

Review

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Small heat shock proteins and neurodegeneration: recent developments

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Abstract: Members of the small heat shock protein (sHSP) family are molecular chaperones with a critical role in the maintenance of cellular homeostasis under unfavorable conditions. The chaperone properties of sHSPs prevent protein aggregation, and sHSP deregulation underlies the pathology of several diseases, including neurodegenerative disorders. Recent evidence suggests that the clientele of sHSPs is broad, and the mechanisms of sHSP-mediated neuroprotection diverse. Nonetheless, the crosstalk of sHSPs with the neurodegeneration-promoting signaling pathways remains poorly understood. Here, we survey recent findings on the role and regulation of sHSPs in neurodegenerative diseases.

Keywords: Alzheimer's disease; Apoptosis; Heat shock response; Huntington's disease; Inflammation; Neurodegenerative pathologies; Parkinson's disease; Protein aggregation; Proteostasis; sHSP.

Introduction

Organisms and cells are constantly exposed to various types of stress, such as environmental, metabolic or pathophysiological stress. These disturbances result in loss of integrity of the proteome, cellular dysfunction and cell death. In the course of evolution, elaborate molecular mechanisms were invented to counteract the detrimental effect of extrinsic and intrinsic stress factors on protein function, and preserve protein homeostasis

(proteostasis). An evolutionary conserved cytoprotective mechanism is the heat shock response pathway [1, 2]. Prominent effectors of the heat shock response pathway are molecular chaperones, which comprise of several families categorized according to their molecular weight: HSP10, HSP40, HSP60, HSP70, HSP90, HSP100 and sHSP. Chaperones are required for the maintenance of the native structure of cellular proteins, protein translocation, assembly of functional protein complexes and protein degradation. sHSPs are ATP-independent molecular chaperones characterized by a small molecular mass ranging from 12 to 42 kDa [3] and a highly conserved domain, called the alpha-crystallin domain [4]. sHSPs display a dynamic behavior, and can exist in the form of monomers, dimers and large multimeric complexes, exhibiting variability in subunit numbers (12 to >48) [5-7].

sHSPs have a wide clientele and sHSP dysfunction results in a broad range of pathologies, including ischemia, myopathies, motor neuron disease, diabetes and cataracts [8-11]. The main function of sHSPs is to bind to hydrophobic regions of aggregation-prone misfolded proteins and prevent the formation of insoluble aggregates [12]. However, association with sHSPs does not lead to substrate refolding to the native state [13-15]. Therefore, the sHSP/substrate complexes act as intermediates that are further processed by the HSP70 and HSP90 chaperones [16-18]. Here, we highlight recent findings regarding the interplay between sHSP function and neurodegeneration.

The role of sHSPs in neurodegenerative pathologies

Many devastating neurological disorders, such as Alzheimer's, Parkinson's, Creutzfeldt Jacobs and amyotrophic lateral sclerosis (ALS), are characterized by aggregation and precipitation of misfolded proteins. sHSPs are key players of the proteostasis network, and their ability to interact with aggregation-prone proteins

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highlights the therapeutic potential of these heat shock proteins [19]. The deposition of fibrillar α -Synuclein (α -syn) into inclusion bodies in the neuronal cell body or processes is a pathological hallmark of neurodegenerative disorders, collectively known as synucleinopathies [20]. The expression of sHSPs is significantly increased in the presence of cellular stress [21], and sHSPs have been colocalized with α -syn in inclusion bodies [22, 23]. The capacity of sHSPs to prevent α -syn aggregation depends on the kinetics of the aggregation process. Factors that increase the rate of aggregation, such as disease-related mutations, gene amplification, or macromolecular crowding, may alter the kinetics of α -syn aggregation in cells, thus overwhelming the protective capacity of sHSPs due to reduced availability of aggregation-combating chaperone subunits [24, 25]. HSPB1 (known also as HSP27) binds along the surface of α -syn fibrils and inhibits their growth by blocking elongation [26]. In addition to HSPB1, HSPB5 (α B-crystallin) also reduces the aggregation of α -syn in *in vitro* assays [27]. Another sHSP, HSPB8 (also known as HSP22) participates in the clearance of several misfolded proteins implicated in neurodegenerative diseases. HSPB8 acts in cooperation with the co-chaperone BAG3 and promotes the clearance of protein aggregates through upregulation of autophagy [28]. These findings show that sHSPs link the different nodes of the proteostasis machinery to counteract the accumulation of misfolded proteins.

