PERSPECTIVE

Excitatory synapse impairment and mitochondrial dysfunction in Huntington's disease: heat shock factor 1 (HSF1) converging mechanisms

Heat shock factor 1 (HSF1) is abnormally degraded in Huntington's disease (HD): HD is a neurodegenerative disorder characterized by severe cognitive and motor impairments. HD is caused by a CAG repeat expansion within exon 1 of the huntingtin (*HTT*) gene (The Huntington's Disease Collaborative Research Group, 1993). These expansions lead to the production of an aberrant mutant huntingtin protein (mHTT) that is prone to misfolding and aggregation. Expression and aggregation of mHTT is present in virtually all cell types in the body but preferentially affects medium spiny neurons of the striatum, a brain region that controls movement and some forms of cognition. Accumulation of mHTT leads to, but not only, transcriptional dysregulation, DNA damage, mitochondrial dysfunction and excitatory synaptic failure ultimately causing neuronal death. However, the molecular mechanisms by which mHTT exerts these defects are still unclear.

Recently, we described that HSF1, a transcription factor that regulates stress protective proteins, energy metabolism, neuronal identity and synapse formation is inappropriately degraded in HD (Gomez-Pastor et al., 2018). Degradation of HSF1 contributes to mitochondrial dysfunction and synaptic deficits caused by mHTT (Gomez-Pastor et al., 2017; Intihar et al., 2019). However, whether these processes are interconnected is still unclear. In mammals, HSF1 belongs to a family of HSFs, comprised by HSF2, HSF4 and HSF5 (reviewed in Gomez-Pastor et al., 2018). HSF2 has also been implicated in HD for its role in protein homeostasis and mHTT aggregation (reviewed in Gomez-Pastor et al., 2018). However, HSF1 is the most studied HSF in the context of HD. Therefore, we will focus this article on HSF1 and how mHTT induced HSF1 loss and mitochondrial dysfunction can converge into mediating excitatory synapse impairment in HD.

HSF1 is subject to several post-translational modifications and protein-protein interactions that regulate HSF1 oligomerization, nuclear translocation, DNA binding and degradation (reviewed in Gomez-Pastor et al., 2018). All these steps are critical for a proper activation of HSF1 under stress conditions. In HD, HSF1 is abnormally degraded by the sequential action of two inappropriately up-regulated proteins, Protein kinase CK2 alpha prime (CK2α') and the E3 ligase Fbxw7 (Gomez-Pastor et al., 2017). CK2α' hyper-phosphorylates HSF1 at serines 303 and 307 and Fbxw7 ubiquitylates phospho-HSF1, signaling the HSF1 for proteasomal degradation. HSF1 degradation contributes to altering the transcriptional profile of numerous genes, including protein chaperones, mitochondrial factors and synaptic proteins, thus aggravating mHTT-mediated cytotoxicity (Gomez-Pastor et al., 2017). HSF1 has proven therapeutic potential and different pharmacological studies aimed at activating HSF1 have been conducted in HD and other human proteinopathies (Gomez-Pastor et al., 2018). However, the use of molecules that activate the HSF1 response have failed to maintain long-term benefits in HD mouse models (reviewed in Gomez-Pastor et al., 2018). These results may in fact be due to the progressive loss of HSF1 protein.

Our previous work demonstrated that lack of one allele of CK2α' in the HD mouse model zQ175 (zQ175;CK2α$^{'+/-}$) was sufficient to prevent HSF1 degradation and increased the expression of several chaperones (Hsp25 and Hsp70) and mitochondrial factors (peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), mitochondrial transcription factor A and cytochrome c somatic) (Gomez-Pastor et al., 2017). Preventing HSF1 degradation also improved long-term HD-like features such as decreased HTT aggregation, ameliorated weight loss, increased medium spiny neurons' spine maturation and enhanced thalamo-striatal excitatory synapse density. These results suggested that preventing HSF1 degradation may be a more effective and long-lasting therapeutic strategy in HD. However, how HSF1 mediates these beneficial effects has yet to be determined.

Mitochondrial dysfunction and HSF1 degradation: Mitochondria are critical organelles in neurons due to their role in controlling Ca^{2+} signaling and energy metabolism. Altered mitochondrial structure and function is a hallmark of HD. Mitochondrial dysfunction results in decreased adenosine triphosphate, altered Ca^{2+} buffering and impaired oxygen consumption. These alterations significantly affect striatal neurons, which are subjected to a higher energy demand than other neuronal types. We have demonstrated that lack of HSF1 in murine immortalized ST*Hdh* striatal cells derived from wild-type mice (ST*Hdh*^{Q7}) negatively altered mitochondrial function by increasing mitochondrial depolarization. This phenotype mirrored the deficits observed in cells derived from a knock-in HD mouse model (ST*Hdh*^{Q111}) (Intihar et al., 2019), suggesting the involvement of HSF1 loss in mitochondrial dysfunction in HD. We have recently reviewed this topic and proposed a crosstalk between the functions of HSF1 and two transcription factors previously associated to mitochondrial dysfunction in HD: tumor suppressor p53 and the mitochondrial biogenesis peroxisomal proliferator co-activator PGC-1α (Intihar et al., 2019).

