Introduction

QRX-704 is a novel antisense oligonucleotide-based therapeutic approach, aiming to mitigate mutant Huntingtin (mHTT) toxicity, while maintaining physiological HTT function. Fragmental cleavage of mHTT generates toxic N-terminal fragments, a cascade of proteolytic cleavages, initiated by caspase-6 cleavage at position D586. Cleavage of mHTT generates toxic N-terminal fragments that are hypothesized to be the main contributor to the pathogenesis of Huntington’s disease (HD). These fragments are formed from a cascade of proteolytic cleavages, initiated by caspase-6 cleavage at position D586. Importantly, HTT cleavage is required for GTPase functions in the N160 mouse model. Moreover, nHTT cleavage in turn increases caspase-6 activity, driving pathology in a toxic forward-feedback loop (Figure 2A). While toxicity stemming from mHTT cleavage is the primary pathogenic mechanism, data indicate that the HTT loss of function exerts secondary pathology. In addition, mHTT and/or nHTT fragment may be critical for toxicity of HTT-targeted therapies when restoring patients for a long period of time.

Objectives

QRX-704 functions at the pre-mRNA level by activating an alternative HTT splice-isoform (HTT Δ12), removing the critical HTT586 caspase-6 cleavage site, thus preventing formation of toxic N-terminal fragments (Figure 18). The aim of the study is to pharmacologically characterize QRX-704 for preclinical development, and describe the novel HTT Δ12 isoform.

Material and Methods

QRX-704 splice-activating activity was tested and optimized in cultured HD fibroblasts (GM04857) and HD-iPSC-derived mature neural cultures (GM04857) and (GM04223), both from Coriell Institute, assessed by droplet digital PCR and western blotting. Biophysical and biochemical HD iPSC-derived mature neural cultures (GM04857) and (ND4223), both from Coriell Institute of Science and Technology (KAIST), Daejeon 34141, Korea, (4) Centre for Molecular Medicine and Therapeutics (CMMT), CFRI, Department of Medical Genetics, University of British Columbia, 950 West 28th Avenue, Vancouver, BC V5Z 4H4 Canada.

Results

QRX-704 sequence optimization in HD fibroblasts and iPSC-neurons

Biophysical and biochemical assays screened and optimized in HD fibroblasts, assessed by droplet digital PCR and western blotting. Biophysical and biochemical characterization of HTT Δ12 was performed using purified protein, with caspase-6 cleavage assays. CD-spectroscopy, and protein-translational modification mapping. Transient transfection assays in Sf9 insect cells expression of QRX-704 in wild type and HD-iPSC mouse model used to study pharmacological activity. Quantitative reverse transcriptase PCR biobidirection assessed by fluorescent in situ hybridization, reactive gliosis was studied by immunohistochemistry, to better quantify HTT Δ12 protein in vivo, isoform-specific antibodies were developed and used to detect HTT Δ12 in HD-iPSC brain tissue.

In vivo evaluation of QRX-704 activity

QRX-704 was administered to FVB-YAC128 mice and sacrificed after 14 days for protein analysis, using crude antibody supernatant, which allowed detecting HTT Δ12 protein. QRX-704 alleviates reactive gliosis, assessed by fluorescent in situ hybridization (Figure 6A). Generation of exon 12 isoform-specific monoclonal and polyclonal antibodies, directed against peptides for mHTT and nHTT isoform (Figure 7A), rescues Western blot analysis (Figure 7B).

Conclusion

QRX-704 constitutes a novel therapeutic approach to HD, potentially preventing toxicity of mHTT while maintaining HTT function.

QRX-704 alleviates all pathologies of YAC128 brain, assessed by novel antisense oligonucleotide therapy designed for HD. Preclinical evaluation of QRX-704 in HD iPSC neural cultures (GM04857), and HD iPSC-derived mature neural cultures (GM04857) and (ND4223). Results were verified in HD-iPSC neural cultures (Figure 28).

In vitro evaluation of QRX-704 activity

QRX-704 was administered to FVB-YAC128 mice and sacrificed after 14 days for protein analysis, using crude antibody supernatant, which allowed detecting HTT Δ12 protein (Figure 6B). In vivo evaluation of QRX-704 activity

QRX-704 alleviates reactive gliosis, assessed by fluorescent in situ hybridization (Figure 6A). Generation of exon 12 isoform-specific monoclonal and polyclonal antibodies, directed against peptides for mHTT and nHTT isoform (Figure 7A), rescues Western blot analysis (Figure 7B).

Conclusion

QRX-704 alleviates reactive gliosis, assessed by fluorescent in situ hybridization (Figure 6A). Generation of exon 12 isoform-specific monoclonal and polyclonal antibodies, directed against peptides for mHTT and nHTT isoform (Figure 7A), rescues Western blot analysis (Figure 7B).

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