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**Review Article**

# Therapeutic Approaches for Inhibition of Protein Aggregation in Huntington's Disease

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Huntington's disease (HD) is a late-onset and progressive neurodegenerative disorder that is caused by aggregation of mutant huntingtin protein which contains expanded-polyglutamine. The molecular chaperones modulate the aggregation in early stage and known for the most potent protector of neurodegeneration in animal models of HD. Over the past decades, a number of studies have demonstrated molecular chaperones alleviate the pathogenic symptoms by polyQ-mediated toxicity. Moreover, chaperone-inducible drugs and anti-aggregation drugs have beneficial effects on symptoms of disease. Here, we focus on the function of molecular chaperone in animal models of HD, and review the recent therapeutic approaches to modulate expression and turn-over of molecular chaperone and to develop anti-aggregation drugs.

**Key words:** protein aggregation, Molecular chaperone, Huntington, anti-aggregation drug

## INTRODUCTION

Huntington' disease (HD) is a fatal and late-onset neurodegenerative disorder with an autosomal dominant manner of inheritance [1]. The most obvious symptoms of HD are chorea, psychiatric impairment and cognitive deficits due to region-specific neuronal degeneration of medium-size spiny GABAergic neurons in striatum and pyramidal neurons in cerebral cortex [2]. It is reported that 5 to 10 per 100,000 people suffers from HD in worldwide [3]. However, no efficacious treatment has been developed with HD yet, for now, there are some medications which can only retard the progression or alleviate symptoms of the disease [4].

HD is a monogenic disorder caused by the expansion of CAG triplet in the first exon of gene which encodes a protein called huntingtin (Htt) [2]. The Htt with abnormally expanded polyglutamine (polyQ) stretch (>36) is prone to be cleaved by caspases or other proteases and it releases N-terminal fragments. These fragments aggregate with each other easily and become toxic [5-9]. The mutant Htt (mHtt) oligomer or aggregate exerts a toxic gain-of-function in transcriptional regulation and axonal transport by sequestering other proteins aberrantly [10]. In HD, aggregation of misfolded mHtt is considered a main cause of pathogenesis, thus it would be rational if we approach to inhibit the misfolding of aggregation-prone proteins to cure the disease. Here, we review recent therapeutic approaches to modulate expression and turnover of molecular chaperone, and anti-aggregation agents to prevent formation of mHtt aggregation or its toxic oligomer.

## THE NORMAL HTT AND MHTT AGGREGATION

Normal Htt is ubiquitously expressed, but its level of expression

is higher in brain. This soluble protein is mainly localized in cytoplasm, and is also found in nucleus and vesicular membranes [11]. Because Htt is a 348-kDa protein which has polyQ and proline-rich region at the N-terminus and numerous protein interaction motifs including HEAT domain, it may act as a scaffold in various cellular mechanisms. *In vitro* molecular studies showed that normal Htt interacts with huntingtin-associated protein 1 (HAP1), which is an important factor for vesicle trafficking in the neuronal cells [12]. Htt also binds to repressor element 1 transcription factor/neuron restrictive silencer factor (REST/NRSF), which is involved in transcriptional repression [13]. In the huntingtin knockout mice study, wild-type Htt is suggested to have an anti-apoptotic function in embryonic development [14].

In contrast to normal Htt, mHtt has longer polyQ (>36) stretch in N-terminus. The length of polyQ stretch shows inverse correlation with the onset of symptom [1]. Recent study suggested that the number of polyQ tract in mHtt is implicated in flexibility, which is required for close proximity between N17 (the first 17 amino acids of Htt) and proline-rich domain [15]. N17 and proline-rich domain have opposite effects on mHtt aggregation [16, 17]. The deletion of proline-rich domain leads to a rapid transition into aggregates, in contrast, the deletion of N17 domain decreases the mHtt aggregation [18]. It seems that the proline-rich domain prevents polyQ aggregation through inhibition of N17 at proximal position. When the flexibility of polyQ tract is reduced because of long polyQ length, N17 is critical to induce mHtt aggregation. In the formation of protein aggregation, different threshold of polyQ length may be determined by different intradomain composition of disease protein in several types of polyQ disorders.