p53 controls different biological processes including DNA repair, mitochondrial metabolism and apoptosis and its protein levels are increased in HD. mHTT has been shown to bind to p53 and it is believed that such pathological interaction leads to p53 protein stabilization, mitochondrial hyperpolarization and increased cell death (Bae et al., 2005). Genetic and pharmacological inhibition of p53 has demonstrated amelioration of mitochondrial, neuropathological and behavioral deficits in HD cells and mouse models (Bae et al., 2005). However, the mechanism by which mHTT mediates p53 stabilization and up-regulation is still unknown. Two main systems control the levels of p53, the E3 ligase Mdm2 and the αβ-crystallin/Fbx4 complex (Jin et al., 2009). Mdm2 directly interacts with p53 and promotes ubiquitylation and degradation of p53. Alternatively, the chaperone αβ-crystallin mediates the degradation of p53 by acting as a bridge protein and recruiting the Fbx4 E3 ligase. αβ-Crystallin$^{-/-}$ cells show increased p53 levels (Jin et al., 2009). Interestingly, αβ-crystallin is a gene target of HSF1 and its expression is decreased in HD (Gomez-Pastor et al., 2017). This suggests that degradation of HSF1 and the consequent down-regulation of αβ-crystallin may contribute to the stabilization and up-regulation of p53 levels and mitochondrial dysfunction in HD. On the other hand, the fact that p53 also binds to and regulates the expression of Fbxw7 in cancer cells allows us to hypothesize that stabilization of p53 by mHTT could contribute to HSF1 degradation in HD (Intihar et al., 2019). These intricate pathways of induced p53 and degraded HSF1 could thereby exacerbate mitochondrial dysfunction in HD.

We have also demonstrated that HSF1 directly binds to and regulates the expression of PGC-1α in STHdh cells (Intihar et al., 2019). We showed that ST*Hdh*^{Q111} cells showed decreased HSF1 binding to the PGC-1α promoter which correlated with decreased PGC-1α expression compared to ST*Hdh*^{Q7} cells. More importantly, our data showed that overexpression of HSF1 in ST*Hdh*^{Q111} cells restored PGC-1α expression. Therefore, expression impairment of PGC-1α and mitochondrial dysfunction in HD could be mediated, at least in part, by the degradation of HSF1. In support of this hypothesis, our previous work demonstrated the enhancement of PGC-1α and its effect on expression of downstream mitochondrial genes after preventing HSF1 degradation in the zQ175;CK2α$^{'+/-}$ mouse model (Gomez-Pastor et al., 2017). However, further investigation using *in vivo* models will be necessary to demonstrate the connection between mitochondrial dysfunction, PGC-1α expression impairment and HSF1 degradation in HD.

HSF1 and excitatory synapse impairment in HD: Glutamate is the main excitatory neurotransmitter in the central nervous system. Imbalances in glutamate levels have been implicated in many neurodegenerative diseases, including HD. Glutamate binds to ionotropic N-methyl-D-aspartic acid and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (NMDARs and AMPARs) on the postsynaptic membrane, causing positive ions to flow into the cell and increase the cell's electrical charge and excitability. Activation of postsynaptic NMDARs is important for controlling synaptic plasticity and memory function and leads to cell protective mechanisms. However, mHTT alters glutamate release and uptake and causes over-activation of the NMDAR signaling (Zeron et al., 2002). Overactivation of NMDARs results in excessive Ca^{2+} influx, altering the mitochondrial membrane potential and causing cell death. This phenomenon is known as glutamate-mediated excitotoxicity.

The striatum receives glutamatergic excitatory input from both the cortex and thalamus. Both cortico- and thalamo-striatal excitatory circuitry are disrupted in HD, although different HD mouse models have shown that thalamo-striatal synapses display changes in function and density at early disease stages (Kolodziejczyk and Raymond, 2016; Gomez-Pastor et al., 2017). Alteration in the levels of NMDAR, AMPAR and specific synaptic scaffolding proteins contribute to striatal excit-