A protective role of HSPB5 has been described in Alzheimer's disease, characterized by A β amyloid fibrils in extracellular plaques [29]. HSPB5 binds and inhibits the elongation of amyloid fibrils [30]. Interestingly, HSPB8 is upregulated specifically in astrocytes of cerebral areas undergoing neurodegeneration [31], suggesting that astrocytic proteostasis is critical for the clearance of aggregates in the neuronal microenvironment. HSPB4 (α A-crystallin) and HSPB5 knockout mouse models are viable and have tissue specific developmental roles [32, 33]. Another sHSP, HSPB3, found in the central and peripheral nervous system, protects against motor neuron cell death [34]. Recent findings suggest that different members of the proteostasis machinery of the cell may prevent aggregation of proteins through distinct mechanisms. HSPB1 delayed tau (a microtubule-associated protein forming aggregates in various dementias) fibril elongation, by weakly interacting with early species during the aggregation process [35]. Interestingly, the phosphorylation dynamics of HSPB1 appears to be critical for the ability of this sHSP to reduce neuronal tau levels [36].

Alexander disease (AxD) is a rare disorder of astrocytes caused by mutations in the gene encoding

for glial fibrillary acidic protein (GFAP). In AxD mouse models, overexpression of HSPB5 has a protective effect [37] and restores the defective proteasomal degradation of mutant GFAP [38], suggesting that this sHSP may serve as a therapeutic target in astropathology. In spinal and bulbar muscular atrophy, a disease characterized by abnormally long polyglutamine tract (polyQ) in mutant androgen receptor (ARpolyQ), HSPB8 promotes motorneuron survival of patients by restoring autophagic flux and removing misfolded aggregates of ARpolyQ [39].

Charcot-Marie-Tooth (CMT) is the most common inherited peripheral neuropathy. Missense mutations in HSPB8 and HSPB1 have been implicated in CMT pathogenesis [40-42]. HSPB8 knockout mice are viable and display normal locomotor performances [43]. The list of mutations affecting the function of these sHSPs continues to grow [44, 45]. Transgenic mice expressing mutant HSPB1 developed features of CMT [46, 47] and displayed decreased α -tubulin acetylation and defective axonal transport of mitochondria [46]. Intriguingly, pharmacological inhibition of histone deacetylase 6 (HDAC6) ameliorated the axonal transport defects caused by mutant HSPB1, underlying the importance of identifying downstream targets of sHSPs. In addition, recent knock-in mouse models expressing mutant HSPB8, recapitulated the CMT patient pathology and displayed reduced autophagy and HSPB8 aggregates [43].

In an animal model of experimental autoimmune encephalomyelitis (EAE), genetic ablation of HSPB5 dramatically increased animal clinical paralysis and induced inflammatory responses [48]. The inflammation was accompanied by an elevated temperature, and therefore heat shock proteins serve as ideal effectors in inflamed tissue. Interestingly, exogenous administration of HSPB5 suppressed inflammation in animals. HSPB5 is a potent modulator of inflammation and sequesters, in a temperature-dependent manner, a variety of pro-inflammatory proteins, therefore affecting both innate and adaptive immune responses [48].

In a systematic approach for the identification of sHSPs with a potential role in cerebral ischemia *in vivo*, investigators observed upregulation of HSPB1 and HSPB5 in neurons in the infarcted cortex [49]. Phosphorylation of those sHSPs is critical for their neuroprotective role during cerebral ischemia [49, 50]. In addition, HSPB1 maintains the integrity of the blood-brain barrier during ischemia/reperfusion injury in experimental stroke, suggesting a potential therapeutic role of HSPB1 in injuries characterized by breach in brain vasculature [51].

