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HspB1 (Hsp 27) Expression and Neuroprotection in the Retina

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Abstract Heat shock proteins (Hsps) are highly conserved proteins that are induced in response to various physiological and environmental stressors. HspB1 (Hsp27) is a prominent member of the small Hsps family and is strongly induced during the stress response. Notably, HspB1 has powerful neuroprotective effects, increasing the survival of cells subjected to cytotoxic stimuli. This is especially relevant to the study of the retina, where cells are subject to death due to retinal disease and injury. While HspB1 shows constitutive expression in some areas of the mammalian retina, of particular interest is the upregulation of the protein in response to ischemia and oxidative stress, traumatic nerve injury, and elevated intraocular pressure and glaucoma. Several mechanisms have been proposed to account for the cytoprotective actions of HspB1, including its role as a molecular chaperone, a stabilizer of the cytoskeleton, and a regulator of apoptosis. This review will focus on the role of HspB1 in the retina, emphasizing effects on retinal ganglion cells, by analyzing the expression, induction by stressors, and mechanisms of its neuroprotective function. Finally, the potential of HspB1 as a clinical therapeutic will be examined.

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Introduction

Discovered as cellular defense mechanisms, stress or heat shock proteins (Hsps) are highly conserved polypeptides that are induced in response to various physiological and environmental stressors. Mammalian Hsps have been classified into five major families based on molecular mass, structure, and function: Hsp 100, Hsp90, Hsp70, Hsp 60, and the small Hsps (15–30 kDa, including HspB1 (Hsp27) and the alpha-crystallins (HspB4 and HspB5)). Some Hsps are expressed constitutively, while the expression of others is induced by cellular exposure to aversive stimuli, such as heat, irradiation, oxidative stress, heavy metals, osmotic stress, and metabolic poisons [1]. In unstressed cells, constitutively expressed Hsps act as molecular chaperones, maintaining protein homeostasis by facilitating the folding, assembly, and transport of polypeptides. In response to the increased amounts of unfolded proteins present after proteotoxic damage, Hsp expression is induced rapidly, leading to protein refolding or degradation, and prevention of irreversible aggregation [2]. Thus, Hsps have powerful cytoprotective functions.

Among the heat shock proteins, HspB1, along with Hsp70, is most strongly induced after stress. A member of the small Hsp family, HspB1 is an ATP-independent chaperone, functioning especially in protection against protein aggregation [3]. The structure of HspB1 consists of a core crystallin box domain, which includes 80–100 amino acids in the C-terminus and an IgG-like fold (see Fig. 1a). The N-terminus contains the WDPF motif that is involved in the oligomerization of HspB1 into large aggregates of up to 800 kDa (see

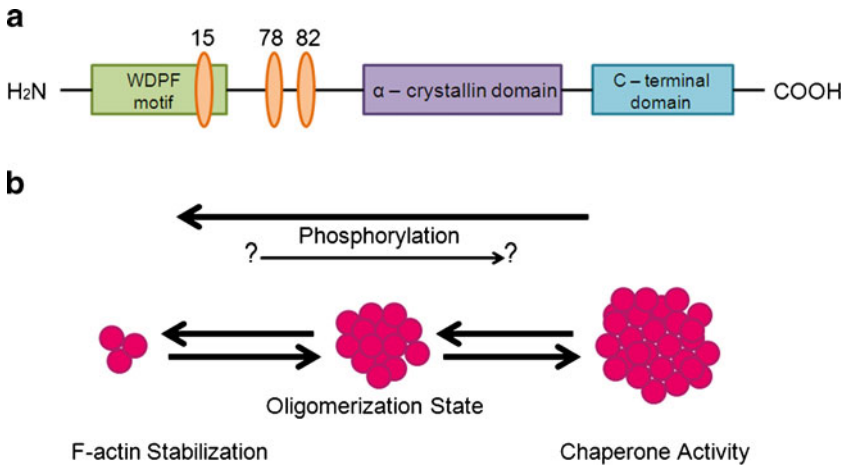

Fig. 1 Structure of HspB1. **a** HspB1 has a core alpha-crystallin domain and a WDPF motif (involved in oligomerization). The phosphorylation sites of HspB1 at serines 15, 78, and 82 are indicated. **b** HspB1 undergoes oligomerization. Phosphorylated small oligomers of HspB1 are involved in F-actin stabilization. Larger HspB1 oligomers have chaperone activity. Phosphorylation may be necessary for HspB1 to move between oligomerization states

