Huntington’s Disease: current therapies and future directions

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Abstract. Huntington’s disease is a neurodegenerative disease that have significantly negative impact to cognitive function. In the worldwide range, approximately 5-10 individuals are affected per 100,000 people. At molecular level, the expanded CAG repeats lead to misfolding and aggregation of the huntington protein, which can interfere with cellular metabolism, including transcription, mitochondrial function, and other important physiological processes. Though scientists already have a well-established theory for the pathology of Huntington’s Disease, no effective cure has been developed due to the heavy genetic base of the disease. Despite the genetic barrier to overcome, many therapies have been created to alleviate the symptoms. In this primer, four main therapies are discussed who reduce the mutant huntington protein amount at post-transcriptional level: Antisense Oligonucleotides, Ethyl-Eicosapentaenoic Acid, autophagy modification, and intrabody based immunotherapy. Within each module, it is described how these therapies can reduce the level of mHHTT in molecular level and correct the symptom. Development history is also touched upon briefly and discussion about the current status of each approach is made. Clinical prospective and future direction is included at the end as well.

Keywords: Huntington’s Disease, Neurodegenerative Disease, Therapy.

1. Introduction
Neurodegenerative disease is one of the biggest challenges in modern society, where patients progressively loss their neuronal cells both structurally and functionally. Among the prevalent ones, Huntington’s disease is one of the most well-known and studied one. In almost all cases, Huntington’s Disease is inherited from parents in an autosomal dominant fashion. The disease can start at any age, but clinical data has shown that most patients usually begin their symptoms in middle age (30-50 years old), and gradually increase the severity. Early symptoms include slight mood or mental disabilities and, as the disease progresses, uncontrollable movements can occur [1].

At molecular level of the disease, mutations occur in huntingtin protein (HTT), which is an essential protein for neuronal development. In all cells types HTT is detected, while its specific functions are unclear. Nonetheless, its interactions with proteins involved in transcription, cell signaling, and intracellular transporting were discovered. Particularly, the gene contains multiple repeats of cytosine-adenine-guanine (CAG) in series, forming a structure called polyglutamine tract. Generally, people have fewer than 27 repeated glutamines in the polyglutamine tract is diagnosed as normal. Once the glutamine repeat exceeds 36 times, this conformation will cause the HTT protein to misfold. As a result, the misfolded protein forms aggregation and ultimately leads to neuronal cell dysfunction. The prevalence of HD varies across different regions around the world, with an average of 5–10 cases per 100,000 people [2]. Though for many years, extensive effort and worked have been done, no effective cure is developed for HD and other similar neurodegenerative diseases. The major obstacle for developing a cure is the genetic basis for the disease and the late onset of symptoms. In medical genetics, many pre-natal genetic screen and risk evaluation does reduce the disease population considerably, but large number of patients still exist in the world, which further raise the importance of developing an effective approach against HD. Fortunately, several approaches are widely accepted to ameliorate the symptoms. In this article, four different types of therapies (antisense oligonucleotide, Ethyl-EPA, autophagy regulation, and intrabody) are discussed in terms of their mechanism, current status, and prospect.
2. Ethyl- eicosapentaenoic acid