The expanded polyQ-tract containing mHtt is easily misfolded, and tends to self-aggregation [19]. It leads to formation of a toxic soluble oligomer, and gradually accumulates into intracellular aggregates, consisting of insoluble β-sheet rich amyloid deposits [20]. Previous studies have shown that mHtt aggregates are detected earlier than pathogenic symptoms in human patients and mouse model of HD [10]. It is doubtful that mHtt aggregates could induce the degeneration of neuron. A number of studies suggest that insoluble aggregates seem to play a protective role, leading to autophagy-mediated clearance [21, 22]. Moreover, R6/2 chimera and shortstop stain of YAC128 HD mouse models show no correlation of mHtt inclusion and pathogenic phenotype [23, 24]. Although there are still controversies which one is more toxic between soluble oligomer and insoluble aggregates, the contributions of both putative toxic insults on HD are reported. Thus, it would be better therapeutic direction to cope with misfolded monomer, which inhibits aggregation with itself.
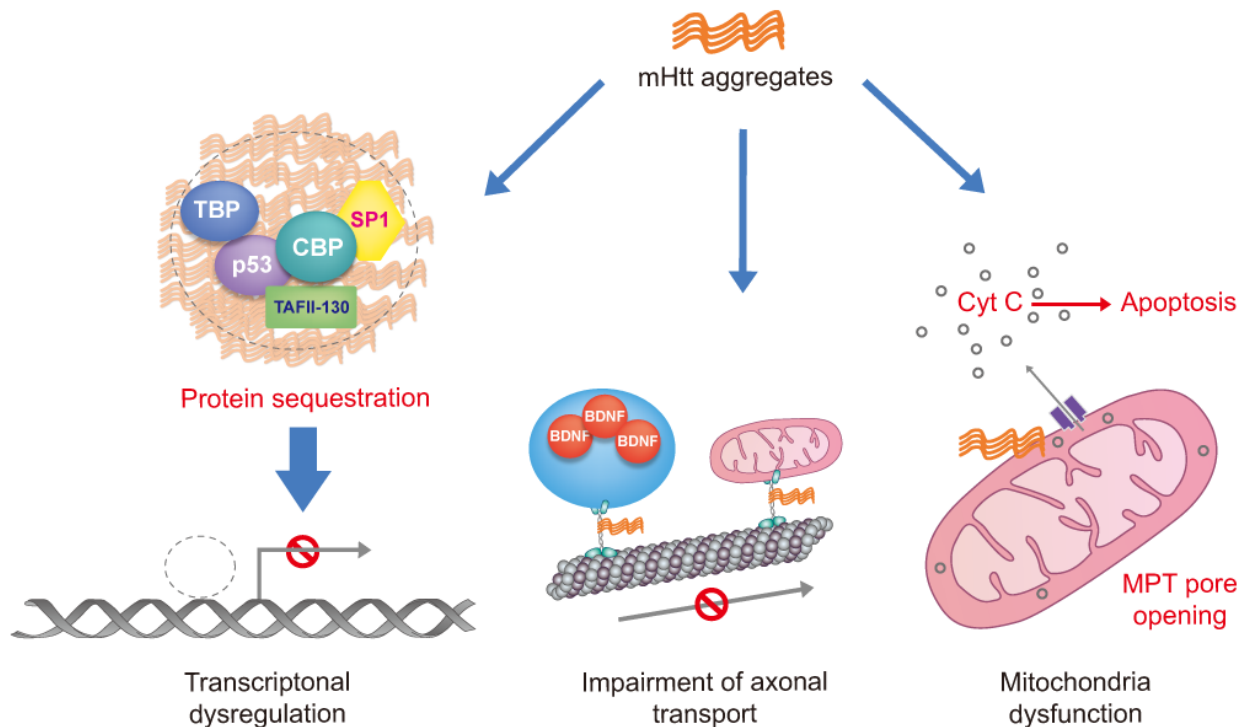
## THE MOLECULAR PATHOGENESIS OF MHTT IN HD

Although mHtt has similar expression and distribution with normal Htt protein in HD patient, toxicity of mHtt is region-specific, especially striatum and cerebral cortex are vulnerable regions. Only a single report explains the striatum-specific neuronal degeneration resulting from mHtt sumoylation by Rhes (Ras homologue enriched in striatum) [25].

A prominent pathological feature of HD is accumulation of mHtt aggregates in neurons. mHtt is toxic to cells by affecting transcription, mitochondrial function, synaptic transmission and axonal transport (Fig. 1) [10]. mHtt interacts aberrantly and sequesters with many other cellular proteins into nuclear aggregates and cytoplasmic inclusion.

