LITERATURE REVIEW

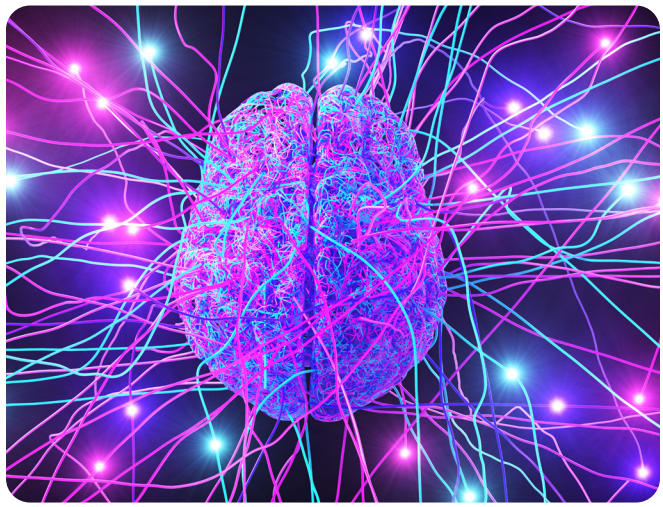
Innovative Therapeutic Strategies Against Huntingtion's Disease

Abstract

Hereditary and currently incurable, Huntington's Disease is associated with the degeneration of neurons in a part of the brain involved in motor, cognitive, and behavioral functions. While the gene whose alteration causes the disease is known, the mechanisms that lead to this neurodegeneration have not yet been elucidated. Various therapeutic avenues are however being explored.

Introduction

Huntington's Disease is a rare hereditary degenerative brain disorder that was described for the first time by George Huntington in 1872. The symptoms include disturbances in the motor (movement), behavioral (mood, anxiety, apathy), and cognitive (such as understanding) systems, generally appearing in the middle of adult life. It is a progressive and fatal neurodegenerative genetic disorder in which an autosomal-dominant mutation is present on either of an individual's two copies of the Huntingtin (HTT) gene coding for the Huntingtin protein, located on chromosome 4. The HTT gene contains a repeat of CAG codon coding for glutamine. If this repeat contains 40 or more repeats, it will lead to the development of Huntington's Disease within a person's normal lifetime. The mutation results in the production of an altered protein and leads to dysfunction and neuronal death in the brain.



There is currently no therapy available to effectively treat the disease, even though there are treatments that can reduce symptoms and improve the quality of life for those affected. However, recent studies have shown that the level of soluble mHTT is closely related to disease pathology, and therefore that lowering soluble mHTT may provide an effective approach to treat HD by ameliorating any downstream toxicity. Subsequently, several drug discovery strategies have been investigated to treat HD and lower levels of mHTT.

