78 A DNAzyme that cleaves CAG repeat RNA in polyglutamine diseases

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Polyglutamine (polyQ) diseases are a group of incurable neurodegenerative disorders that are charactertic of CAG repeat expasnions in coding regions of unrelated proteins, including Huntington's disease (HD), spinocerebellar ataxia (SCA types 1, 2, 3, 6, 7 and 17), dentatorbropallidoluysian atrophy (DRPLA), and spinobulbar muscular atrophy (SBMA). The mutant polyQ proteins form nuclear and cytoplasmic aggregates that have been proposed to cause DNA strand breaks, impaired protein homeostasis, repeat-associated non-ATG (RAN) translation, transcription dysregulation, dsiruption of nucleocytoplasmic transport and endpalsmic reticulum architecture, autophagic defects, and mitochondrial dysfunction. The aggregation related toxicity may become dominant as disease deteriorates; thus, eliminating or reducing the mutant polyQ proteni load has become a major disease intervention that has great impacts on ameliorating downstram pathological defects. Human and animal studies using CRISPR-Cas or antisense oligonucleotide (ASO) therapeis indicate that the therapeutic efficacy may vary from disease to disease. However, targeting both mutant and normal copies of the polyQ protein does not associate with any pathology (at least in the case of HD and SCA3). In addition, repeat-based ASOs may favorably target the mutant polyQ RNA in an HD mouse model. These observations clearly outlined the possibility and benefit of using a single repeat-based agent to reduce mutant polyQ RNA and protein load across mulitple polyQ diseases.

In this study, we developed an RNA-cleaving DNAzyme that binds to the repeat CAG RNA and cleaves at each CAG repeat unit. By optimizing binding arm lengths and incorporating chemical modifications, we demonstrate that the DNAzyme can cleave in vitro transcribed CAG RNA at physiologically relevant ionic concentrations. The DNAzyme can be packaged in our proprietary liposome formulation that facilitates the crossing of the blood brain barrier endothelial cells and the uptake by neuroblastoma cells. We demonstrate the DNAzyme can eliminate or knockdown a panel of expanded polyQ proteins in HEK293 cells and induced pluripotent stem cell-derived neurons and favorable reduces the mutant polyQ protein in patient derived fibroblasts. This study highlights the therapeutic applicability of the developed DNAzyme in treating polyQ diseases.