In HD patients and mouse models of HD, transcriptional dysregulation is an early feature in pathogenic mechanism. The transcriptions of key neuronal genes are progressively repressed by sequestering selective transcription factors and co-activator, including cAMP response element binding protein (CREB) binding protein (CBP), TATA-binding protein (TBP), p53, SP1 and TAFII-130 into aggregates of mHtt [26-28]. The mHtt also loses its ability to retain of cytoplasmic REST/NRSF, leading to transcriptional repression of neuron restrictive silencing element (NRSE) containing gene such as brain-derived neurotrophic factor (BDNF) [13, 29]. Nuclear localized REST/NRSF controls transcriptional repression of NRSE containing target genes through recruitment of co-repressor mSin3a, HDAC1 and HDAC2. Consistent with microarray data, ChIP-seq analysis reveals that the histone acetylation at promoter of key neuronal gene is reduced [30]. H3K4 trimethylation at transcriptionally repressed promoters is also decreased in human HD and in brain of HD model mice [31]. Thus therapeutic approach targeting histone methylation and acetylation is thought to rescue transcriptional dysregulation by mHtt. Many of HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA), sodium butyrate (SB) and trichostatin A (TSA) have been addressed to mouse model of HD, and have been demonstrated alleviation of mHtt toxicity [32-34].

The aberrant interaction of mHtt also affects axonal-transport of vesicle and cellular organelles. Cortical BDNF which is transported to striatum is critical to striatal neuronal activity implicated in cortico-striatal connection, but mHtt aberrantly interacts with HAP1 and p150Glued subunit of dynactin leads to impairment of retrograde transport of BDNF [35]. The mHtt not only damages retrograde transport, but also impairs anterograde transport due to reduction of α-tubulin acetylation, which is important for kinesin 1 binding to microtubules [36, 37].

**Fig. 1.** Potential molecular pathogenesis of toxicity of mHtt aggregates. Mutant huntingtin may affect the aberrant interaction with or sequester transcription factors leading to transcriptional dysregulation of many genes. Moreover, mutant huntingtin causes defects in trafficking of vesicle and cellular organelle such as mitochondria through long dendritic and axonal projections by affecting both molecular motors and microtubules. Finally, mutant huntingtin directly influence to decrease the $Ca^{2+}$ threshold for MPT pore opening by interaction with the outer mitochondrial membrane, leading to Cyt c release and apoptosis. Mutant huntingtin (mHtt), cAMP response element binding protein (CREB) binding protein (CBP), TATA-binding protein (TBP), specificity protein 1 (SP1) and TBP-associated factor, 135 kDa (TAFII-130), brain-derived neurotrophic factor (BDNF), mitochondrial permeability transition (MPT), Cytochrome c (Cyt c).

In HD pathogenesis, mitochondrial dysfunction is one of risk factors for neuronal survival. Mitochondria produce ATP, which is the cellular energy source, using oxidative phosphorylation, and mitochondria handle the calcium homeostasis in the brain. The mHtt not only inhibits the axonal-transport of mitochondria by affecting both molecular motors and microtubules [36], but also represses the expression of PPARγ co-activator-1α (PGC-1α) via interfering with CREB function [38]. The transcriptions of nuclear-encoded mitochondrial genes related to mitochondrial biogenesis and respiration are downregulated by impaired PGC-1α transcriptional activity in mouse model of HD. The mHtt also affects the outer mitochondrial membrane, and induces mitochondrial permeability transition (MPT) pore opening via reduction of $Ca^{2+}$ threshold to trigger opening, which is implicated in excitotoxicity mediated neuronal cell death [39].

**MOLECULAR CHAPERONE IN HD**

Within cells, misfolded proteins are refolded into their correct conformation by molecular chaperones or are degraded by proteasomal and lysosomal pathway. Molecular chaperones recognize misfolded protein through exposed hydrophobic surfaces and capture it to refolding. Molecular chaperones are also the first line of defense against misfolded protein aggregates. The molecular chaperones prevent inappropriate interactions between misfolded proteins or aberrant interactions with nearby proteins [40-42].

