Hunting diseases messages in the brain: emerging gene silencing approaches as novel therapeutic strategies for the treatment of Huntington's disease.

Delivering a disease-modifying treatment for Huntington's disease

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Huntington's disease (HD) is an incurable genetic neurodegenerative disorder that leads to motor and cognitive decline. It is caused by an expanded polyglutamine tract within the Huntingtin (HTT) gene, which translates into a toxic mutant HTT (muHTT) protein. Although no cure has yet been discovered, novel therapeutic strategies, such as RNA interference (RNAi), antisense oligonucleotides (ASOs), ribozymes, DNA enzymes, and genome-editing approaches, aimed at silencing or repairing the muHTT gene hold great promise. Indeed, several preclinical studies have demonstrated the utility of such strategies to improve HD neuropathology and symptoms. In this review, we critically summarise the main advances and limitations of each gene-silencing technology as an effective therapeutic tool for the treatment of HD.

Introduction

HD is an autosomal dominant neurodegenerative disease caused by a CAG triplet mutation within the HTT gene and affects approximately 5–10 in 100 000 people in European, Australasian and American populations [1,2]. In general, symptoms strike during middle age and include chorea (involuntary choreiform rapid movements), progressive motor and cognitive impairment, depressive-like behaviour, and mood alterations, usually leading to death 15–18 years after onset of clinical manifestations [3,4]. Unfortunately, current pharmacotherapy is only able to provide temporary symptomatic relief and fails to treat the underlying cause and the progression of the disease [5]. Therefore, the development of new therapeutic strategies to stop disease progression is crucial to improve the standard of care for patients with HD.

More than two decades have now passed since the identification of the causative mutation by The Collaborative Huntington's Research Group, and it is now well known that HD is caused by the expression of a muHTT protein with an abnormally long polyglutamine (polyQ) tract (>40 Q) close to its N terminus [6]. In addition, an increasing body of knowledge demonstrates that the disease is caused by a toxic 'gain-of-function' mechanism rather than merely by a loss of function of the wild-type HTT (wtHTT) protein. Indeed, muHTT has been shown to interact with many

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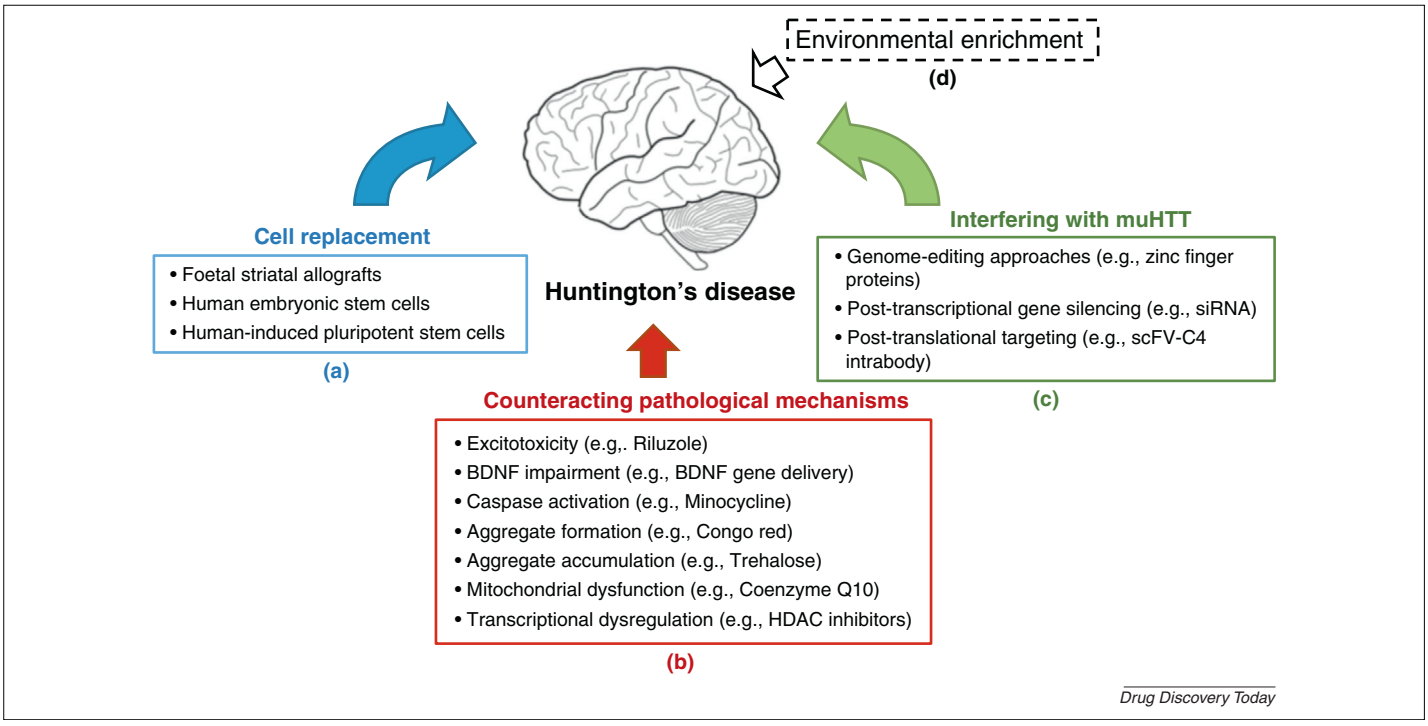

