Identification of processes involved in CAG repeat contraction following double strand break induction in the *HTT* gene

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Abstract

Expansions of CAG repeats are the cause of many neurodegenerative, hereditary, and currently incurable human diseases (polyQ diseases). One such condition is Huntington's disease (HD). An innovative therapeutic strategy dedicated to this disease is the controlled shortening of the repeat tract using genetic engineering tools such as CRISPR-Cas9. Induction of double-strand breaks (DSBs) within CAG repeat tracts leads to their instability, resulting in both expansions and contractions of the tract. However, the mechanisms underlying this observed instability are not fully understood. Previous studies suggest the involvement of certain DNA repair pathways in this process. Nevertheless, there is a lack of studies on DSBs repair mechanisms within CAG repeats in an endogenous locus in a human cell model.

The aim of this study was to better understand the mechanisms of DSBs repair in CAG repeat regions at an endogenous *locus* in human cells. The results of this work revealed the complexity of DSBs repair in regions containing CAG repeats. They enabled the identification of factors and mechanisms involved in this process at various stages and contributed to the development of models of DNA repair in regions with CAG repeats in humans. This study demonstrated the involvement of DNA end resection in DSBs repair within CAG repeat regions and presented evidence suggesting a correlation between the extent of resection (and consequently, the choice of DNA repair mechanism and its outcome) and the length of the repeat tract. A strong influence of the DSB position relative to the CAG repeats and the flanking sequences on the choice of DNA repair pathway was observed. It was shown that repair of a DSB induced at the beginning of the CAG repeat tract involves the NHEJ mechanism and DNA polymerase theta (POLθ), which participates in the deletion of the entire repeat tract. It was found that induction of a DSB within the repeat tract results in its contraction while preserving the reading frame, a process involving the NHEJ pathway and a mechanism utilizing extensive resection and homologous recombination proteins such as RAD51.

As a result, this work contributed to a better understanding of the phenomena responsible for the instability of CAG repeat tracts. Expanding knowledge in this field may aid in the development of effective and safe therapies for polyQ diseases using genome editing technologies such as CRISPR-Cas9.