The common features of several types of neurodegeneration are the aggregation of misfolded causative proteins. Because most of neurodegenerative diseases occur in late-onset manners, many researchers consider that the accumulation of aggregates in neurons is attributed to reduction of functional capacity of molecular chaperones and proteasomal activity during the normal aging process. The level of molecular chaperones shows a biphasic response in neurodegenerative disease. In early stage, the expression of molecular chaperones is increased by aggregates-induced cellular stress, whereas amount of chaperones are decreased due to sequestration into aggregates in the late

pathogenesis, leading to progressive accumulation of protein aggregation [43-45]. Because molecular chaperone functions in prevention of the earliest aberrant protein interactions, which trigger pathogenic cascades, therapeutic approaches modulating chaperone expression and function can be promising for treatment of neurodegenerative diseases.
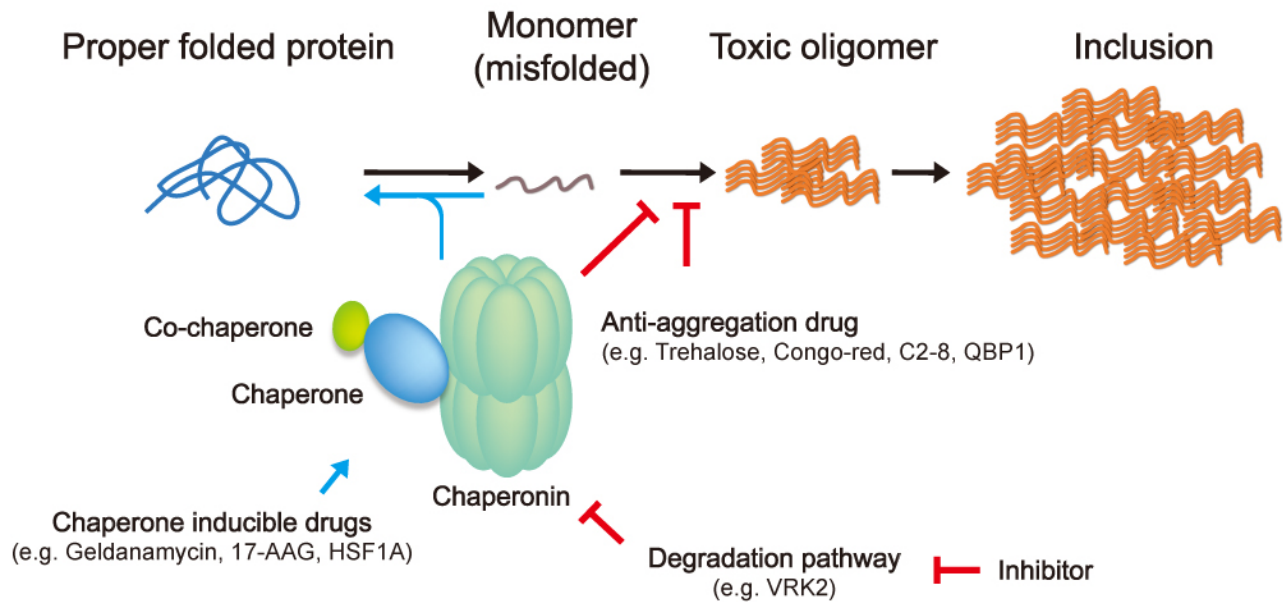
Several types of molecular chaperones are implicated in suppression of protein aggregation in HD model systems. In *Drosophila* HD disease models, HSP70 and HSP40 have been identified as genetic suppression factors against the neurotoxicity caused by mHtt [46, 47]. These studies have demonstrated that Hsp70 and Hsp40 directly interact with polyQ-containing exon1 of mHtt *in vivo* and *in vitro*. However, the formation of polyQ containing aggregates is not reduced by overexpression of Hsp70 in fly eyes. In the yeast system, polyQ-induced neurodegeneration is alleviated by co-expression of Hsp70 or Hsp40 with mHtt, and the results clearly showed that the inhibition of large-insoluble polyQ aggregates [47]. In the mouse model of HD, R6/2 HD transgenic mice was crossed with Hsp70-overexpressing transgenic mice, and the resulting R6/2-Hsp70 transgenic mouse exhibited the modest effects on disease progression [48]. Conversely, the deletion of Hsp70 in R6/2 transgenic HD mice exacerbates the behavioral and neuropathological defects, including decreased lifespan, weight loss, tremor, limb clasping and motor dysfunction. Although the lack of Hsp70 have no correlation with levels of fibrillar aggregates, the size of inclusion bodies formed by mHtt is increased in the neocortex of R6/2$^{tg/-}$ -Hsp70$^{-/-}$ mice [49]. Similarly, Hsp104 overexpressed transgenic mice with expressing the first 171 residues of mHtt not only ameliorate neurotoxicity via reduction of aggregate formation, but also prolong lifespan of HD mice by 20% [50]. In the lentivirus-based rat model of HD, Hsp104 and Hsp27 rescue the down-regulated dopamine and cAMP-regulated phosphoprotein 32 (DARPP-32) levels that lead to prevent striatal neuronal degeneration [51]. Other molecular chaperones have been reported to suppress detrimental effects induced mHtt aggregation. HSP84 co-localizes with mHtt aggregate *in vitro* and *in vivo*, and reduces the polyQ-mediated cellular toxicity [52]. ER chaperone glucose-regulated protein 78 (GRP78) inhibits the formation of mHtt aggregates and blocks cell death via inhibition of caspase-12 activation [53]. Prefoldin also reduces aggregates and cell death by mHtt through suppression of aggregation at the small oligomer stage [54].