FIGURE 1

Novel emerging therapeutic approaches for Huntington's disease (HD). Several strategies are being considered as therapeutic alternatives to current symptom management: **(a)** cell replacement approaches, which aim to compensate for neuronal loss that occurs mainly in the striatum; **(b)** strategies that counteract the underlying pathological mechanisms of HD, which try to avoid neuronal dysfunction and loss; and **(c)** directly interfering with the cause of the disease by targeting the mutant Huntingtin (muHTT) at the genomic level, post-transcriptionally or at post-translational level. Additionally, **(d)** environmental enrichment has also proven to be effective in delaying the progression of HD in animal models and could be used as a complementary approach to a pharmacological therapy in humans. *Abbreviations*: BDNF, brain-derived neurotrophic factor; HDAC, histone deacetylase; muHTT, mutant HTT; siRNA, short interfering RNA.

intracellular targets, disrupting their normal function and consequently leading to neuronal dysfunction and loss in the striatum, but also in other structures of the brain, such as the cortex [7]. Based on these understandings of HD neuropathology, several therapeutic approaches have been advanced (Fig. 1). In addition, a variety of animal models have been developed to evaluate the pathophysiological mechanisms of the disease and the success of emerging therapeutic modalities (Box 1, Table 1). These novel therapeutic modalities include neuroprotective strategies targeting the underlying pathologic mechanisms of muHTT, and cell replacement therapies focussed on counteracting neuronal loss in the brain [7]. Additionally, preclinical evidence has also shown that environmental enrichment improves HD neuropathology in transgenic rodent models of HD [8,9], which in turn suggests that this strategy may play a role in improving patients' quality of life. However, and despite being potential alternatives to current pharmacotherapy, these strategies are aimed at downstream effects of muHTT and do not specifically target the root cause of the disease [7]. By contrast, oligonucleotide therapeutic approaches that directly interfere with muHTT by abrogating or reducing its expression have also been considered and presented encouraging results [10]. Among such strategies are genome-editing techniques and post-transcriptional gene silencing approaches using ribozymes and DNA enzymes, ASOs and RNAi, all of which enable a specific reduction of the synthesis of muHTT. In fact, these approaches target upstream processes of disease and might enable therapeutic intervention even before cellular damage arises [10]. Given their potential as therapeutic strategies, lately they have received significant attention from the scientific community and the field has rapidly progressed. Therefore, here we aim to not only capture such significant development, but also identify limitations and hurdles that need to be overcome for these concepts to reach the clinical setting.

Post-transcriptional gene silencing: therapeutic potential for HD

Post-transcriptional gene-silencing approaches for HD have undergone considerable research and include nucleic acids with catalytic capabilities (ribozymes and DNA enzymes), ASOs, and RNAi [10,11]. These nucleic acids have been shown to modulate the translational efficiency through a process that involves cleavage, degradation, or translational suppression of the target messenger RNA (mRNA). Although they share the common concept of reducing muHTT mRNA (and, consequently impacting on muHTT protein load) to block or reverse HD neuropathology and symptoms, their mechanisms of action differ significantly (Fig. 2).

Catalytic nucleic acid approach

Catalytic nucleic acids include ribozymes and DNA enzymes (DNAzymes), and are aimed at the elimination or repair of target mRNA transcripts.

Ribozymes: mechanism of action and advances towards a potential therapeutic approach for HD

Ribozymes are naturally occurring RNA molecules with self-cleaving capabilities that comprise an effector catalytic core and two flanking sequences that enable specific binding to the mRNA