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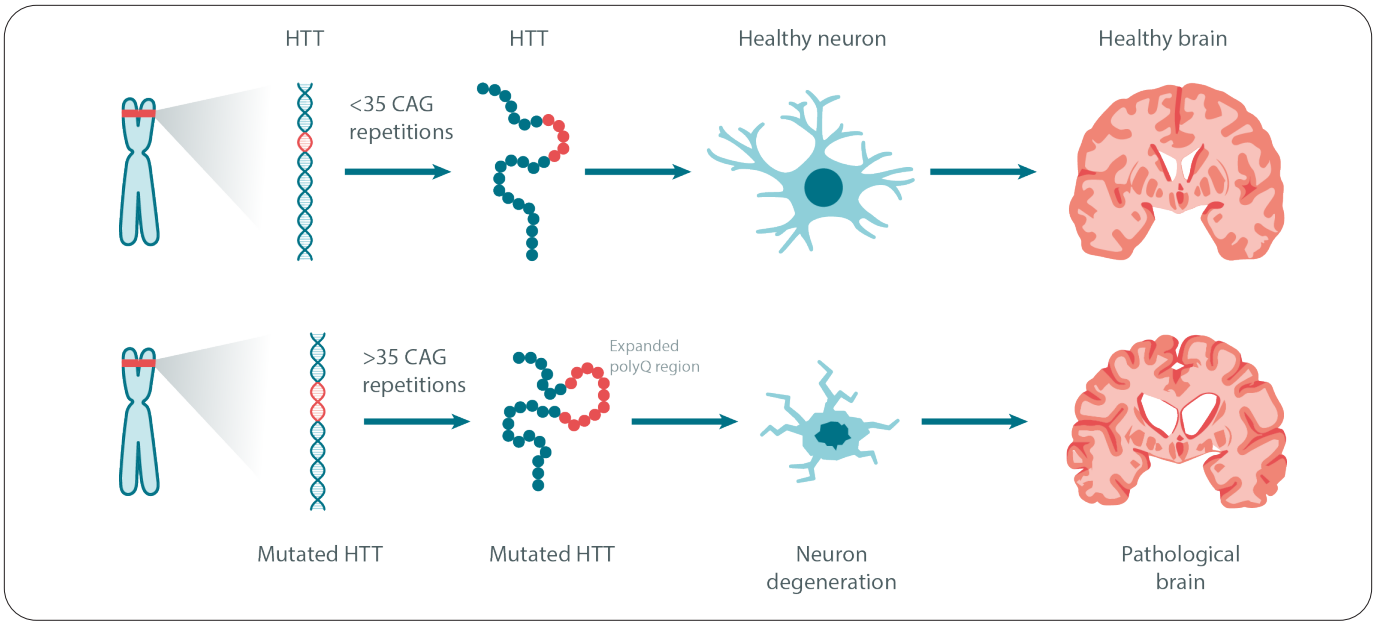

Figure 1: Representation of the molecular genesis of Huntington's Disease.

Autophagy mechanism to counter mHTT aggregation

Li et al. identified four small molecule compounds that link both mHTT and LC3 (not wtHTT), triggering the selective decrease of mutant HTT levels.[1]

In this study, HTRF was used to assess the effects of the compounds in primary cells from patients affected with HD. HTRF technology consists of a signal that is generated through fluorescent resonance energy transfer between a donor and an acceptor molecule when near each other. The HTRF assay used in this study included specific antibodies: 2B7, that recognizes HTT protein in Nterm; 2166, targeting the region around amino acid 44; and MW1, that binds the polyQ epitope. The antibody pairs 2B7/2166 and 2B7/MW1 enable the specific assessment of wt and mutated HTT respectively (Figure 3).

Using HTRF technology, researchers showed that lowered mHTT protein levels were due to autophagy induction through the action of the four compounds in fibroblasts from Huntington's Disease patients, and induced pluripotent cell-derived neurons (iPS cells).

Interestingly, the four compounds did not have any effect on wtHTT levels in fibroblasts from healthy donors or from patients with Parkinson's Disease. These first results showed the specificity of linker action to mHTT proteins in primary cells from patients suffering from HD.

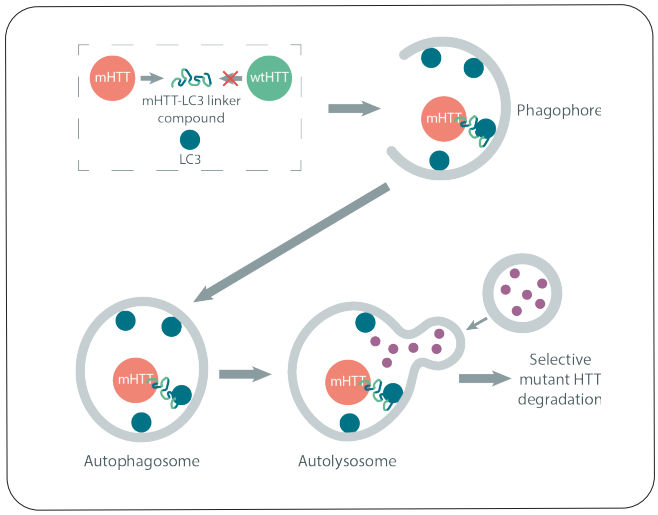

Figure 2: Model showing how mHTT-LC3 linker compounds may induce mutated HTT degradation. This diagram illustrates the concept of lowering target protein levels using autophagosome-tethering compounds.