Previous studies have reported efficacy of ethyl-eicosapentaenoic acid (Ethyl-EPA) in alleviating the severity of many neuronal diseases. Ethyl-EPA is a polyunsaturated fatty acid derived from the omega-3 fatty acid eicosapentaenoic acid (EPA) and was approved by FDA in 2012 for the first time. It was conventionally designed as a medication for dyslipidemia and hypertriglyceridemia [3], and is now being investigated for ability of treating Huntington’s disease. Ethyl-EPA is enzymatically modified by esterases and liberate the free acid EPA, acting as pro-drug. Similar to other fatty acid based drug, ethyl-EPA had a potential efficacy in altering lipid metabolism in HD patients, especially in affecting mitochondrial function. It has now been widely accepted that mitochondrial dysfunction is the one of the most important pathophysiological bases of Huntington’s disease and ultimately lead to apoptosis. In HD patients, mitochondrial toxins, such as 3-NPA and cyanide, can induce changes of the striatum. As been described by H Murck et al., c-Jun amino-terminal kinases (JNK) pathway and NF-kB-pathway are the two main pathway. In JNK pathway, stress-signal-kinase1 (SEK1) was exclusively observed in pathological cell lines containing 48 and 89 repeated CAG, while the 16 CAG repeat cell lines show negative results in this pathway [4]. Notably, another paper concluded a direct correlation of HD severity with CAG repeat number. After delivery of Ethyl-EPA, both NF-kB and JNK pathway can be suppressed. More specifically, EPA plays a role in reducing the P65 component of NF-kB heterodimer, thus inhibiting the activity of NF-kB [5]. In terms of JNK pathway, there is increasing evidence that activation of the c-Jun by N-terminal phosphorylation can is key to JNK mediated apoptosis. Upon receiving EPA, activity JNK-AP-1 is suppressed immediately and the whole pathway is blocked in the middle. Worth noticing, little effect was observed in human fibroblasts. In 2015, JJ Ferreira et al. carried out a clinical trial (randomized, double-blind, placebo-controlled), where 290 HD subjects ranging from mild to moderate symptoms were tested for the efficacy of EPA. Interestingly, mixed results were gained, in which no significant benefits were observed in the primary outcome, patients with less than 45 CAG repeats showed stable or improvement on the Total Motor Score – 4 (TMS–4) after treatment [6]. Consistent with what was found by Puri et al, little therapeutic effect was observed in the 6-month clinical trial, and meta-analysis revealed that only after 12 months do beneficial impact occurred. Regardless of the conflicting results, there’s no doubt that Ethyl-EPA can produce neuroprotective effect. Therefore, many physicians are still assessing the efficacy of this type of drug and actively investigating its role in treating Huntington’s disease.

3. Selective Antisense Oligonucleotide Mediated Modification

Previous data reveals that the severity of HD symptoms is closely associated with mHTT expression [7]. Several trials have been done in mice models to reduce the mHTT level at post-transcriptional level, including delivery of siRNA, miRNA or other small molecules via viral based vectors. The results were quite encouraging at short terms, which shows efficacy of reduction in both wild type and mHTT in vivo and creation of clinical benefits. However, loss of WT HTT protein can lead to series side effect. Prenatal depletion of hdh (mouse homologue of human HTT) in mice model leads to unviable embryo or, if embryo is viable, perinatal lethality and impaired neurodevelopment [8]. Therefore, considering the neuroprotective nature of wild type HTT protein, selective inhibition of mHTT by synthetic antisense oligonucleotides (ASOs) has become a popular therapeutic approach. In most cases, ASOs lower the mHTT level by either targeting the expanded CAG-tract directly or its linked polymorphism, rendering them the capacity of discrimination between mutant form and wild type. More importantly, any SNP-related locus in the pre-mRNA can be the potential target for ASOs, including introns. Therefore, much more access for pre-mRNA modification is provided, comparing to siRNA-based therapy. In 2014, Xin et al. published a paper describing phosphorodiamidate morpholino oligomers (PMOs), ASOs that are highly stable and non-toxic, that selectively inhibit mHTT mRNA. Phosphorodiamidate morpholino oligomers are short single-stranded DNA analogs (around 25 nucleotides long) whose backbone is composed of morpholine
rings and connected by phosphorodiamidate linkages [9]. In the experiment, Xin et al. designed two groups of PMOs in total, one (CTG22, CTG25 and CTG28) that targets expanded CAG repeat tracts directly, and the other (HTTex1a and HTTex1b) that targets sequences who flank the CAG repeat. With respect to the CAG targeted PMOs, the results show that the as the number of CTG triplets increases, its specificity rises, but efficacy reduces correspondingly. Of note, PMO CTG25, who contain medium length of CTG repeats, can reduce the total level of HTT to less than half of untreated cells in disease fibroblasts containing 44 CAG triplets. Besides the relationship between selectivity and efficacy, the authors also proposed that off-target effect has to be minimized in order to make an ideal ASO therapy. By using ATXN3 as a biomarker, the authors found both repeat length and structure can influence off-target effects. Therefore, the data suggests that the difference in CAG repeat genes expression and intracellular distribution of ASOs across different tissues should be considered before choosing the subtype of CTG PMOs. Notably, the lack of an electrical charge, independence of RNase H activity and low non-specific toxicity have provided PMOs with advantages over other approaches. However, several other RNase H dependent therapies also show promising efficacy, such as Tricyclo-DNA (tcDNA) Antisense Oligonucleotides and IONIS-HTTRx (also known as ISIS 443139 and RG6042). TcDNA-ASO is a structure containing central DNA nucleotides modified by tcDNA at both ends. This conformation enables the ASO to recruit RNase H and mediate subsequent degradation. The authors used patient-derived fibroblast cell lines to test the efficacy of tcDNA-ASO and the data revealed a strong reduction in HTT mRNA and protein levels, comparing to control. In the other half of the experiments, YAC128 mice models (HTT mRNA contains 128 CAG repeat expansion) were also infected with tcDNA-ASO and similar decrease in HTT expression was observed in the cortex, hippocampus, striatum, and cerebellum. These results indicate the efficacy of tcDNA-ASO both in vivo and in vitro. IONIS-HTTRx act via similar mechanism to tcDNA-ASO through triggering the activity of RNase H. Both approaches show sustained amelioration of Huntington’s disease after long term administration. Besides the canonical silencing function of ASO based therapy, Evers M. M et al. proposed a modification mechanism that can detoxify the mHTT protein. Previous studies have reported that N-terminal huntingtin fragments are more toxic than full-length protein, and the proteolytic cleavage is mediated by the cleavage site located upon exon 12 [10]. The authors thus tested the capacity of exon skipping through 2′O-methyl modified ASO. Upon binding, the key cleavage site is masked by the antisense nucleotide chain and resulted in depletion of 552 caspase-3 and 586 caspase-6 cleavage sites. Also, ASO is able to induce cryptic cleavage site to simply remove the intrinsic cleavage site. The results suggest that ASO mediated exon skipping can shorten the mHTT both in vivo and in vitro. This is a novel approach and its main advantage is that the total transcript level are unaltered. As mentioned above, over suppression of HTT protein can significantly have native impact to cellular metabolism. Hence, detoxification can ameliorate the symptom, while leave the cell in a healthy state. Comparing to vitro, the lower efficacy in vivo is partly due to the difficulty in passing the blood-brain barrier. Therefore, a better delivery method is still under exploration.