Fig. 1b). In this large oligomerized state, HspB1 acts as a molecular chaperone [4]. In contrast, small HspB1 monomers or small oligomers bind and stabilize filamentous actin (F-actin) [5–7]. The dynamic process of oligomerization is partially dependent upon the phosphorylation state of the protein, which can be induced by various stimuli, including differentiating agents, mitogens, inflammatory cytokines, oxidants, and heat shock [8]. Human HspB1 can be phosphorylated at three serine residues: serines 15, 78, and 82 [9, 10], whereas the murine homologue of HspB1, Hsp 25, can be phosphorylated at serines 15 and 86 [11]. This phosphorylation is primarily caused by MAPK-activating protein (MAPKAP) 2/3, which is activated by phosphorylation of p38 MAP kinase [10]. HspB1 is dephosphorylated by protein phosphatase 2A [8]. Previous research indicated that phosphorylation results in oligomeric complex dissociation, the redistribution of the protein into small oligomers, and the subsequent loss of chaperone activity [5–7]. It remains to be determined whether phosphorylation at specific serine residues regulates HspB1 transition between oligomerization states.

In addition to its role as a molecular chaperone, HspB1 is cytoprotective through interactions with various intrinsic and extrinsic apoptotic proteins and with the cytoskeleton. Cytoprotection, or more specifically neuroprotection, is of particular interest to those studying the mammalian retina. As retinal ganglion cells (RGCs) are embryologically derived from the central nervous system (CNS), they have been used for studying mechanisms of neuron survival that may be relevant in the treatment of various neurological disorders, such as neurodegenerative diseases, stroke, and trauma. Furthermore, death of RGCs resulting in loss of vision is of clinical significance in conditions such as glaucoma, macular degeneration, and diabetic retinopathy. This review will focus on the role of HspB1 in the retina, emphasizing effects on RGCs, by analyzing the expression, induction by stressors, and mechanisms of its neuroprotective function. Finally, the potential of HspB1 as a clinical therapeutic will be examined.

Retinal HspB1 Expression

Expression of HspB1 in Development

While they are usually associated with expression in response to injury, the highly inducible Hsps, including HspB1, are also expressed during development. In the neonatal rat retina, developmental expression of HspB1 is observed in a subset of RGCs at postnatal day 3 (P3), peaking from P6 to P12, with little expression after this point. Interestingly, this expression does not correlate with early postnatal RGC apoptosis during P1–P3, but it does correlate with two developmental events in the visual system: the arrival of late-projecting RGC axons to the superior colliculus at P6 and the onset of spontaneous retinotectal activity from P6 to P12 [12]. In addition, in the neonatal (P1) pig retina, HspB1 is observed in RGCs in the ganglion cell layer and in some processes of astrocytes in the innermost nerve fiber layer. HspB1 is also weakly detected in the inner plexiform, inner nuclear, outer plexiform, and photoreceptor layers. In comparison with neonatal retinas, HspB1 levels across all layers are significantly enhanced in 6-month-old (adult) pig retinas [13].

Constitutive Expression of HspB1

Constitutive expression of HspB1 occurs in some regions of the normal retina. In general, HspB1 immunoreactivity is low in the rat retina; however, results as to where HspB1 is present have varied. HspB1 has been detected in the rat ganglion cell layer [14–16], and HspB1 has been detected in RGCs of the adult pig [13]. In rats, HspB1 immunoreactivity also occurs in the inner plexiform layer, inner nuclear layer, outer limiting membrane and adjacent photoreceptor nuclei, in the inner segments of photoreceptors, and at the tips of photoreceptor outer segments, possibly including processes of retinal pigment epithelial cells. HspB1 was also present in retinal blood vessels [14].

In addition to the ganglion cell layer, HspB1 was also expressed highly in photoreceptor outer segments and in retinal pigment epithelium cells [15] and in the nerve fiber layer [16]. However, one study reported basal expression observed only in cells of the choroidal and retinal vasculature [17]. HspB1 immunoreactivity was not detected in back-labeled RGCs of control rat retinas [18, 19]. In addition, only faint immunostaining for HspB1 in a few RGCs of normal human retinas, with no staining in the nerve fiber layer, was observed, although clearly positive staining in the vascular wall was present [20].