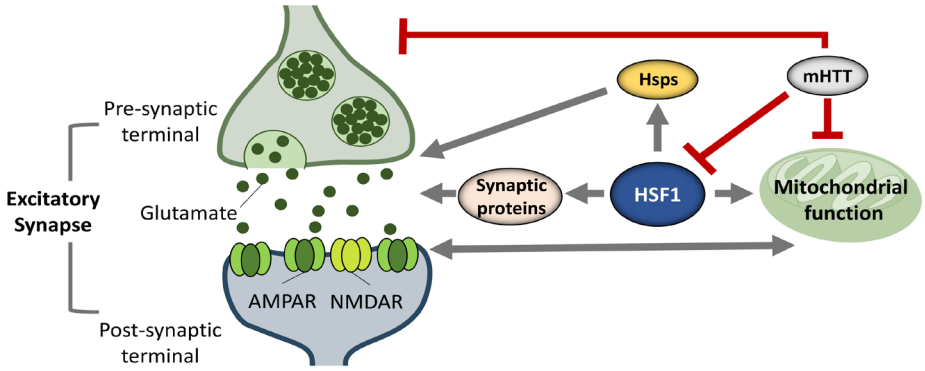

Figure 1 Working model for synapse impairment and mitochondrial dysfunction in Huntington's disease. Mutant HTT (mHTT) alters excitatory synaptic transmission and mitochondrial function and promotes heat shock factor 1 (HSF1) degradation. HSF1 regulates the expression of synaptic proteins, chaperones (Hsps) and mitochondrial factors and its degradation contributes to the synaptic and mitochondrial deficits in Huntington's disease. AMPAR: A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDAR: N-methyl-D-aspartic acid receptor.

atory dysfunction in HD. Our previous work showed that preventing HSF1 degradation in the zQ175 mouse model increased thalamo-striatal synapse density although no changes in cortico-striatal synapses were observed (Gomez-Pastor et al., 2017). These results suggest a specialized role of HSF1 in regulating different synaptic circuits.

The benefit of restoring HSF1 levels in HD has been demonstrated in various neuropathological aspects of the disease, however it is still unknown how HSF1 contributes to the enhancement of excitatory synapses in zQ175;CK2α'$^{+/-}$ mice. Several hypotheses can be considered. It is known that chaperones serve as a protective mechanism for excitatory synapses. Synapses rely on efficient protein homeostatic mechanisms to preserve their structure and function and require local chaperone activity to modulate neurotransmission and synaptic assembling (Gorenberg and Chandra, 2017). Chaperones like Hsp70 also participate in protecting glutamatergic synaptic transmission from glutamate excitotoxicity. Therefore, although up-regulation of chaperones in the zQ175;CK2α' mouse is an HSF1-dependent effect, the direct action of chaperones would be the determining factor in controlling excitatory synapses in HD. However, this hypothesis does not completely explain the selectivity of inducing one circuitry or another.

On the other hand, HSF1 has been previously related to the direct regulation of synaptic proteins in different contexts. Hsf1$^{-/-}$ mice have shown decreased dendrite length in the hippocampus and decreased expression of the postsynaptic scaffolding protein postsynaptic density-95, required to anchor NMDARs and AMPARs to the membrane, and the polysialic acid-neural cell adhesion molecule (Uchida et al., 2011), demonstrating the role of HSF1 in neuronal development. In addition, activation of HSF1 using 17-AAG, an Hsp90 inhibitor, in a mouse model of Alzheimer's disease resulted in the upregulation of different synaptic proteins such as the presynaptic markers synapsin I and synaptophysin, and postsynaptic density-95 (Chen et al., 2014). Although it is still unknown whether loss of HSF1 is directly responsible for the transcriptional alterations of synaptic proteins in HD, it is reasonable to hypothesize that enhancement of excitatory synapse density in the zQ175;CK2α'$^{+/-}$ mouse may be directly influenced by HSF1-dependent transcriptional regulation of pre and postsynaptic proteins. Expression of different synaptic proteins could display synapse specificity in their actions and therefore explain the differential role of HSF1 in regulating a subset of excitatory synapses.

Finally, HSF1 could influence excitatory synapses by modulating mitochondrial function. It is known that mitochondria support synaptic function by providing adenosine triphosphate and maintaining synaptic ion homeostasis. Mitochondrial function extends to neurotransmitter synthesis and storage as well as synaptic vesicle recycling. Dendritic spines of glutamatergic synapses require high amounts of Ca^{2+} influx through NMDARs and therefore mitochondrial activity is essential to modulate excitability (Zeron et al., 2002). Recently, the role of mitochondrial dysfunction, altered Ca^{2+} metabolism and accumulation of mitochondrial reactive oxygen species in mediating synaptic transmission deregulation in Alzheimer's disease has been discussed (Guo et al., 2017). Since synaptic mitochondrial dysfunction occurs prior to synaptic deficits in early stage Alzheimer's disease, it implies that mitochondrial deregulation could promote synaptic stress. We have shown that HSF1 influences the levels of PGC-1α, mitochondrial function and excitatory synapse density in HD (Gomez-Pastor et al., 2017; Intihar et al., 2019), and based on previous studies, it is reasonable to hypothesize that all of these processes may be interconnected (**Figure 1**). However, further studies are required to determine how HSF1 regulates the formation and integrity of excitatory synapses and whether HSF1-dependent mitochondrial dysfunction contributes to synaptic failure in HD.

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