and versatile tool to correct different types of splice defects that are caused by *ABCA4* mutations. Given the promising data obtained so far in a clinical trial using AONs for the treatment of *CEP290*-associated LCA, AONs may serve as a broadly applicable therapeutic strategy, not only for Stargardt disease but potentially also for other genetic subtypes of inherited retinal disease caused by splicing defects.