Review

Drugging unconventional targets: insights from Huntington's disease

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Classical targeted drug discovery is based on targeting druggable targets, typically kinases and receptors of which the function can be agonized or antagonized. This strategy meets difficulties in cases such as Huntington's disease (HD) and similar neurodegenerative disorders, where the pathological function of the protein causing the disease is not clear. HD is caused by mutant HTT protein (mHTT) containing an expanded polyglutamine (polyQ) stretch, but the function of mHTT and how mHTT causes HD are unknown, thus preventing efforts to screen for mHTT 'inhibitors'. However, HD is appealing for drug discovery because the genetic mutation is clear, as compared with other major neurodegenerative disorders. Although mHTT is not a conventional 'druggable' target, one approach that appears promising is lowering its level, which might be applicable to other neurodegenerative disorders and proteinopathies linked to aberrant accumulation of proteins. Here we review mHTT lowering strategies that might provide promising avenues for drugging such diseases.

Protein accumulation and diseases

A hallmark of most neurodegenerative disorders is the accumulation and aggregation of misfolded disease-causing proteins, such as amyloid β in Alzheimer's disease [1], α-synuclein in Parkinson's disease [2], and TDP-43 in amyotrophic lateral sclerosis [3]. The function of these proteins and the exact etiology about how these proteins cause the diseases are unclear. As a result, it is impossible to establish functional assays to identify 'inhibitors' that inhibit the pathological functions of these proteins. By contrast, reducing levels of disease-causing proteins would in theory suppress all downstream toxic effects and thus provide a promising approach for disease treatment. This concept has recently been studied in polyglutamine (polyQ) diseases (see Glossary), especially Huntington's disease (HD).

HD is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in exon 1 of the Huntingtin gene (*HTT*), conferring mutant huntingtin protein (mHTT) with cellular toxicity [4] (Figure 1). Because of its simplicity in genetics and its relatively high prevalence [5], HD represents a robust model of polyQ and, possibly, other neurodegenerative diseases. Understanding the pathology of the disease, however, is confounded by the unclear cellular functions of HTT, various protein forms adopted in cells, and its intricate interactions with hundreds of proteins [6–8]. Although wild type HTT (wtHTT) is neuroprotective and possibly plays a partial role in HD pathogenesis [9], the disease appears to be dominated by a gain of mHTT function, rather than the loss of wtHTT function [10]. Several studies indicate that mHTT abundance and proteostasis are predictors of neurodegeneration and disease severity [11,12]. Furthermore, a continuous synthesis of mHTT is critical for disease symptom maintenance, whereas the HD-relevant phenotypes are reversed by stopping the expression of mHTT fragments [13]. This strongly suggests that HD could be reversed if mHTT levels are reduced (Figure 1). Indeed, as we discuss in this review article, reducing mHTT protein levels by various

Glossary

Antisense oligonucleotides (ASOs): single-stranded antisense DNA or RNA that could suppress protein translation of certain mRNA with complementary sequences.
Duplex RNAs: double-stranded RNA molecules mediating target gene silence, such as shRNA and siRNA.
Intrathecal pump: a device delivering small molecules into the space between the spinal cord and the protective sheath surrounding it, normally at the waist position.
Polyglutamine (polyQ) diseases: diseases caused by CAG trinucleotide repeat expansion in the coding regions of certain genes; resulting in expression of mutant proteins with an abnormal polyQ tract. The discovered polyQ diseases include Huntington's disease, spinocerebellar ataxia 1,2,3,6,7, and 17, dentatorubropallidoluysian atrophy, and spinobulbar muscular atrophy.
Reactive oxygen species (ROS): chemically reactive molecules formed as byproducts of the metabolism of oxygen, including oxygen ions, peroxides, etc.
RNA-induced silencing complex (RISC): a protein complex with one strand of siRNA or microRNA activates RNase that cleaves the target RNA with complementary sequences.
Short hairpin RNA (shRNA): a type of RNA with a tight hairpin turn that could block target gene expression through RNA interference. Its expression is typically through delivery of plasmids or through viral or bacterial plasmids.
Small interfering RNA (siRNA): a type of double-stranded RNA that is typically 19–23 base pairs in length and capable of silencing the expression of the target gene via RNA interference.