HspB1 expression was observed prominently in retinal glial cells [14, 18, 21], including processes of Müller cells in the outer nuclear layer [16]. Constitutive expression of HspB1 was detected in Müller cells of the pig retina [13] and in human type 1B astrocytes from the optic nerve head [22]. HspB1 immunoreactivity increases in the optic nerve head, with intense expression in the retina-optic nerve transition region and the optic nerve proper. Furthermore, there is a greater prominence of HspB1 in the rat optic nerve, where astrocytes are abundant, than in the retina [14].

Induced Expression of HspB1

HspB1 expression is highly inducible in response to cellular stress. This induced upregulation has been observed in the retina due to stressors, including ischemia/hypoxia, oxidative stress, nerve injury, and elevated intraocular pressure.

Induction of HspB1 by Ischemia and Oxidative Stress

Rat retinal ischemic preconditioning via clamping of the optic nerve and associated blood vessels for a period of 5 min caused levels of both HspB1 messenger RNA (mRNA) and protein to be elevated 5 h post-preconditioning. They reached an increase of 200% over basal levels 24 h post-ischemia and did not return to control levels until 120 h (mRNA) and 168 h (protein) post-ischemia. These increases were localized to the ganglion cell and inner plexiform layers of the retina, as well as some cell bodies of the inner nuclear layer [17]. Ischemic preconditioning may regulate HspB1 gene expression through hypoxia-inducible factor-1. In addition, in rats treated with $CoCl_2$, a mimicker of hypoxia, HspB1 was upregulated in the retina from 24 to 120 h post-treatment [23].

HspB1 levels in the ganglion cell layer were significantly increased after four different forms of ischemic injury: transient middle cerebral artery occlusion, permanent middle cerebral artery occlusion, cortical photothrombosis of the sensorimotor cortex, and bilateral common carotid artery occlusion, all compared with sham-treated rats [16]. Myeloperoxidase-mediated oxidative injury increased levels of HspB1 in differentiated ARPE-19 (human retinal epithelial cell line) cells [15] and oxidative stress due to hydrogen peroxide increased HspB1 expression in human optic nerve head astrocytes [24].

Induction of HspB1 by Optic Nerve Injury

While no HspB1 in the ganglion cell layer of control rats was detectable by immunohistochemistry, by 4 days post-optic nerve transection, HspB1 was detected in a subset of RGCs in the retina. The percentage of HspB1-positive RGCs increases up to 28 days following injury. HspB1 was detected through Western analysis in control retinal and superior colliculus tissue, and increased levels were detected at both sites 10 days post-optic nerve transection. Expression was also increased in the nerve fiber layer, optic nerve head, and in glial cells of the optic tract 4 days following transection [18, 19].

Induction of HspB1 by Elevated Pressure or Glaucoma Models

Elevated intraocular pressure causes increased HspB1 and HspB1 phosphorylation in the rat retina, which is observed in the nerve fiber layer and colocalizes with glial fibrillary acidic protein and vimentin, suggesting that glial cells contribute to the increases [25]. Also of note, human type 1B astrocytes exposed to elevated hydrostatic pressure display increased synthesis and immunoreactivity of HspB1 [22]. In eyes from the DBA/2J mouse model of glaucoma, those with elevated intraocular pressure had higher levels of HspB1 expression and also expressed phosphorylated HspB1 [25]. Furthermore, increased HspB1 expression is observed in RGCs and astrocytes of rats with laser-induced elevated intraocular pressure, with the highest level of expression observed in RGCs 2 weeks post-treatment [21]. In human glaucomatous eyes with either primary open-angle glaucoma or normal-pressure glaucoma, there is prominent increased immunostaining for HspB1 in RGCs and astrocytes of the nerve fiber layer, as well as in the optic nerve head [20].

HspB1-Mediated Neuroprotection

In the retina, HspB1 confers neuroprotection against various stressors known to cause cell death, including ischemia and oxidative stress, traumatic nerve injury, and elevated intraocular pressure.

Enhanced Neuronal Survival Following Ischemia and Oxidative Stress

Ischemia is believed to cause neuronal cell death in the retina by prompting oxidative stress, glutamate excitotoxicity, and intracellular Ca^{2+} overload [26–28]. In the rat retina, there is

a strong correlation between HspB1 expression induced by ischemic preconditioning and protection of retinal function after subsequent ischemic injury, as indicated by electroretinography [17]. In rats pretreated with $CoCl_2$ injection to mimic hypoxia, the upregulation of HspB1 was protective against subsequent complete retinal ischemia [23]. Furthermore, HspB1 protects RGC-5 cells from stress associated with ischemia and Ca^{2+} overload, further supporting the idea that an increase in HspB1 expression can protect retinal cells from ischemic injury [29]. Consistent with this, HspB1 protein delivered into RGCs by electroporation increased their survival rate following ischemia–reperfusion injury [30].