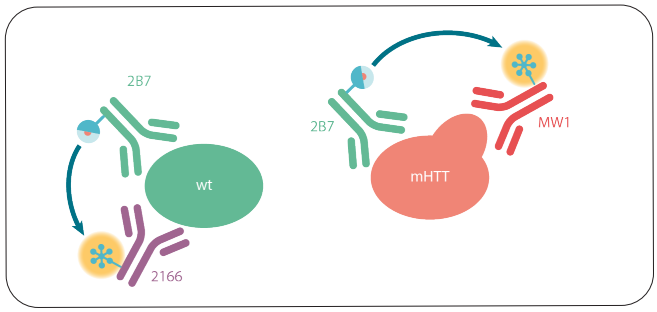

Figure 3: Diagram of the HTRF method used to determine mHTT and HTT cellular levels

Suppression of specific kinase activities reduces mHTT levels

Yu et al. studied the involvement of two kinases, HIPK3 and MAPK11, as positive modulators of mHTT levels both in cells and *in vivo*.[2] These two kinases regulate mHTT levels through their kinase activities, suggesting that inhibiting this activity could result in new opportunities for drug development. To identify potential regulators for mHTT levels, an RNAi screen was performed in immortalized HD patient fibroblasts from patients expressing mHTT proteins (Q45 and Q68). This screen used a siRNA library mostly targeting genes expressing enzymes and receptors. mHTT levels were measured by HTRF assays using the 2B7:MW1 antibody pair, which specifically detects mHTT levels (Figure 3). Among the 11 identified putative mHTT modulators, a pool of MAPK-related proteins was identified: MAP2K6 and MAPK11. These two proteins belong to the same signaling pathway. MAP2K6 phosphorylates MAPK11; and HIPK3, which is regulated by JNK1 and JNK2. This cluster of genes for MAPK-related proteins suggested that these kinases may have a role as mHTT modulators, and MAPK11, MAP2K6, and HIPK3 have been prioritized for further validation. To go into this characterization more deeply, mHTT and wtHTT levels were measured on iPSC-derived neurons from HD patients using HTRF assays.

siRNAs knock down experiments on the different regulators identified lead to reduced mHTT levels in the iPSC-derived neurons (Figure 4). This result confirms their efficacy in the human HD neuronal model expressing mHTT.

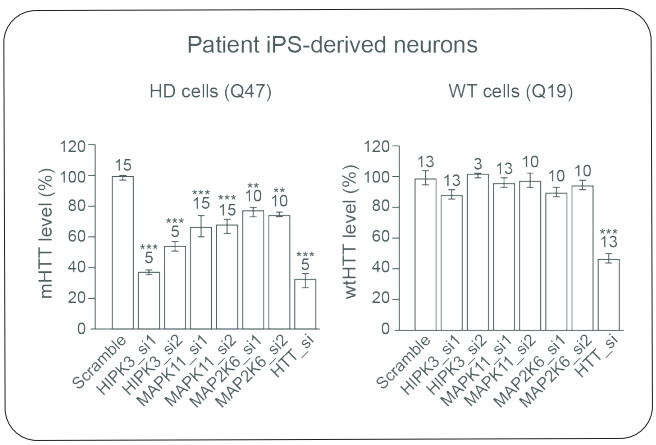

Figure 4: mHTT and wtHTT specific levels measured by HTRF assays in HD patient iPSC-derived neurons.

Yu, M. et al. Suppression of MAPK11 or HIPK3 reduces mutant Huntingtin levels in Huntington's disease models. Cell Res. 27, 1441-1465 (2017).