sHSPs in the presence of external stress

In addition to neurodegeneration caused by detrimental protein aggregation, our research uncovered a protective role of sHSPs against necrotic cell death and neurodegeneration triggered by hyperthermia [52, 53]. We found that during the preconditioning of animals at mildly elevated temperatures, heat shock transcription factor 1 (HSF-1) upregulated the expression of HSP-16.1, which protects neurons against subsequent heat-induced damage. HSP-16.1 exerts its protective role through the $\text{Ca}^{2+}/\text{Mn}^{2+}$ ATPase PMR-1 in the Golgi. Importantly, this protective mechanism is evolutionary conserved and protects murine neurons against heat stroke-inflicted neurodegeneration [52]. These data suggest that organelle-specific Ca^{2+} channels and pumps might be important targets of sHSPs, and the protection of the function of these proteins under stress conditions preserves organelle, and consequently cellular ionostasis [53]. In a *Drosophila* model, external heat stress disrupted proteostasis and led to degeneration of muscle, motor neurons and associated glia. Intriguingly, muscle-specific overexpression of the sHSP HSP23 also had a protective effect on neurons and glia, suggesting the presence of a sophisticated intercellular proteostasis network [54]. Extracellular HSPB4 and HSPB5 protected astrocytes from various harmful insults, such as staurosporine and serum-starvation, through activation of pro-survival signaling pathways [55]. Finally, in the presence of external stress, overexpression of HSPB5 preserved the dendritic architecture in rat hippocampal neurons [56].

The multifaceted action of sHSPs

Inhibition of the apoptotic machinery is a key mechanism accounting for the cytoprotective effect of sHSPs. sHSPs participate in both intrinsic and extrinsic apoptotic signaling pathways. HSPB1 abolishes the release of cytochrome c from mitochondria [57], and protects against apoptosis through AKT activation and subsequent Bax inhibition [58]. HSPB1 knockout mice are viable and fertile [59, 60]. Interestingly, HSPB1 binds to cytochrome c released from mitochondria and prevents apoptosome formation [61]. In addition, HSPB6 and HSPB8 suppress apoptosis by inhibiting cytochrome c release from mitochondria [62]. In the extrinsic pathway, HSPB2 suppresses the activation of caspases-8 and 10 therefore blocking downstream apoptotic events, such as caspase-3

activation [63]. Also, HSPB4/5 have anti-apoptotic effects against Bax-induced apoptosis by sequestering Bax in the cytoplasm and preventing its translocation to the mitochondria [64]. sHSPs are critical for the survival of adult mammalian neurons. The fate determinant transcription factor Pax6 maintains homeostasis by directly regulating the expression of HSPB4, which inhibits the activation of procaspase-3 and apoptosis [65]. Therefore, sHSPs exert their anti-apoptotic role through blocking critical nodes in the cell death pathway, such as the release of cytochrome c from mitochondria and the activation of caspases.

Several sHSPs show organelle specificity [66-69]. Phosphorylation of HSPB5 results in localization at SC35 speckles, a nuclear compartment involved in the storage and recycling of splicing factors [66]. Further investigation will show whether this sHSP has a role in speckle stability and regulation of gene expression. HSPB8 was found to localize to stress granules, membrane-less compartments that form upon proteotoxic stress and sequester ribonucleoprotein complexes. Importantly, the disassembly of stress granules depends on the concerted action of the HSPB8-BAG3-HSP70 complex, suggesting that sHSPs are critical mediators of protein quality surveillance mechanisms of the cell [70, 71]. These findings may have clinical significance given the implication of stress granules in ALS pathology. Several sHSPs exert their function in mitochondria, possibly through preservation of membrane integrity. HSP25 (the murine homologue of HSPB1) localizes to the mitochondria and protects PC12 cells (rat pheochromocytoma) against 6-Hydroxydopamine (6-OHDA), a neurotoxin that targets dopaminergic neurons [72]. Better understanding of the organelle-specific roles of sHSPs will uncover the molecular mechanisms deregulated in pathological conditions caused by sHSPs malfunction.

In addition to binding to proteins and preventing their denaturation [73], sHSPs are also active in the extracellular matrix and alter gene expression through membrane receptor signaling [74]. sHSPs extracellular concentration increases in several neurodegenerative conditions [75]. Serum levels of HSPB1 have been shown to correlate with acute attack phases in patients with multiple sclerosis [76]. In contrast to secreted proteins that typically exit cells via the endoplasmic reticulum-Golgi network, HSPs lack an N-terminal signal peptide that marks the protein for secretion. In addition to sHSP accumulation into circulation in response to either cell damage or cellular stress, another mechanism for the release of HSPB1 to the extracellular space is through exosome secretion [77]. Specifically, HSPB1 was detected in membrane fractions of