Enhanced Neuronal Survival Following Optic Nerve Injury

Neuronal injury by intra-orbital axotomy of the optic nerve leads to apoptosis of RGCs, resulting in death of approximately 90% by 14 days post-injury [31]. Optic nerve transection leads to increased expression of c-Jun [32], the pro-apoptotic protein Bax [33], and increased activation of the apoptotic mediator caspase-3 [34] in RGCs. Following transection of the optic nerve, HspB1 expression is induced in a subset of RGCs. Of the surviving RGCs, there is a significant increase in those that express HspB1 by 28 days, indicating a slower death rate compared with the HspB1-negative population. This suggests that HspB1 may promote the survival of this subset following axotomy [18, 19]. In addition, HspB1 may play a role in axonal regeneration following injury. There is a significant positive correlation between the presence of HspB1 in injured RGCs after optic nerve transection and evidence of axonal regeneration into peripheral nerve autografts [35].

HspB1 Neuroprotection against Elevated Intraocular Pressure and Glaucoma

Glaucoma causes RGC pathology and a progressive loss of vision. While the exact mechanism of glaucomatous optic neuropathy, characterized by elevated or normal intraocular pressure depending on the form of the disease, is unknown, studies suggest that specific forms may arise from an autoimmune response [36, 37]. HspB1 may play a role in the disease, as human studies have found elevated serum titers of antibodies to HspB1 in glaucoma patients compared with controls [38, 39]. Furthermore, HspB1 immunization in the rat induces RGC degeneration and axonal loss, suggesting that HspB1 may act as an antigenic stimulus, activating an immune response during glaucomatous neurodegeneration [40]. The increased immunostaining of HspB1 in the retina and optic nerve head of human glaucomatous eyes suggests that it functions as a defense mechanism of stressed or injured neurons in glaucoma [20].

Mechanisms of HspB1 Neuroprotection

The actions of HspB1 are complex and are mediated by its ability to function in various roles within the cell. Since the activation of one or more of multiple intracellular pathways by various stressors can influence cell survival, there are several points where HspB1 could stimulate protective functions or inhibit cell death mechanisms. The actions of HspB1 as a molecular chaperone, in preventing the stress-induced disruption of the cytoskeleton, in modulating intracellular redox potential, and in inhibiting apoptosis all contribute to neuroprotective effects.

HspB1 as a Molecular Chaperone

As a member of the Hsp family, HspB1 acts as a molecular chaperone, facilitating the refolding of misfolded proteins. Since ATP levels can be significantly reduced in stressed cells, large oligomers of unphosphorylated HspB1 [41, 42], as ATP-independent chaperones, form complexes with damaged proteins to prevent their non-specific aggregation [41]. As the cells begin to recover, HspB1 cooperates with ATP-dependent Hsp chaperones, such as Hsp70, as they restore the native structure of denatured proteins or direct their proteolytic destruction [43]. Large aggregates of misfolded proteins can signal apoptosis to begin in the cell [44]. Therefore, the induced upregulation of HspB1 expression is thought to help cells recover from the accumulation of aggregates and prevent the triggering of apoptosis.

HspB1 Stabilization of the Cytoskeleton

The neuronal cytoskeleton is composed of various filamentous and tubular polymers, including microtubules, microfilaments, and neurofilaments, which function to determine cell shape, partitioning of organelles and molecules by transport mechanisms, division, and motility. Proper organization of cytoskeletal elements is vital to the health of the cell, and disruption of these elements can be catastrophic [45]. Stress causes disruption of the integrity of filamentous actin structures, leading to potential damage of neuron form. Overexpression of HspB1 increases the stability of F-actin microfilaments during stress caused by hyperthermia [46], oxidants [47], and cytochalasin D [48]. This increased stability may prevent the disaggregation of the cytoskeleton, therefore preventing the potential induction of apoptosis due to cytoskeletal breakdown. Phosphorylated, small oligomers of HspB1 mediate this effect as this form binds to actin [48–50], and HspB1 has been shown to inhibit actin polymerization [51–53]. Internalization of exogenously applied HspB1 antibody by RGCs leads to a decreased ability of endogenous HspB1 to stabilize the actin cytoskeleton, thereby facilitating apoptosis [54].

