Development of an allele-selective therapeutic strategy for polyglutamine diseases using vector-based RNA interference technology tools

MSc Anna Kotowska-Zimmer

Abstract

Polyglutamine diseases (polyQ) are a group of genetic, neurodegenerative disorders that share a common feature of the presence of a mutation associated with the expansion of CAG repeats in specific genes. This mutation leads to the formation of toxic protein containing an abnormally elongated polyglutamine tract. To the group of polyQ diseases, we can include Huntington's Disease (HD), spinocerebellar ataxias type 1, 2, 3, 6, 7, and 17 (SCA), dentatorubral-pallidoluysian atrophy (DRPLA), and spinal and bulbar muscular atrophy (SBMA). Depending on the disease, neurons degenerate in different regions of the brain, and thus various neurological and psychological symptoms occur in most cases in the fourth decade of life. The occurrence of the diseases is influenced by the number of CAG repeats in the certain genes, which depends on the disease, e.g. for HD it is >40 CAG repeats, and for SCA3 >60. Unfortunately, despite many years of research, these diseases are incurable and only their symptoms are alleviated. One of the most promising therapeutic strategies that aim to reduce the development of these diseases is RNA interference (RNAi). Results of many *in vitro* and *in vivo* experiments confirmed the effectiveness of RNAi tools, such as siRNA (small interfering RNA), shRNA (short-hairpin RNA), and amiRNA (artificial miRNA) in the silencing of the expression of mutant genes.

Therefore, the main goal of my dissertation was to develop a therapeutic strategy that selectively silences the expression of mutant genes which cause polyQ diseases, with the use of vector-based tools of RNAi technology targeting CAG repeats.

In the first step of the study, I designed a series of shRNAs with embedded siRNAs targeting repeats and containing mismatches at certain positions to the target sequence, which allowed for an allele-selective effect by inhibiting translation.

I tested their efficiency and allele-selectivity in cellular models of polyQ diseases such as HD, SCA3, SCA7, and DRPLA. I showed that they are precisely processed in cells, cause a specific silencing of mutant proteins, and do not cause overt toxicity in cell lines. Due to the fact, that

the literature shows the toxicity of shRNAs *in vivo*, in the next steps of my study I undertook to investigate and analyze the knowledge of amiRNA as well as design and test my amiRNAs. In the review, in which I am the main author, the most up-to-date knowledge of miRNA biogenesis, structural and sequence features of pri-miRNAs affecting their cell processing, as well as the rules of designing amiRNAs and the use of this type of tools in potential therapies of neurodegenerative, cancer and viral diseases were collected. The acquired knowledge allowed me to design amiRNAs that were embedded into 4 different backbones to silence the level of mutant HTT in the HD fibroblast. The most efficient and selective molecule contained the same siRNA insert as the most effective shRNA containing A substitution at the 8^{th} position from the 5' end of the molecule. This amiRNA was embedded into the pri-miR-136 backbone, which shows the homogenous processing in cells. I tested both molecules, shRNA, and amiRNA in the mouse model of HD, YAC128. I showed that both of them cause efficient and allele-selective reduction of mutant HTT. I also confirmed that shRNAs can be toxic in mice. In the case of amiRNA, it did not cause any overt symptoms of toxicity. The immunofluorescence stainings of mouse brain sections did not show micro- and astrogliosis after mice injection with AAV5 carrying amiRNA. Moreover, this molecule led to a reduction in the number of HTT aggregates in the mice striatum. A partial improvement in some motor and learning deficits was also observed.

In conclusion, CAG-targeting shRNAs and amiRNAs designed by me, efficiently and allele-selectively silence the expression of mutant proteins. In addition, amiRNAs compared to shRNAs are safe in mice and lead to the partial improvement of the YAC128 phenotype.