exosomes released from rat astrocytes upon treatment with amyloid- β . The precise localization of sHSPs in exosomes is still under investigation and depends on the type of sHSP, the cells from which the exosome is released and the stimuli that induce the release. Extracellular application of HSPB4/5 activated the PI3K/Akt/mTOR signaling pathway and promoted survival of olfactory ensheathing cells [78]. HSPB5 was also secreted via exosomes by highly polarized human retinal pigment epithelial cells (RPE). HSPB5 uptake from apical photoreceptors facing neural retina conferred neuroprotection in the presence of oxidative stress [79]. In addition to the release of HSPB5 via exosomes, a recent study demonstrated that HSPB5 is also critical for exosome biogenesis [80]. Administration of HSPB4 peptides inhibited protein insolubilization and opacification in selenite-induced cataract in mice [81]. Similarly, HSPB1 and HSPB6 peptides inhibited protein aggregation and oxidative stress, and prevented selenite-induced cataract formation in rats [82]. Extracellular HSPB5 neutralized misfolded oligomers and protected cells by shielding the reactive surfaces [83]. The mechanisms by which extracellular sHSPs exert beneficial functions need further investigation and may be stress-specific.

Inflammation underlies the pathology of many neurodegenerative diseases [8]. Prolonged inflammation results in cytotoxicity in the nervous system. sHSPs have the ability to control neuroinflammation through activation of macrophages [84]. Moreover, HSPB4/B5 prevent the accumulation of proinflammatory mediators and suppress ROS and NO production [48, 73, 85]. Also, in a mouse model of acute spinal cord injury, administration of human recombinant HSPB5 improved locomotor skills and ameliorated secondary tissue damage. Mechanistically, HSPB5 modulated an inflammatory response in the injured spinal cord, promoted granulocytes infiltration and reduced accumulation of inflammatory macrophages [86]. The therapeutic potential of HSPB5 is also evident by the increased survival of retinal ganglion cells and the inhibition of retinal microglial cells in the rat model of optic nerve crush [87]. Importantly, HSPB5 administration inhibits the expression of TNF α and iNOS in this model. Treatment of an EAE mouse model with HSPB1 reduced the paralytic symptoms through modulation of inflammatory cytokines [88]. Pro-inflammatory cytokines induce the formation of ROS and NO, and subsequently downregulate the levels of HSPB1 and promote apoptosis of retinal capillary endothelial cells [89]. HSPB8, HSP20 (HSPB6) and HSPB2 are associated with cerebral amyloid angiopathy (CAA) in hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D). Importantly, these sHSPs induce interleukin-6 production in cultured

astrocytes and pericytes, suggesting that sHSPs may be critical mediators of local inflammatory response in HCHWA-D [90]. In Alzheimer's disease, the majority of patients are characterized by some degree of CAA, and HSP20, HSPB8 and HSPB2B3 colocalize with CAA and induce production of interleukin-8, intercellular adhesion molecule 1 (ICAM-1) and monocyte chemoattractant protein by human brain astrocytes, reinforcing the role of sHSPs in neuroinflammation in Alzheimer's disease [91]. These findings, in their totality, suggest that exogenous administration of sHSPs has a protective effect in many diseases involving cell death, inflammation and protein aggregation.

The transcriptional regulation of sHSPs

In addition to a plethora of post-translational modifications, including phosphorylation, isomerization, deamidation and glycation [92, 93], sHSPs also undergo transcriptional regulation. Although the transcriptional regulation of sHSPs during development of various tissues is well described [92], understanding of the gene expression regulation in disease is lacking. Historically, the transcription of heat shock proteins, including sHSPs, has been attributed to the heat shock transcription factors (HSFs) [1]. However, the crosstalk of HSFs with disease-promoting signaling pathways and other transcription factors is poorly understood. Recent findings have implicated signaling pathways, with a critical role in disease, in the regulation of HSF1 [2, 94, 95]. In neurons, the HSF1 amino acids Ser303 and Ser307, two critical residues for the stability of HSF1 [94], are phosphorylated by the casein kinase 2 (CK2). These phosphorylation events link HSF1 to the E3 ligase FBXW7 which ubiquitylates HSF1, resulting in proteasomal-mediated degradation [2, 96]. In Huntington's disease, the CK2 α catalytic subunit and FBXW7 are elevated. Subsequently, phosphorylated HSF1 interacts with FBXW7 and is degraded in the proteasome leading to protein aggregation, neuronal dysfunction and death [96]. Future experiments will reveal whether exogenous administration of sHSPs is sufficient to prevent aggregate formation in this model and in neurodegenerative disorders characterized by HSF1 reduced protein stability. Interestingly, decreased activity of HSF1 has been reported in several neurodegenerative disorders, including Parkinson's, Alzheimer's disease and ALS.

