

Optimization of CRISPR-Cas9 technology in the context of targeting repetitive sequences

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SUMMARY

Polyglutamine disorders (polyQ) are rare neurodegenerative genetic diseases caused by the expansion of CAG repeats in associated genes. Huntington's disease (HD) occurs most frequently among polyQ disorders. Although the *HTT* gene responsible for this disease has been well characterized, aspects of HD pathogenesis are still unknown. Therefore, it is one of the reasons for the lack of potential therapy.

The CRISPR-Cas9 technology gives a broad range of opportunities to create new cellular models and establish potential therapies. Nevertheless, applying the CRISPR-Cas9 technology to explore repetitive sequences is still challenging due to a long CAG sequence which can form secondary structures of DNA and RNA, and *slipped strands*. Therefore, long repetitive stretches are problematic for the majority of methods used in molecular biology.

The doctoral thesis aimed to optimize the CRISPR-Cas9 technology in the context of targeting repetitive sequences. In my research, I used the CRISPR-Cas9 system and its modifications to induce DNA breaks in sequences flanking CAG repeats and within a repetitive sequence in the *HTT* gene. These studies have contributed to establishing a new potential therapeutic approach that caused "seamless" CAG repeat excision. To better explore the editing outcome within the CAG repeat sequence, we created a new method in the genome engineering field called Eva-CRISPR. This method is dedicated to detecting specific and unspecific cuts and distinguishing the DNA repair events. Moreover, the doctoral thesis included issues related to CRISPR-Cas9-based creating new cellular models of HD. In this work, we utilized new cell lines to test the therapeutic potential of drugs and to explore some aspects of HD pathogenesis.

The results obtained from these studies contributed to improving the CRISPR-Cas9 technology in the context of its activity within repetitive sequences and to increasing the efficacy and specificity of DNA modification in the *HTT* gene. Moreover, exploring editing effects within CAG repeats tract arose new questions: which DNA repair mechanisms are involved in repetitive sequences repair and how to manipulate them? A new method established during Ph.D. studies can be utilized to determine the cleavage specificity of CRISPR-Cas9 components, including new proteins belonging to the CRISPR family. New cellular HD models can be utilized to explore the effect of the CAG repeats expansion on cellular function and phenotype. Moreover, they are necessary to study various aspects of HD pathogenesis.