Since the genome-wide RNA interference analysis identified the genes that suppress the polyQ aggregation in *C. elegans*, the eukaryotic chaperonin TCP-1 Ring Complex [TRiC, also called to chaperonin containing TCP-1 (CCT)], a member of HSP60 family, have been suggested to have a significant role in protecting against

polyQ aggregation [55]. Unlike HSP70, TRiC/CCT consists of eight subunits and forms ring-shaped complex that sequesters non-native state of protein in the cavity and properly folds in an ATP-dependent manner [56]. Several lines of evidence suggested that TRiC/CCT inhibits polyQ aggregation and alleviates the cytotoxicity of mHtt during the early stage of the aggregation process. In mammalian cell system, disruption of TRiC/CCT by RNA interference results in cellular toxicity caused by the appearance of soluble mHtt aggregates [57]. In yeast model system, knockdown of TRiC/CCT also increases the mHtt aggregation and toxicity. Notably, TRiC/CCT cooperates with the Hsp70 system to promote the assembly of mHtt into soluble oligomers about 500 kDa [58]. Interestingly, specific TRiC/CCT subunit, including CCT1 and CCT4, modulates the polyQ aggregation to non-pathogenic conformations. Moreover, overexpression of a single TRiC/CCT subunit CCT1 is sufficient to rescue mHtt-aggregate formation [59]. Apical domain of CCT1 directly interacts with N17 domain of mHtt and prevents the aggregation with inter- and intramolecular interactions within mHtt [18]. Recently, it is demonstrated that exogenous apical domain of CCT1 (ApiCCT1) is delivered to striatal neuronal cells prepared from full-length knock-in HD mice, and ApiCCT1 is sufficient to alleviate mHtt-mediated toxicity and delays the onset of inclusion body formation [60]. In addition to subunit specific effect, TRiC/CCT complex also affects the aggregation of mHtt via capture of the smaller mHtt oligomers within its cavity [61].

## THERAPEUTIC APPROACH TO INDUCE THE MOLECULAR CHAPERONE LEVELS

Over the past decade, a number of studies have revealed that molecular chaperones alleviate neurodegeneration through modulation of the aberrant protein interactions by mHtt in the early stage of aggregation (Fig. 2). Heat-shock factor protein 1 (HSF1) is known to induce a set of HSP proteins under the stress condition such as heat-shock [62]. Overexpression of active form of HSF1 elevates the expression of HSP proteins and suppresses the mHtt aggregates formation in cultured cells, and HSF1-overexpression transgenic mouse showed prolonged lifespan and restoration of weight loss [63]. Pharmacological agents have been reported to potentiate chaperone expression by HSF1 activation. It is well-characterized that these drugs inhibit the HSP90 action against negative regulation of HSF1 activation. Geldanamycin, known as a HSP90 inhibitor, binds to ATP-binding site of HSP90 and blocks the interaction between HSP90 and HSF1, leading to HSF1 trimerization and activation of HSPs synthesis [64, 65]. Additionally, 17-allylamino-17-demethoxygeldanamycin (17-

