The use of artificial miRNAs in the therapy of Huntington's Disease

Huntington's disease is a neurodegenerative polyglutamine (polyQ) disease caused by the expansion of unstable CAG triplet repeats in the *HTT* gene coding huntingtin. Other diseases caused by this type of mutation and belonging to the group of polyQ diseases include dentatorubral-pallidoluysian atrophy (DRPLA), spinocerebellar ataxias (SCA) type 1,2,3,6,7,17 and spinal and bulbar muscular atrophy (SBMA). Expansion of CAG repeats results in an abnormal protein with elongated polyglutamine domain and forms intracellular aggregates leading to a degeneration of neurons. Currently, there is no therapy that stops or slows the neurodegeneration process. For many years, research has been conducted to select the best therapy for patients. One of the very promising approaches is the use of RNA interference (RNAi) technology, especially artificial miRNAs (amiRNAs). In our laboratory, RNAi molecules targeting CAG repeats were designed and patented. These molecules are delivered to the cells by viral vectors, which allows for long-lasting and stable silencing of mutant proteins after a single treatment. Furthermore, we use a strategy that allows for reducing only mutant protein, leaving normal protein intact (selective strategy). Artificial miRNAs resemble naturally occurring primary miRNAs (pri-miRNAs), but contain the introduced sequence which targets a specific gene, in our case *HTT*. We showed that this reagent significantly reduces mutant huntingtin in patient-derived cells and a mouse model of HD. However, before proceeding with clinical trials, the safety of the proposed therapy should be carefully assessed, as it is known that the risk of side effects depends on the amount of the administered therapeutic molecule.

Therefore, the question is whether the efficiency of amiRNA can be increased so as to obtain a similar therapeutic effect using a lower vector dose? How to achieve this effect without altering the selectivity of the amiRNA?

Hence, the goal of this project is to increase the efficiency of our amiRNAin silencing of mutant huntingtin. One of the approaches will be to modify the sequence of the molecule in order to release more insert in the cell. Moreover, there will be used combinatorial strategy in which additional molecules will be designed. They will be delivered to cells with our amiRNA to increase the blockade of toxic protein production.

Recent years were abundant in research conducted on HD, which allowed to start clinical trials and gave hope to patients. However, most of these studies use a non-selective strategy, which results in the silencing of mutant and normal protein. What is more, molecules that are used have to be administered repeatedly and not one time, as in the vector-based strategy. Therefore, the development of selective and universal strategy of reducing polyQ proteins is greatly important for patients suffering from rare diseases.