In a model of Huntington's disease, HSF1 and nuclear factor of activated T cells (NFAT) cooperatively regulate the expression of HSPB5 [97]. Also, HSF2 regulates proteostasis in a mouse modeling Huntington's disease, partially through cooperative interaction with HSF1 upon the regulation of expression of HSPB5 [98]. Hsf2 genetic ablation results in reduced lifespan and increased protein aggregation in the striatum of this disease mouse model [98]. The specific interactions between stress transcription factors in different neuronal cell types will uncover the expression programs activated against aggregation in neurodegenerative disorders.

Activating transcription factor 3 (ATF-3) is induced in the presence of neuronal injury and, in cooperation with c-Jun, upregulates the expression of the anti-apoptotic chaperone HSPB1 [99]. HSF4, the predominant stress factor expressed in ocular lens tissue, protects lens epithelial cells against cytotoxic drug-induced apoptosis, partially through upregulation of HSPB5 [100]. Moreover, HSF4 controls the selective expression of heat shock proteins in the lens tissue by promoting HSF1 protein degradation and selectively binding the promoters of stress response genes [101]. Mechanistically, HSF4 interacts with the ATP-dependent DEXD/H-box RNA helicase UAP56, coupling the transcriptional and post-transcriptional machinery [102].

The importance of efficient transcriptional regulation of sHSPs is exemplified by the identification of a mutation in the promoter of HSPB1 in a cohort of sporadic ALS patients. This mutation effects a conserved nucleotide in the heat shock element (HSE), the sequence recognized by HSF1, resulting in impaired promoter activity [103]. HSPB1 is upregulated in a mouse model of ALS overexpressing human mutant SOD1, suggesting an involvement of this sHSP in the ALS pathogenesis [104]. However, HSPB1 overexpression alone is not sufficient to protect against chronic motor neuron injury in an ALS mouse model overexpressing human mutant SOD1 [105].

Future perspectives

Preservation of protein homeostasis is critical for many cellular processes and underlies the pathology of diseases as diverse as cancer, neurodegeneration and cardiovascular disease. Over the past few years, findings suggest that the regulation of stress transcription factors and their effectors is cell-type and disease specific. It will be important to gain a systematic understanding of the disease signaling that converge on altered expression of effectors of the proteostasis machinery, including sHSPs

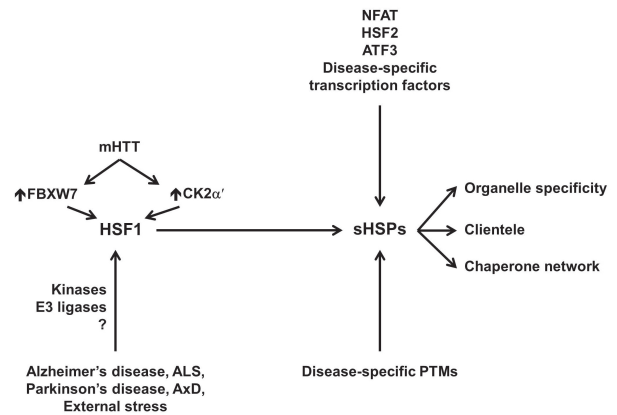


Figure 1: Multiple levels of regulation of small heat shock proteins in neurodegeneration. In Huntington disease mutant huntingtin protein (mHTT) increases the levels of CK2 α and FBXW7 leading to altered HSF1 protein levels. Future studies will reveal whether disease-specific molecular pathways or unfavorable external conditions affect the stability and function of HSF1. Also, better understanding of the cooperation of HSF1 with critical for disease transcription factors on the regulation of sHSPs transcription will uncover the stress-induced transcriptional network. In addition to transcriptional regulation, future analysis will identify the sHSPs' post-translational modifications and how they affect sHSP functionality. Systematic functional approaches will catalogue the sHSP clientele, organelle tropism and participation in chaperone networks in disease-specific context.

(Figure 1). Moreover, the recent advances in genome-wide methods and high-resolution chromatin analysis techniques [106] will allow the investigators to uncover the mechanisms of nucleosome remodeling, chromatin states and transcription factor synergy on the regulation of sHSPs in neurodegeneration. The beneficial effects of sHSPs in neurodegeneration animal models and human clinical trials is emerging [107-109]. To exploit sHSPs therapeutically, it is imperative that we decipher the regulation and targets of these multifaceted molecular chaperones.

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