HspB1 Modulation of Intracellular Redox Potential

Normal cellular processes involving interactions with oxygen will sometimes form reactive oxygen species (ROS) as by-products. However, when high levels of ROS accumulate intracellularly after exposure to toxic stimuli, such as tumor necrosis factor-α (TNF-α) and hydrogen peroxide, they cause oxidative damage to the cell and contribute to pathological disease processes including ischemia [55]. As HspB1 protects cells against ROS and oxidative stress, it follows that HspB1 could play a role in modulating intracellular redox status. Rather than acting to directly detoxify ROS, HspB1 likely exerts this effect by increasing intracellular levels of glutathione [56], a protein that functions to detoxify ROS and regulate cell death. HspB1 can also maintain glutathione in its reducing form in conjunction with increasing the activity of glucose-6-phosphate dehydrogenase, a key enzyme in cellular reduction processes [57]. In addition, HspB1 expression is correlated with greatly decreased intracellular iron levels, attenuating the generation of hydroxyl radicals (OH·) and therefore decreasing levels of oxidized proteins [58]. It is unknown whether modulation of glucose-6-phosphate dehydrogenase by HspB1 requires HspB1 to be in a particular structural organization; however, large unphosphorylated oligomers (>400 kDa) are purported to control intracellular ROS and glutathione levels [59].

HspB1 Inhibition of Apoptosis

Much of the cytoprotective effect of HspB1 is due to the inhibition of apoptosis or programmed, controlled, energy-dependent cell death. In several diseases, including glaucoma, age-related macular degeneration, and diabetic retinopathy, retinal cell death occurs via this process. Apoptosis can be induced by several stimuli, including cytokines, cytotoxic drugs, oxidative stress, and ionizing radiation [60]. Two different signaling transduction pathways lead to apoptosis: the extrinsic and the intrinsic pathways. Notably, mitochondria serve as a control point for cross-talk between the pathways. For example, pro-caspase-8 activated by the extrinsic pathway can facilitate cleavage of the BH3 domain-only death agonist protein (Bid), creating a pro-apoptotic fragment that translocates to the mitochondria and induces cytochrome *c* release in the intrinsic pathway [61, 62]. HspB1 directly interacts with several components of these pathways (see Fig. 2). The extrinsic pathway is activated by the binding of ligands, such as TNF superfamily receptor 6 ligand (FasL) and TNF-α, to plasma membrane death receptors including Fas and TNF receptor. This leads to the activation of pro-caspase-8 through the adaptor protein Fas-associated protein with death domain, with downstream activation of pro-caspase-3 [63]. Small oligomers of phosphorylated HspB1 interact with the Fas death domain-associated protein (Daxx), blocking its interaction with the Fas receptor and apoptosis signal regulating kinase 1 (Ask1), preventing caspase-independent apoptosis [64]. Furthermore, HspB1 can bind to activated Ask1, leading to the suppression of mitogen-activated protein kinase kinase 4/Jun N-terminal kinase (MKK4/JNK) signaling, ultimately preventing cell death [65].

In contrast, the intrinsic pathway involves the release of pro-apoptotic molecules from the mitochondria and the activation of the caspase cascade. Cytochrome *c* interacts with cytosolic apoptosis protease-activating factor-1 (Apaf-1) and caspase-9 to form the apoptosome, a complex that activates caspase-3 [66]. HspB1 directly inhibits pro-caspase-9 [67], preventing the formation of the apoptosome and thus the subsequent activation of caspases [67–69]. HspB1 also functions to sequester cytochrome *c* at its release into the cytosol [68, 69] in addition to blocking its release [70]. HspB1 can inhibit caspase-3 activity through its interaction with pro-caspase-3 as well [69]. Overexpression of rat HspB1 in RGC-5 cells blocked caspase-3 activation induced by intracellular Ca^{2+} overload, although this was not sufficient to prevent neurotoxicity, suggesting that HspB1 protected the cells by both caspase-dependent and caspase-independent mechanisms [29]. As transection of the optic nerve leads to increased caspase-3 activation, HspB1 may directly block caspase activation in a subset of RGCs that survive following this injury [18]. However, some studies have demonstrated little or no direct interaction between HspB1 and cytochrome *c* or caspase-3 [71, 72]. HspB1 can inhibit the mitochondrial release of the second mitochondria-derived activator of caspase (Smac) [73], a protein that neutralizes the inhibitory activity of inhibitory apoptotic proteins that interact with caspases [74].