4. Autophagy Regulation

Potentially, there’re two ways to degrade the protein in human cells, which is proteosome and autophagy. Autophagy is an cellular degradation system where unwanted cargo is digested and recycled [11]. The autophagy process was by Christian de Duve in 1963. It involves the sequestration of unwanted cellular cargo into autophagosomes and their degradation occurs after the fusion with lysosome (degradation mediated by lysosomal hydrolases). Autophagy in eukaryotes comprises of macroautophagy, chaperone-mediated autophagy, and microautophagy, but macroautophagy remains the most extensively studied one to date. Existing data reports dysfunction in macroautophagic activity in cell lines under Parkinson’s disease, indicating a possible role of macroautophagy in HD. Recent studies indicated that a chemical called genistein stimulates the lysosomal biogenesis, which is a key step of autophagy. To test the effect of genistein in ameliorating HD, K Pierzynowska et al.
chose immortalized human embryonic kidney cells (HEK-293) as model organism and transfected the cell with two types of plasmids: plasmids pEGFPQ74 encodes a fragment of huntingtin protein with 74 CAG repeats fused to EGFP protein (this is acting as the mutant group), while pEGFP-Q23 only contain 23 CAG repeats and is acting as the wild-type control. Following a 48h long genistein treatment, both size and level of mHTT were significantly decreased comparing to untreated cells (Figure 1). When they use western blotting to assess the LC3 level (biomarker for autophagy), LC3-II in both groups of cells reduced considerably, but results were more remarkable in mutant cell culture (Figure 2). K Pierzynowska et al. did a follow-up experiment, in which chloroquine was added to inhibit the lysosomal function. As expected, they found the addition of chloroquine can restore the disease symptom. In other words, the effect of genistein is impairecd. The results revealed the genistein’s ability to correct the HD phenotype in cellular model, predominantly by activating the autophagy. At relatively low concentration (30μM) of genistein, autophagy appears to be the predominant process responsible for the mHTT degradation, if not the only one. At higher genistein concentrations (60 and 100 μM), in the contrast, the efficacy became less effective comparing to low concentration. Therefore, the researchers suspect that some other mechanisms may be activated by high concentration of genistein, but remain elusive.

![Figure 1](image.png)

**Figure 1.** Reduction in aggregate level by Genistein. Panel A: HEK-293 cells with EGFP-Q74. Panel B: HEK-293 cells with EGFP-Q23. mHTT aggregates is represented by the sharp fluorescence in the figure. Quantification measurement is summarized in the right half.