The kinase activity of MAPK11 could have an effect on the HTT protein level. This kinase activity is known to be activated by MAP2K6. To follow this up, the study found that dominant negative MAPK11 (DN-MAPK11) reduced HTT levels when over-expressed in HD cells. Furthermore, over-expression of MAPK11 but not DN-MAPK11 countered the HTT lowering effect of Mapk11 knockdown. This result suggests a role of MAPK11 kinase activity in HTT level modulation. In the same way, over-expression of Hipk3 cDNA increased endogenous HTT levels. However, over-expression of the kinase dead Hipk3 cDNAs resulted in a low reduction in HTT.

These results show that this regulation is also kinase activity dependent. To take the investigation further, they studied the effect of the HIPK3 AST487 kinase inhibitor (Figure 5A and B). AST487 effectively decreased mHTT levels, and this effect is reduced by knocking down HIPK3.

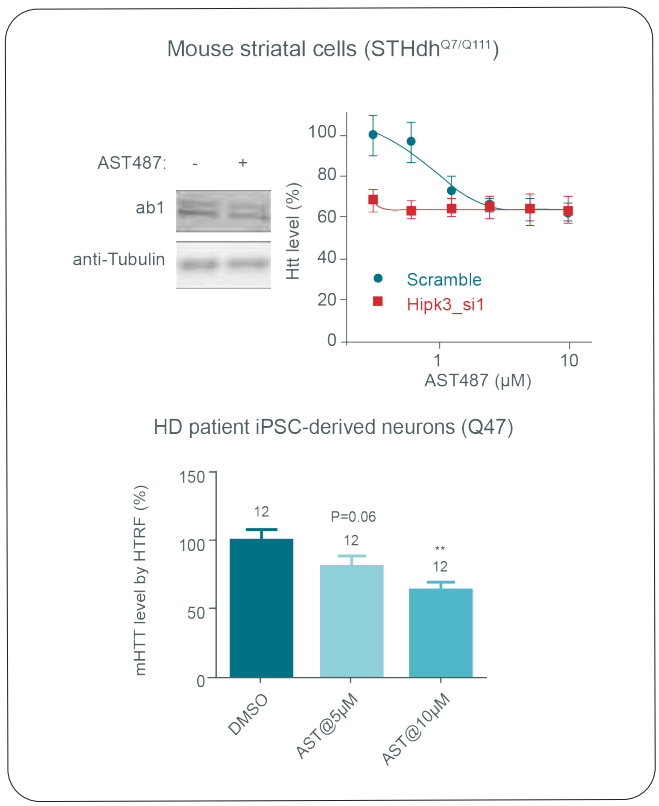

Figure 5: Mapk11 and Hipk3 regulate HTT levels via their kinase activities.

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The results strongly suggest that AST487 reduces mHTT levels through the inhibition of HIPK3. Moreover, AST487 decreased mHTT levels in iPSC-derived neurons from HD patients (Figure 5B). These results are in good agreement with those found previously on a human neuronal cellular model that recapitulate the HD phenotype with HTT polyQ extension.

In conclusion, this study showed that MAPK11 and HIPK3 directly influence mHTT. The different results showed that MAPK11- and HIPK3-mediated effects are dependent on their kinase activities. Furthermore, the HIPK3 kinase inhibitor AST487 effectively decreased mHTT levels, and this effect is reduced by knocking down HIPK3. This confirms that AST487 reduces mHTT levels through HIPK3 inhibition.

Aptamer binding as a way to reduce mutated HTT activity

Another way to counter Huntington's Disease is to use aptamers to specifically target mHTT. Baehyun Shin et al. have identified four aptamers that bind to mutated Huntington in the C-terminal domain region.[3] This specific binding reduces the polycomb repressive complex 2 (PRC2) activity conferred by the polyQ tract of mHTT. It has been shown that endogenous mutated Huntingtin is associated with an increased stimulation of PRC2. Moreover, PRC2-dependent transcriptional changes were reported to play a prominent role on progressive neurodegeneration in adult mice neurons.

In order to determine cellular PRC2 activity in human HD neuronal progenitor cells (HD604i and HD17m8), an AlphaLISA H3K27me3 assay was used (Figure 6). This assay enables the determination of the cellular PRC2 activity by assessing tri-methylated histone H3 lysine 27 in cellular extracts that is affected by CAG expansion mutation in mHTT.