HspB1 also regulates upstream signaling pathways. Apoptosis can be inhibited by the activation of the protective phosphatidylinositol 3-kinase (PI3-K) pathway, as it phosphorylates inositol lipids in the plasma membrane that attract protein kinase B, also known as Akt (Akt/PKB). Akt/PKB inhibits apoptotic proteins, such as Bcl-xL/Bcl-2 associated death promoter (Bad) and caspase-9 [75, 76]. The binding of HspB1 to Akt/PKB is necessary for Akt activation in stressed cells [77]. Furthermore, the activated form of the stress-activated kinase, p38, is detected in RGCs following optic nerve transection [78]. As p38 is an upstream regulator of HspB1 phosphorylation [10], this kinase may factor in neuroprotection after optic nerve injury.

Little is known concerning which phosphorylation and oligomerization states of HspB1 are required to mediate these anti-apoptotic effects. However, recent work suggests that varying degrees of HspB1 phosphorylation and oligomerization may be stimulated in an inducer-dependent manner. In

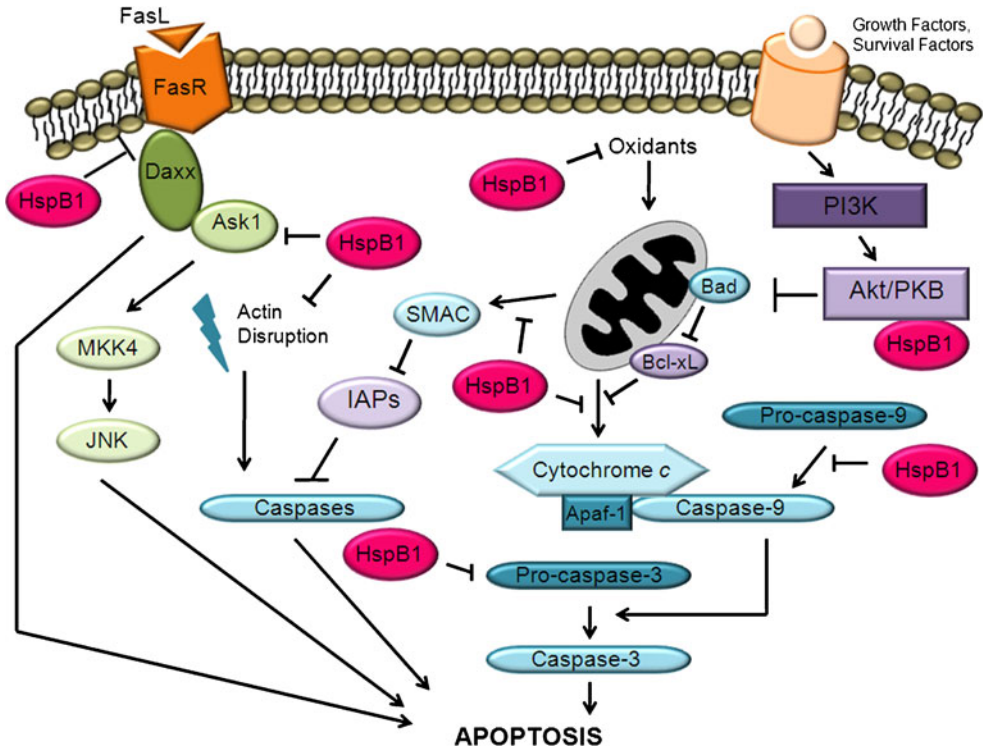

Fig. 2 Role of HspB1 in apoptosis. In extrinsic apoptotic pathways, phosphorylated HspB1 inhibits Daxx from interacting with the Fas receptor and Ask1, preventing caspase-independent apoptosis. HspB1 also binds to Ask1 directly, inhibiting Ask1 activity, thus leading to the suppression of MKK4/JNK signaling and cell death. In intrinsic pathways, HspB1 sequesters cytochrome *c* in mitochondria, preventing it from being released. HspB1 inhibits pro-caspase-9, preventing caspase-9 from forming the apoptosome with cytochrome *c* and Apaf-1. HspB1 inhibits pro-caspase-3, leading to inhibition of caspase-3 activity. HspB1 inhibits the release of Smac from mitochondria, so it cannot block inhibitory apoptotic proteins. HspB1 forms a complex with Akt/PKB, leading to protective effects against apoptosis. HspB1 increases levels of glutathione and maintains it in its reducing form, leading to reduced levels of reactive oxygen species. HspB1 stabilizes the cytoskeleton, preventing induction of apoptosis due to cytoskeletal breakdown