Another well-known autophagy inducer is called Rapamycin, who inhibit the activity of mTOR to facilitate intracellular protein degradation. At cellular level, mTOR is responsible for a wide range of
processes, mainly cell growth and proliferation. Once impaired, the dysfunctional pathway of mTOR can cause, neurodegenerative diseases, or even cancer [12]. At molecular level, mTOR is a serine/threonine kinase which sense environmental cues to promote downstream effect. Upon combination with other accessory proteins, mTOR can form two distinct complexes: mTOR complex 1 (mTORC1) and mTORC2. Since decades ago, it has been described that inhibition of mTORC1 enables autophagy induction. In mammalian cells, mTORC1 phosphorylates Ser758 of ULK1, thus block the access of AMPK to ULK1, resulting in no interaction and phosphorylation. Therefore, the initiation of autophagy by ULK1 is inhibited. Rapamycin impairs mTOR activity by binding with its intracellular receptor FKBP12. This binding cause conformational change in FKBP12-Rapamycin Binding (FRB) domain of mTOR, thus inhibiting its activity [13]. The first experiment that proof the potential of rapamycin in reducing protein aggregates were conducted by using Q74 and A19 cell lines, who contain 74 glutamines repeats and 19 alanine repeats respectively. After treatment with rapamycin for 48h, A19 cell lines showed significant reduction is aggregation size, while Q74 cell lines had little effect. This indicate that Q74 might form aggregates more firmly and quickly, since the 48h reported size is larger compared to that in 24h. To confirm the impact of rapamycin Q74 cell lines, the authors repeated the experiment but set the treatment time to 24h. Different result was gained as both aggregation and cell deaths were reduced. These promising results inspired other researchers to further test its efficacy. Berger Z et al. conducted experiments to examine whether the rapamycin could promote the clearance of other mutant aggregate proteins. During in vitro experiment, they transfected COS-7 cell lines with synthesized constructs that contain different types and lengths of repeats. It turned out that, after treatment of rapamycin, all types of mutant cell lines underwent decrease in aggregation size, comparing to control. In vivo, the authors transfected Drosophila with polyalanine tract (EGFP-NLS-A37) and gained similar results as in vitro experiment. Next, to eliminate the confounding that the reduction is due to the characteristic of polyalanine, the authors used Drosophila that express mutant tau protein, comparing to wild type. Dara suggests that rapamycin is still efficient in clearing the tau aggregation [14]. As proposed by the researchers, all results were consistent with previous finding that the species with higher susceptibility to aggregation formation tends to have a greater dependence on autophagy of the degradation system.

Figure 2. Genistein promote autophagy activities. HEK-293 cells were transfected with eitherEGFP-Q74 or EGFP-Q23 plasmids. Levels of LC3 and LC3-II were measured through western blotting 48h after treatment of genistein. Band width was controlled against B-actin. Quantification measurement was summarized in the bottom half of the figure.
5. Antibody-based suppression of mutant Huntington protein

As a variety of different therapies are developed in recent years, many researchers have begun to shift their focus from alleviating symptoms to disease modifying. Among the newly emerged approaches, antibody-based therapy is one of the most well studied treatment, as the monogenic nature of Huntington’s disease made antibody possible for being a potential treatment. While other gene-based therapies aim to suppress the expression of predominantly the intracellular mHTT protein, antibody is able to tackle another aspect like mHTT in free form. In recent years, increasing evidence pointed out that, regardless of the intracellular aggregation, mHTT also exist as free form in large amount, mainly present cerebrospinal fluid, the plasma, and the extracellular matrix. In another aspect, accumulating data reveals the migration and seeding capacities of mHTT, reminding researchers to pay attention to pathology of both intracellular and extracellular form. To date, several roles of free form mHTT has been investigated: 1) the peripheral mHTT load is associated with immune system and can subsequently influence CNS features; 2) the migration and seeding capacity; 3) the prion-like behavior of mHTT. In a study carried out by Weiss et al. in 2012, they reported the existence of mHTT in peripheral monocytes and the presence of mHTT is closely associated with disease progression. Similarly, in 2008, Bjorkqvist et al. showed that alterations in cytokine level can be detected as early as 16 years prior of HD onset. Although the toxicity of mHTT aggregation has been well established for decades, the toxicity of soluble form hasn’t been suggested until the observation made by Cicchetti et al. in 2012. In their experiment, they did a follow up research in a HD patients who underwent fetal striatal transplants many years ago, and mHTT aggregation was detected within the transplant. As a immune-based therapeutic approach, intrabody has been the most well studied one since they were first developed in 1990s [15]. In current stages, intrabodies mainly target PolyQ domain, PolyP domain and N-terminal exon 1 domain as the most effective approach. After entering the cell, the sFv intrabody can bind stoichiometrically to the target protein and block the toxic effects of pathogenic agents. In vivo experiment using Drosophila as a model and C4 sFv intrabody as therapy, the researchers found coexpression of C4 sFv intrabody with mHTT exon-1-Q93 completely rescued survival to adult emergence. Also, after administration of C4 sFv Ab, the aggregation progression is slowed down, as well as the neurodegenerative speed.

