Targeting for success: mechanistic insights into microRNA-based gene therapy for Huntington disease

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Appendix &

English Summary

Nederlandse samenvatting

Resumen en Español

List of abbreviations

Curriculum vitae

List of publications

Acknowledgements

English Summary

Huntington Disease (HD) is a fatal neurodegenerative disease caused by a CAG repeat expansion in the exon 1 of the huntingtin (HTT) gene. This mutation is translated into a polyglutamine tract in the mutant HTT protein which confers a toxic gain-of-function inducing aggregation and cell death. Due to its monogenic cause and undeniably polyglutamine-dependent toxicity, mutant HTT protein became the ideal target of choice for many therapies in development.

One of the most advanced HTT lowering approaches it is a miRNA-based gene therapy, consisting of an engineered microRNA targeting HTT (miHTT), delivered by adeno-associated virus (AAV), referred from now on as "AAV-miHTT". The direct administration of AAV-miHTT in the striatum of animal models has demonstrated efficacy in lowering the mutant HTT protein and rescuing HD phenotypes, and safety in toxicology studies in nonhuman primates. However, during the last years, relevant studies in the field have demonstrated that HD pathology is rather complex and new hypothesis are being debated as the cause of onset and progression of the disease. Moreover, the lack of efficacy in first HTT lowering clinical trials suggests that the lowering of the mutant HTT protein might not be sufficient to delay the progression of the disease.

This thesis describes novel mechanistic insights of miRNA-based gene therapies including the targeting of different HTT species, the therapeutic spread between neuronal cells and the development of translational biomarkers to monitor its effect in the affected brain regions.

Chapter 1 is a general introduction to the field of HD and includes an up-to-date comprehensive analysis of the most relevant questions and challenges for the development of successful therapies. We describe w*hat, how,* and *where* to target and treat the disease and the main outcomes to assess the efficacy (*how good*) and toxicity (*how bad)* to ensure optimal translation to patients.

Increasing evidence indicates that, besides the mutant full-length HTT protein, exon 1 HTT (HTTex1) fragments generated by aberrant splicing are highly prone to aggregate and contribute to HD pathology. Generation of pathogenic HTTex1 highly correlates with CAG repeat expansion and somatic instability and the slow but steady accumulation over time might explain why HD patients develop neurodegeneration and symptoms in adult life. This finding also suggests that reducing the expression of HTTex1 transcripts might achieve a greater therapeutic benefit than targeting only the full-length mutant HTT and conversely, strategies that exclusively target full-length HTT might not prevent HD pathogenesis. In **chapter 2,** we evaluated the ability of AAV5-miHTT to reduce the levels of aberrantly spliced HTTex1 in the brain of two mouse models of HD. Intrastriatal administration of AAV5-miHTT

resulted in dose-dependent significant lowering of both full-length mutant HTT and HTTex1 in striatum and cortex of Q175 knock-in and humanized Hu128/21 mice at two and four months, respectively. These results demonstrate that AAV5-miHTT gene therapy is an efficient approach to lower both full-length HTT and the highly pathogenic HTTex1 levels, and support the added therapeutic benefit of exon 1-targeting therapeutics for HD.

One of the biggest challenges in the treatment of brain diseases is to achieve adequate biodistribution and coverage of all affected brain areas, as well as sustained effect in time. For this, preclinical studies in large animals are important for successful translation into patients. In **chapter 3** we investigated the transability and long-term efficacy of AAV5-miHTT administered in the striatum in transgenic HD minipigs. We demonstrated that direct intrastriatal delivery of AAV-miHTT therapy results in widespread and persistent HTT protein lowering in all brain areas that are known to be affected in up to one year. Potential reliable biomarkers to monitor expression and therapeutic efficacy were also investigated. This study, together with separate toxicology studies, was essential to support the clinical development of AAV5-miHTT into the clinic.

Regarding the mechanism of action of AAV-miHTT, we have proposed a novel mechanism of secretion and dissemination of gene therapeutics mediated by extracellular vesicles (EV). In **chapter 4** we investigated the secretion of engineered miRNAs and its potential use as suitable markers to monitor the expression and durability of gene therapies in the brain. Moreover, measurable engineered miRNA levels enriched in EVs were detected in the CSF of nonhuman primates up to 2 years after intrastriatal infusion. These results support the use of EV-associated miRNAs as novel translational pharmacokinetic markers in ongoing clinical trials of gene therapies for neurodegenerative diseases.

Mechanisms that diffuse therapeutics beyond initially transduced cells might contribute to elimination of intracellular disease-causing proteins in all affected brain cells and regions and eventually prevent disease progression. In **chapter 5**, we investigated the EV-mediated functional transfer of AAV-delivered therapeutic microRNA molecules from AAV-miRNA-corrected neuronal cells to neighboring cells. We demonstrated the uptake of EVs by neuronal cells and the efficacy of miRNAs to lower target genes upon transfer to recipient cell.

To finalize, **chapter 6** is a general discussion of the main findings of this thesis in the context of HD field and miRNA-based gene therapies for neurodegenerative diseases. We further discuss the needs and future perspectives for a successful gene therapy to treat HD as well as other neurodegenerative diseases.