response to etoposide and Fas antibody stimulation, populations of HspB1 molecules tended to shift from small oligomers to large oligomeric structures. In contrast, staurosporine and cytochalasin D promoted a population shift toward small oligomers followed by increased amounts of large oligomers over time. In response to the inducers, phosphorylation of HspB1 increased transiently until approximately half of the total cellular HspB1 was phosphorylated. Notably though, each inducer promoted different phosphorylation patterns at the three phosphoserine sites, indicating that each site may play a specific role in promoting the observed functions of HspB1 [79]. As the dynamic structural organization of HspB1 is so interestingly complex, it allows for the protein to have multiple strategies to combat a range of stressors and may serve to explain the diverse protective effects. For instance, phosphorylation may regulate the oligomerization state and directly or indirectly the cellular location of HspB1 molecules, all of which will undoubtedly influence HspB1 function. Finally, HspB1 function, cellular localization, and oligomerization state will vary depending on whether it is constitutive and mature, or newly synthesized.

Potential as a Clinical Therapeutic

The retinal stressors discussed in this review have broad clinical relevance. For instance, retinal ischemia is involved in many ocular diseases involving the retina, including diabetic retinopathy, central retinal artery occlusion, and anterior ischemic optic neuropathy. Furthermore, injury to the optic nerve leads to RGC death, leading to potential vision loss. Glaucoma is an optic neuropathy, which affects nearly 60 million people and is the second leading cause of blindness worldwide [80]. A progressive degeneration of RGCs is proposed to contribute to the disease; however, the precise cause is unknown [81]. Therefore, protection of RGCs conferred by HspB1 has direct implications for preventing vision loss in various forms. However, lessons learned from the role of HspB1 in RGC survival are not limited to retinal/ocular disease. This is especially relevant given an aging population and the limited capacity of the CNS for regeneration. Gaining an understanding of cellular events responsible for cellular degeneration is of principal importance in devising therapeutic strategies for CNS diseases.

HspB1 has multiple targets and functions that comprise the complex mechanisms of its neuroprotective role, indicating that multiple types and combinations of treatments may be developed. Since the upregulation of HspB1 induced by ischemic preconditioning is associated with protection against subsequent ischemia [17, 23], there is potential for HspB1 to be used in a preventative manner to protect patients at risk for ischemic injury, such as prior to surgery. Previously, HspB1 that was exogenously delivered via a herpes simplex virus-based vector to cultured neuronal cells protected them from apoptosis [82]. Similarly, the delivery of attenuated herpes simplex virus carrying HspB1 significantly reduced lesion volume in a rat middle cerebral artery occlusion model of reversible focal cerebral ischemia [83]. Electroinjection of HspB1 into RGCs protected them from apoptosis after ischemia–reperfusion injury [30]. Furthermore, a PEP-1-HspB1 fusion protein expression vector was used to directly transduce this fusion protein into primary neuronal cells, resulting in increased cell viability in response to hydrogen peroxide-induced oxidative stress [84]. It is possible, therefore, that the use of gene therapy to deliver exogenous HspB1 to the retina may help treat retinal diseases.

Accumulating evidence of the cyto- and neuroprotective roles of HspB1 in the retina indicates that it has substantial potential to be a useful clinical therapeutic. However, further investigation involving the targeting of molecular pathways specific to individual disease processes is required.

Conclusion

Clearly, HspB1 plays an important role in the mammalian retina. Levels of HspB1 are upregulated in response to stress caused by ischemia and oxidative stress, traumatic nerve injury, and elevated intraocular pressure and glaucoma. HspB1 increases cell survival in response to these retinal cytotoxic stimuli. HspB1 acts as a molecular chaperone, stabilizes the actin cytoskeleton, and prevents apoptosis by interacting with various members of the apoptotic cascade. Consequently, HspB1 is a molecule with the potential to play a pivotal role in protecting retinal and other CNS neurons. Further research will need to focus on developing strategies to incorporate the cytoprotective effects of this protein into viable therapeutic treatments for neuronal injury and disease.

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