Although sFv intrabody has shown huge advantages, its neuroprotective outcome gradually diminishes both with the disease progression and age increase [16]. To compensate for this drawback, a novel bifunctional intrabody was developed, who has a PEST region binding to it. Protein containing PEST region is designated for proteasomal degradation and will have a shorter half-life. Specialized study of PEST region reported that Deletion of the C-terminal PEST motif from Mouse Ornithine Decarboxylase (mODC) extends its half-life and leads to a more stable structure. In the contrast, adding the C-terminal PEST motif to other stable protein can promote accelerating degradation. Both deletion and addition show no detrimental effect [17]. Experiments proofed that Anti htt scFv-C4-PEST intrabody enhances degradation of httex1-72Q fragments. Live cell images (Figure 3) revealed that, though scFv alone is able to suppress the aggregation, scFv-C4-PEST intrabody gained a more pronounced effect. Another interesting achievement in the field of intrabody therapy was made by DW Colby et al. years ago. In their published article, they developed single-domain intracellular antibody in the absence of disulfide bond stabilization. This innovation overcomes the fact that disulfide bonds are not as stable as it should be in the reducing environment of the cytoplasm. In other words, common intrabodies are destabilized more quickly in the cytoplasm and require a higher administration level to reach therapeutic effect. After elimination of anti-htt VL (light chain only) intrabody’s disulfide bond, the aggregation inhibition property is not affected, though the binding affinity is reduced. However, the binding affinity can be restored by reengineering or editing. Though this approach still needs more exploration, but it has provided much inspiration to engineering the existing intrabody for higher efficiency.
Figure 3. scFv-C4-PEST intrabody enhances aggregation degradation. Two cell lines (httex1-25Q and htex1-72Q) were transfected with empty vector, C4, and C4-PEST. Aggregations were tagged by green fluorescence and captured by live cell imaging. Quantification measurements were collected in the bottom part of the figure, by SDS-PAGE Western blot.

6. Conclusions

The core characteristic that physicians concern is selective inhibition of the target. In other words, treatments that achieve the best therapeutic goal while minimize the toxic effects should be in highest priority. This aspect should be considered carefully in terms of antisense oligonucleotide therapy, since researchers haven’t found complete disease allele selectivity to date. The development of RNase H independent subgroup of AOS shows great flexible potential to the public. Another approach posing high flexibility is antibody-based therapy, more specifically, the intrabody mediated mHTT suppression. While designing the epitope of the antibody, it’s more convenient to target the mutation-associated region of mHTT exclusively, such as the expanded polyglutamine tract or the N-terminal region. Another advantage intrabody offer is its capacity of modifying disease phenotype without altering the gene expression. Like other immunotherapy, scientists can introduce antibody by either passive (direct administration) or active (activate immune system to produce antibody) pathway, which further increase the potential of antibody therapy. In the contrast, autophagy regulation has lower flexibility as there’re limited steps in the signaling pathway we can manipulate to make an impact. However, since autophagy is a common process in all tissues, both in vivo and in vitro experiment reports little toxicity and side effect. Ethyl-EPA as a novel approach, previous data has proved its neuroprotective nature, but clinical trials have gained controversial results. Therefore, the efficacy of Ethyl-EPA still needs further examination. Generally, the data has shown promising future in the field of neurodegenerative diseases. Many clinical trials suggest the capacity of these novel approaches to reduce the mHTT level to negligible amount. Despite the intricate obstacle in curing...
the disease, the newly developed approaches are already able to extend the patients’ lifespan for years, or even bring them back to normal people’s life. In other aspects, current therapies can also work as inspirations to encourage other researchers to come forward with other unique possibilities and contributions. As for the future direction, selective inhibition, low side effect, and comparatively affordable price is three factors that need to be considered in higher priority.

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