ASO-mediated modulation of HTT 3’UTR polyadenylation alters the level of huntingtin in human cell lines

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Background: Huntington’s disease (HD) is an incurable neurodegenerative disorder caused by a mutation in the HTT gene that leads to the production of a protein (huntingtin) with an abnormally elongated polyglutamine tract. Disease-modifying therapeutic strategies aim to downregulate HTT expression. HTT mRNA contains two major polyadenylation signals (PASs) in the 3'UTR that determine the 3’end of the transcript. Selection of proximal or distal PAS - alternative polyadenylation (APA) - can affect mRNA abundance, stability, localization, and also the encoded protein level. Antisense oligonucleotides (ASOs) can modulate polyadenylation by sterically blocking recognition of the PAS. Method: We aimed to determine the ratio of HTT 3’UTR isoforms in various human cell lines, including HD cells, and to investigate how the modulation of polyadenylation patterns affects huntingtin level. ASOs (morpholino oligomers, PMOs), targeting the region of proximal or distal PAS in HTT mRNA, were tested in the HEK293T line, HD fibroblasts, and HD neural stem cells (NSCs). We analyzed the effects of ASOs on HTT expression using RTqPCR and western blot, and applied polysome profiling and RNA-seq to assess the molecular effects of HTT APA. Results: ASOs efficiently modulated HTT polyadenylation in all tested cell lines. Lengthening of the HTT 3'UTR significantly decreased huntingtin levels without changing HTT mRNA levels. In contrast, ASO-mediated shortening of the HTT 3’UTR had a tendency to increase huntingtin levels. RNA-seq analysis performed in HD NSCs showed that the tested ASOs did not induce significant transcriptomic changes, except for activation of the MSANTD1 gene, due to its genomic location directly downstream from the HTT gene. Conclusion: Our results identify APA as a post-transcriptional mechanism regulating HTT expression and establish polyadenylation modulation as a promising strategy for regulating huntingtin levels. Funding: Medical Research Agency, Poland [2021/ABM/05/00004] Patent application: [WIPO ST 10/C PL455513