Genetic Therapy for Dominant-Negative Disorders

Internship project Otorhinolaryngology/Genetics (Erik de Vrieze & Erwin van Wijk)

Background:

Genetic therapies for recessively inherited diseases are rapidly emerging. However, there are no therapies that arrest disease progression of dominant non-haploinsufficiency disorders. These disorders, caused by mutations with a dominant-negative disease mechanism or deleterious gain-of-function effect, include several types of autosomal dominant hearing impairment (e.g. DFNA9, DFNA11), Huntington's disease, osteogenesis imperfecta and familial exudative vitreoretinopathy (FEVR). Gene augmentation therapy will not abrogate the genetic defect and gene repair therapy is risky due to potential deleterious off-target effects or unwanted damaging of the healthy wildtype allele. Silencing of the mutated allele has high therapeutic value but has never been used to develop treatments for genetic disorders with a dominant-negative mechanism. Clinical development of currently available strategies, silencing gene expression at the mRNA level, is hindered by the limited options for allele-specific targeting and potential off-target effects.

Here, we take inherited deafness DFNA9, caused by mutations in the *COCH* gene, as example of dominant non-haploinsufficiency disorders. Patients with *COCH* mutations suffer from a rapidly progressive form of hearing impairment. The age of onset of DFNA9 is strongly variable, and ranges from 30 to 60 years of age.

The dominant disease mechanism of DFNA9 is well established. The *COCH* gene encodes the protein Cochlin, an extracellular matrix protein in the cochlea. Heterozygous missense mutations in *COCH* result in the accumulation of toxic Cochlin deposits in the cochlea, in which also wildtype Cochlin is sequestered. The *COCH* gene contains a prevalent founder mutation (c.208C>T; p.Pro51Ser) that underlies hearing loss in ~1000 patients in the Dutch/Belgian population. Using long-read targeted genome sequencing, we identified multiple deep-intronic variants that are specific for the mutant allele. Using different genetic therapy approaches, we aim to exploit these mutant-allele specific variants to develop a therapeutic strategy that is able to silence specifically the mutant copy of the COCH gene.

Research question/goal

Development of a save and efficient allele-specific therapeutic strategy to block mutant, but not wildtype Cochlin expression.

Techniques

- Cell culture and sorting
- iPS-to-otic progenitor cells differentiation
- Antisense oligonucleotides
- CRISPR-Cas9
- (q)PCR

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