ABSTRACT

Abstract

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder resulting from a polymorphic CAG triplet repeat expansion in the Huntingtin gene, which leads to an abnormal polyglutamine (polyQ) expansion in the Huntingtin (HTT) protein. At the cellular level, HD is characterized by the preferential degeneration and death of striatal medium spiny neurons (MSNs) as well as cortical pyramidal neurons. The toxicity of mutant HTT and its fragments has been demonstrated both *in vitro* and *in vivo*, with numerous studies suggesting that post-translational modifications (PTMs) of the expanded HTT protein play a significant role in modulating HD pathogenesis.

Huntingtin has been shown to interact with polycomb repressive complex -2 (PRC2) and facilitates its activity. PRC2 complex plays a vital role as transcriptional regulator and mediates gene silencing by Histone H3-Lysine-27-tri-methylation. Mutant huntingtin facilitates this activity significantly higher compared to the normal Huntingtin. Further, previous reports have also shown that mutant huntingtin alters cellular metabolism and results in lower ATP/ADP ratio in *STHdh*$^{Q111/Q111}$ knock-in mouse striatal cells. Some reports have also suggested that huntingtin phosphorylation at Ser13/Ser16 can directly or indirectly prevent the toxic consequences associated with expanded polyQ in mutant huntingtin.

Currently, there is no effective treatment available for HD however some of the strategies like silencing of the mutant allele by siRNA, RNAi, Zinc-finger nucleases and targeting huntingtin post-translational modifications are being employed in animal models of HD, with limited success. Recently, a human trial with HD patients employing the mutant huntingtin specific antisense oligonucleotide Tominersen (previously IONIS-HTTRx and RG6042) revealed a significant reduction in mutant huntingtin protein expression, However, the trial was halted after interim data from the Phase 3 GENERATION HD1 clinical trial indicated the therapy was not benefiting participants. Further, very few mechanistic studies have been done to determine beneficial effects of phosphorylation for mutant huntingtin. Based on these observations, it is imperative that any intervention which can abrogate mutant huntingtin mediated hyper-activation of PRC2 and/or restore dysregulated pathways can be of potential therapeutic interest.

This PhD Thesis investigates the molecular and cellular mechanisms underlying HD, focusing on the phosphorylation of mHTT at Ser13 and Ser16 and its effects on various cellular processes. We employed site-directed mutagenesis to create phosphoresistant and phosphomimetic variants of

these sites in full-length huntingtin proteins, expressed them using the *Sf9* insect cell-Baculovirus system and purified by FLAG affinity chromatography.

Our studies reveal that mHTT with expanded poly-Q repeats enhances Polycomb Repressive Complex 2 (PRC2)-mediated H3K27 trimethylation (H3K27me3) activity, a key epigenetic modification. Interestingly, phosphomimetic variants exhibited significantly reduced PRC2 activity, suggesting potential therapeutic implications of manipulating HTT phosphorylation. Treatments with Kinetin, BMS 345541, and Bay 11-7082 were found to increase mHTT phosphorylation at Ser13 and Ser16, alter PRC2 interactions, and decrease H3K27me3, indicating these compounds' ability to modulate epigenetic regulation in HD.

Furthermore, these compounds prevented mHTT aggregation, rescued mitochondrial dysfunctions, alleviated endoplasmic reticulum (ER) stress, and improved cell viability in HD150Q cells, underscoring their therapeutic potential in mitigating HD pathology. Our label-free quantitative proteomics approach identified differentially expressed proteins, highlighting key pathways and biological processes altered by HD and normalized post-treatment, including translation, peptide biosynthesis, and proteasome function.

Additionally, we examined the peripheral effects of mHTT expression in HD lymphoblastoid cell lines, revealing altered pro- and anti-inflammatory marker expression, which was ameliorated by the treatments. The secretome from HD cells also impacted immune cell gene expression and metabolic functions, further illustrating the widespread effects of mHTT.

In conclusion, this comprehensive study provides significant insights into the molecular mechanisms of HD and highlights the therapeutic potential of targeting mHTT phosphorylation and its downstream effects. The detailed proteomic, epigenetic, and cellular analyses offer a robust framework for future research and therapeutic development in Huntington's Disease.