The activity of MS3, one of the most active aptamer was determined in human neuronal progenitor cells (NPCs) from an individual with CAG expansion mutation (HD60i4) and from a normal individual (HD17m8) (Figure 7). The AlphaLISA H3K27me3 assay enabled the determination of increased signal/cell for mock-transfected HD60i4 NPCs, which express mutant Huntingtin, compared to mock-transfected HD17m8 NPCs, which express only normal Huntingtin. In this experiment, the transfection of MS3 aptamer in HD60i4 cells led to a decreased signal/cell compared to mock transfection, while the signal/cell was similar in the MS3-transfected HD17m8 cells. As a control, the GCdx aptamer did not lead to a decrease in the AlphaLISA signal for both normal and mutant NPCs.

In this study, the different results showed that MS3 aptamer interacts preferentially with endogenous mutant Huntingtin and negatively modulate PRC2 enzymatic activity, which is one of the functional consequences of the impact of the polyQ tract on the mutated protein.

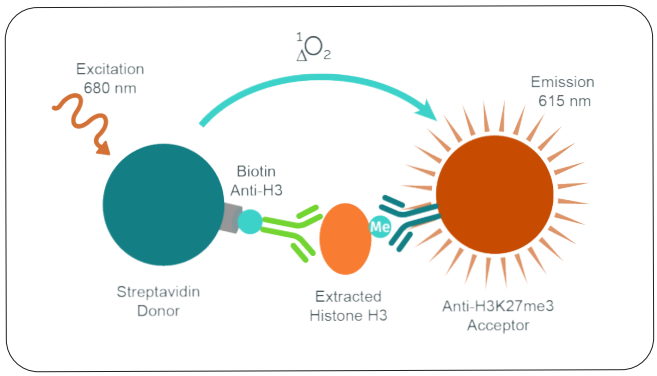

Figure 6: This AlphaLISA® immunodetection assay monitors changes in the levels of tri-methylated histone H3 lysine 27 (H3K27me3) in cellular extracts

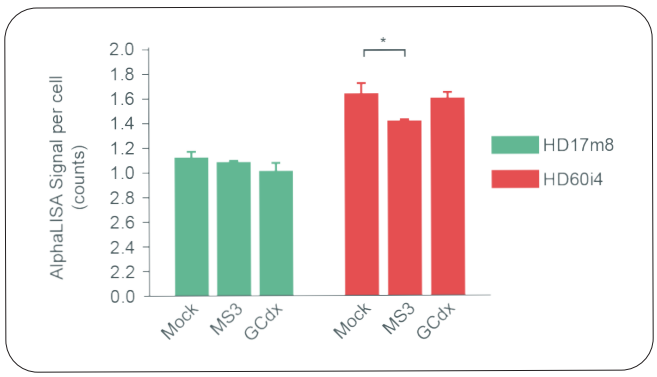

Figure 7: MS3 Aptamer reduces Cellular PRC2 Activity in HD60i4 and HD17m8 NPCs.

Shin, B. et al. Novel DNA Aptamers that Bind to Mutant Huntingtin and Modify Its Activity. Mol. Ther. Nucleic Acids 11, 416-428 (2018).

Conclusion

In this literature review, several cellular pathways were investigated for their role in the pathogenesis of Huntington's Disease. These studies showed that reducing the levels of mHTT may provide an active solution for HD treatment and give hope for new drug discoveries.

References

1. Li, Z. et al. Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. Nature 575, 203–209 (2019).

2. Yu, M. et al. Suppression of MAPK11 or HIPK3 reduces mutant Huntingtin levels in Huntington's disease models. Cell Res. 27, 1441–1465 (2017).

3. Shin, B. et al. Novel DNA Aptamers that Bind to Mutant Huntingtin and Modify Its Activity. Mol. Ther. Nucleic Acids 11, 416–428 (2018).

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