**Fig. 2.** Therapeutic approach to inhibit mHtt aggregation. An initiating event in aggregation may conversion of mutant huntingtin to an abnormal conformation. It leads to progress through oligomeric intermediates to the formation of large aggregates. Although there are still controversies which one is more toxic between soluble oligomer and insoluble aggregates, inhibition early in the aggregation pathway would be beneficial to the cells because it may prevent the formation of putative toxic insults. Accordingly, molecular chaperones and anti-aggregation drugs are shed the light in this therapeutic intervention. Particularly, molecular chaperones not only induce proper folding of misfolded proteins by interacting with exposed hydrophobic surfaces, but also inhibit aggregation with mutant huntingtin itself. A number of HSF1 activating drugs have been developed to induce the Heat-shock proteins, but transcription of TRiC/CCT was not affected by HSF1. Thus, another pathway is necessary to modulate the TRiC/CCT levels such as inhibition of degradation pathway. Recently, one report reveals that VRK2 facilitates the TRiC/CCT protein degradation through increase of its ubiquitination. 17-allylamino-17-demethoxygeldanamycin (17-AAG), polyglutamine binding peptide 1 (QBP1), vaccinia-related kinase 2 (VRK2).

AAG), a geldanamycin derivative, suppresses neurodegeneration in a fly HD model [66]. Recently, HSF1A, small benzyl pyrazole-based molecule, has been developed as an activator of HSF1 without inhibition of HSP90 to avoid undesirable proteotoxic activity [67]. Since TRiC/CCT expression is not affected by HSF1, it is necessary to develop another approach elevating the TRiC/CCT protein levels such as inhibition of degradation pathway. Although the turnover mechanism of TRiC/CCT still remains unclear, TRiC/CCT is degraded by ubiquitin-proteasome system [68]. Interestingly, a recent study demonstrated that vaccinia-related kinase 2 (VRK2) has a role in degradation of TRiC/CCT, which was dependent on its kinase activity and enhanced the accumulation of mHtt aggregates in cultured cell lines [69]. It would be possible approach to develop therapeutic inhibitors targeting VRK2 for HD.

**ANTI-AGGREGATION AGENTS**

A number of studies to search small molecules inhibiting oligomerization of β-sheet containing peptide have demonstrated that these molecules can successfully alleviate the symptoms of

disease (Fig. 2). When the azo-dye congo-red was intraperitoneally injected to R6/2 HD transgenic mice after onset of symptoms, it promoted the clearance of mHtt aggregates *in vivo* and exerted protective effects on survival, weight loss and motor function [70]. The disaccharide trehalose also prevented the polyQ aggregation *in vitro* and *in vivo*, and had beneficial effects on striatal atrophy, weight loss, survival and motor function in R6/2 transgenic mouse model [71]. Because both congo-red and trehalose cannot penetrate blood-brain barrier (BBB), some reports said difficulties to confirm the beneficial effects of congo-red [72] and trehalose when administrated high concentration (2% in water) to HD mouse. To solve this problem, cell-penetrating peptide like guanidine residues have been introduced to trehalose. Accordingly, this trehalose derivative crosses BBB even at lower concentration (0.4% in water) and shows the similar beneficial effects on disease symptoms [73]. Moreover, BBB-permeable C2-8 small inhibitor has also been screened using yeast-based aggregation assay, and it improves the motor functions and reduces harmful effects on neuronal atrophy in HD model mouse at non-toxic dose [74]. In addition to small molecules, polyglutamine binding peptide 1 (QBP1) also significantly suppresses polyQ aggregation and

neurodegeneration in fly HD model [75].

## CONCLUSIONS

There are promising evidences leading us to understand the effects of mHtt, and the critical step of HD pathogenesis would be aggregation of mHtt, which results in a number of neuronal protein sequestrations into intranuclear and cytoplasmic aggregates by aberrant interaction. Accordingly, depletion of transcriptional factor and molecular motor cause the transcriptional dysregulation of important neuronal genes such as BDNF, neurotransmitters and its cognate receptors, and axonal transport of vesicles and mitochondria, leading to neurodegeneration. Post-mitotic neurons are vulnerable against toxic aggregates because neuron cannot dilute the toxic insults during cell division, consequently, it will be a heavy load to protein quality control machinery.

According to our understating of the pathogenesis, it would be rational therapeutic approach to treat HD through inhibition of mHtt aggregation, followed by subsequent alleviation of its downstream harmful effects. Molecular chaperones may have a central role in this process, and anti-aggregation drugs are also shed the light in this approach. Since molecular chaperone inducing drugs have been demonstrated beneficial effects on neurodegeneration in animal models, it is necessary to understand the upstream pathway of modulating the molecular chaperone for the first effective treatment for HD.

## ACKNOWLEDGEMENTS

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