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Development of novel artificial microRNA variants for selective reduction of mutant protein level as a therapeutic approach for Huntington's disease

Huntington's disease (HD) and other disorders caused by CAG repeat expansions belong to a group of uncurable neurodegenerative diseases. One of the most promising therapeutic strategies involves usage of the natural mechanism of RNA interference (RNAi). However, a key challenge in this approach lies in balancing therapeutic efficacy with safety, particularly with regard to minimizing off-target effects. A previously developed artificial microRNA, amiR136-A2, targeting CAG repeats and containing a single mismatch at position 8 of the guide strand, demonstrated good *HTT* silencing efficiency and allele-selectivity. Nevertheless, its further development was limited by certain drawbacks, such as reduced activity in the cerebral cortex and the presence of fully complementary off-target sequences.

The aim of this doctoral dissertation was to improve the previously designed amiR136-A2 by introducing an additional nucleotide substitution to enhance safety while maintaining or improving efficiency. Among the variants tested, the molecule amiR136-13A, featuring an extra mismatch at position 13, showed increased silencing efficiency against mutant *HTT* in HD cell models and *in vivo*, achieving a comparable therapeutic effect at half the AAV5 vector dose compared to the original construct.

amiR136-13A exhibited a reduced number of potential off-target sequences, a favorable cellular processing profile, increased guide strand release compared to amiR136-A2, and high 5′-end homogeneity in the striatum of YAC128 mice. In the *in vivo* model, the molecule was well tolerated up to 28 weeks post-injection, did not trigger microglial or astroglial activation, did not increase serum levels of neurofilament light polypeptide (NfL), and demonstrated no off-target effects.

Furthermore, in a cell model with inducible *HTT1a* expression, amiR136-13A effectively reduced levels of the toxic, truncated huntingtin isoform, which is known to significantly contribute to HD pathogenesis.

Taken together, the results indicate that amiR136-13A is a safer and more effective alternative to amiR136-A2, and represents a promising candidate for further development as a potential RNAi-based therapeutic tool for HD and other polyglutamine disorders.