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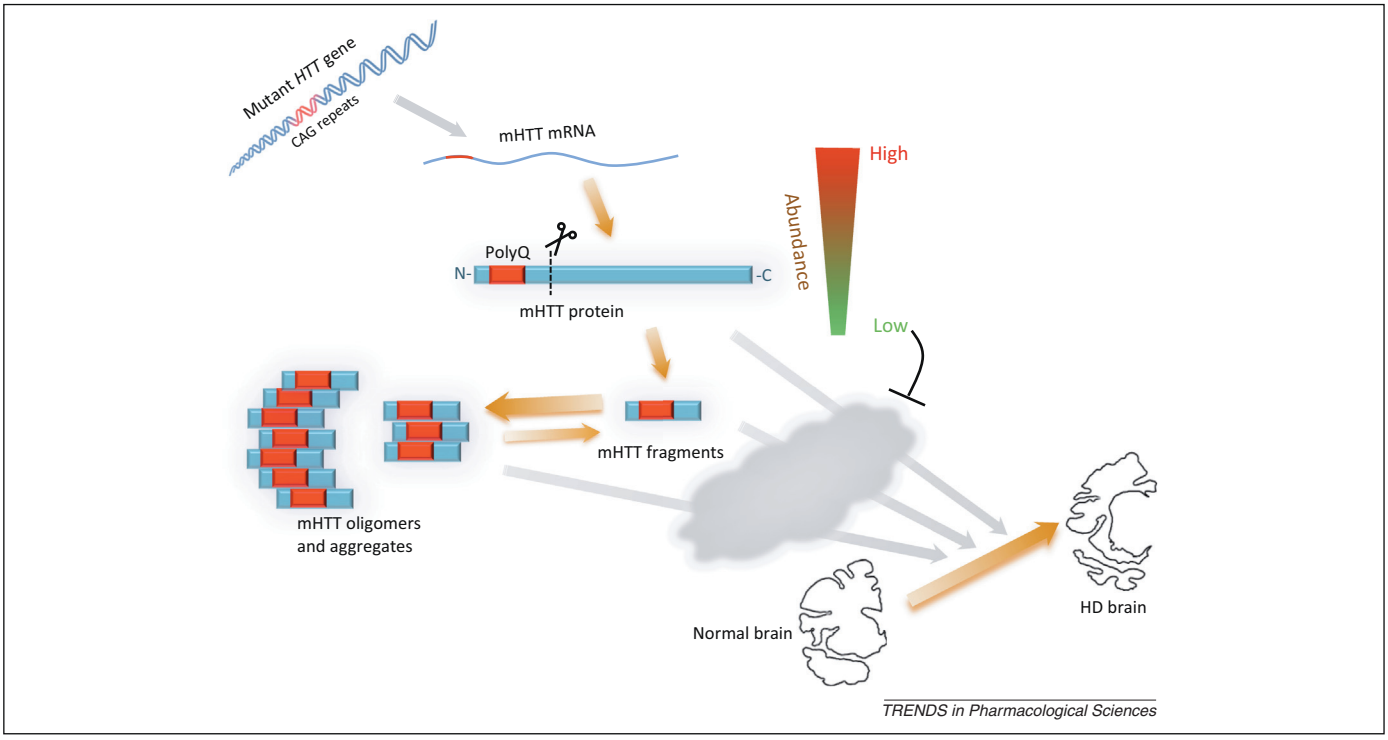

Figure 1. The rationale for targeting mHTT levels directly for HD. HD is a monogenetic disease caused by the mutant *HTT* gene with expanded CAG repeats, resulting in expression of the mHTT protein with the expanded polyQ stretch. mHTT is cleaved and forms oligomers and aggregates. The toxic gain of function of mHTT in its different forms (fragments, oligomers, aggregates) is the major cause of the disease, whereas the mechanism is unknown (the gray cloud), preventing potential discovery of mHTT 'inhibitors'. Meanwhile, the disease phenotypes could be reverted by reducing mHTT levels, which in theory mitigates all of its downstream toxicity effects. Thus, targeting mHTT levels directly is a promising therapeutic approach for HD treatment. Abbreviations: HD, Huntington's disease; polyQ, polyglutamine; mHTT, mutant huntingtin protein.

approaches has been shown to rescue HD-relevant phenotypes in various models. Interestingly, knocking out Htt in the embryos leads to lethality [14,15], whereas knocking down Htt in adult animals seems to be well tolerated [16,17]. Encouragingly, in a human embryonic stem cell (ESC)-derived neuronal model, a ~10–20% reduction of the soluble mHTT alone is sufficient to show a significant reduction of toxicity, whereas reducing wtHTT by up to ~90% seems to be safe [18]. This suggests that lowering mHTT at the expense of a partial loss of wtHTT is probably acceptable. Based on the evidence above, lowering mHTT levels appears to be an effective approach for therapeutic interventions, and two overarching strategic approaches are discussed in the following sections.

Targeting mHTT mRNA

HTT mRNA plays an indispensable role in mHTT protein synthesis. Reducing *HTT* mRNA levels and inhibiting its translation are two major mRNA targeting strategies. RNA interference (RNAi) provides a robust and effective tool for reducing target mRNA levels. Various efforts have been made to reduce mHTT levels via RNAi in the hope of achieving therapeutic effects (Figure 2). Delivery of Huntingtin-specific short hairpin RNA (shRNA) or small interfering RNA (siRNA) effectively attenuated neuropathology in mouse models and human ESC-derived HD neuronal models [16,18–21]. Notably, allele-selective suppression of mHTT could be achieved by duplex RNAs either based on single nucleotide polymorphism (SNP) differences between mutant *HTT* and wild type *HTT* allele populations, or complementary to the CAG expansion with one or more mismatched bases [22–25].

Single-stranded RNAs (ssRNAs) have also been demonstrated to produce potent and specific reduction of mHTT. Antisense oligonucleotides (ASOs) are able to effectively and selectively reduce mHTT levels [26,27], and transient ASO infusion into the central nervous system (CNS) produces sustained phenotypic reversal in HD mouse models [28]. In addition, ss-siRNAs with chemical modifications have been demonstrated to generate potent and selective inhibition of mHTT throughout the brain via intraventricular infusion in a HD mouse model [29].

Targeting mRNA is a very appealing concept because of its comparatively mature techniques and direct effects. Another attractive feature is that the strategies targeting mRNA are capable of having allele-specific clearance, which overcomes the potential risk of losing the wtHTT protein. Unfortunately, delivery is a huge hurdle for these strategies. Direct intraparenchymal injections have been used in many studies, but the therapeutic effects only occurred in the very limited adjacent region of the injection sites [16,19,21,30,31]. In several cases, intraventricular infusion with ASO or ss-siRNA successfully produced mHTT silencing in multiple brain regions and showed sustained therapeutic effects [28,29]. However, delivery in HD patients probably requires intrathecal pumps based on studies in non-human primates [28]. This approach is painful, expensive, and difficult to install (Figure 2), and thus far fails to achieve efficient delivery to the striatum [28], which is the major brain region that is affected in HD