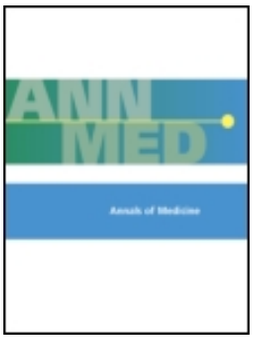

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: informahealthcare.com/journals/iann20

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To cite this article: Ines Dierick, Joy Irobi, Peter De Jonghe & Vincent Timmerman (2005) Small heat shock proteins in inherited peripheral neuropathies, Annals of Medicine, 37:6, 413-422, DOI: 10.1080/07853890500296410

To link to this article: https://doi.org/10.1080/07853890500296410

Annals of Medicine. 2005; 37: 413–422

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REVIEW ARTICLE

Small heat shock proteins in inherited peripheral neuropathies

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Abstract
Small heat shock proteins (small HSPs) are molecular chaperones that protect cells against stress by assisting in the correct folding of denatured proteins and thus prevent aggregation of misfolded proteins. Small HSPs also modulate apoptotic pathways by interacting with components of programmed cell death. Furthermore, some small HSPs interact with the cytoskeleton to assist in spatial organization and dynamics of its structural elements. The role of small HSPs has been studied in many disorders, including neurodegenerative disease. Recently, mutations in *HSPB1* (*HSP27*) and *HSPB8* (*HSP22*), two members of the small HSP superfamily, have been associated with inherited peripheral neuropathies. In this review, we will summarize the current knowledge of small HSPs, in particular HSPB1 and HSPB8, and discuss their role in health and disease.

Key words: *Neurodegeneration, peripheral neuropathy, small heat shock proteins*

Introduction

Heat shock proteins (HSP) act as molecular chaperones and protect intracellular components during stress conditions. Even in unstressed cells, HSPs are constitutively expressed to properly fold and assemble polypeptides. The name 'heat shock protein' in fact refers to the rapid induction of these proteins in response to elevated temperatures, although other stress stimuli (e.g. oxidative stress, heavy metals and ischemia injury) are also capable of inducing expression of HSPs. HSPs are classified according to their molecular weight and currently five major classes are recognized: HSP100, HSP90, HSP70, HSP60 and the small heat shock proteins (small HSPs), which have a monomer size between 12 and 43 kDa. In this review we will focus on the small HSPs. *In vitro* these small HSPs have a chaperone-like activity, i.e. the capacity to interact with partially denatured proteins and to prevent protein misfolding and aggregation. They are also involved in diverse cellular activities such as modulation of actin and intermediate filament dynamics (1,2), cellular growth and differentiation (3,4) and apoptosis (5).

Genes encoding small HSPs are found in all organisms, from bacteria to vertebrates. In addition, all members of the small HSP superfamily share a conserved C-terminal α-crystallin domain, containing 80–100 amino acid residues, a more variable N-terminal sequence and a short variable C-terminal tail. The combination of a conserved domain and their universal presence, indicate a crucial, evolutionary role in cellular homeostasis. In human, the ten genes encoding small HSPs are named *HSPB1* to *HSPB10* according to the HUGO Gene Nomenclature Committee (6,7). Recently, five novel small HSPs were identified in other vertebrates, HSPB11–15, but these do not have a mammalian orthologue (8). In retrospect, the first members of the small HSP superfamily, αA-crystallin/HSPB4 and αB-crystallin/HSPB5, were identified more than a century ago as 'α-crystallins' (9). Only in 1982 the relationship of these proteins with the small HSP superfamily was recognized (10). HSPB4 is almost exclusively expressed in the lens and is not heat-inducible, while HSPB5 is distributed ubiquitously and is heat-inducible (11). HSPB1 (HSP27), a heat-inducible small HSP that was identified 20 years

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ISSN 0785-3890 print/ISSN 1365-2060 online
DOI: 10.1080/07853890500296410

ago, is ubiquitously expressed in human tissues and interacts with HSPB8, another small HSP (12). HSPB8, also known as HSP22, is a heat-inducible small HSP, expressed in various tissues including muscle, motor and sensory neurons (13–16). The remaining small HSPs will not be discussed in detail since so far they have not been implicated in human disorders.

During the last decade, the involvement of small HSPs has been studied in many disorders, including neurodegenerative diseases. The first studies focused on the potential protective effect of upregulation of HSP expression. Increased levels of wild-type small HSPs, especially HSPB5 and HSPB1, were indeed found in individuals with neurodegenerative diseases, such as Alzheimer (17), Parkinson (18), Alexander disease (19) and amyotrophic lateral sclerosis (ALS) (20). These neurodegenerative disorders are all marked by the deposition of improperly folded proteins, inclusion bodies or plaques in the nervous system.

On the other hand, mutations in four small HSPs have been associated with autosomal dominant and recessive hereditary disorders. Mutations in the crystallin genes, *HSPB4* and *HSPB5*, are associated with congenital cataract and desmin-related myopathy (21,22). Mutations in the interacting small HSPs, *HSPB1* and *HSPB8*, have been shown to cause distal hereditary motor neuropathy (distal HMN type II) and Charcot-Marie-Tooth disease type 2 (CMT2) (Table I) (23–25). It is not clear what the underlying pathological mechanism is in these disorders: misfolding and aggregation of the mutant HSP, a defective cytoprotective activity leading to misfolding and aggregation of other proteins, a dysfunction in cell death pathways, or a combination of these effects.

Because mutations in *HSPB1* and *HSPB8* were only recently identified as a cause of inherited peripheral neuropathies, we will concentrate on these two small HSPs and discuss how mutations in these genes affect motor and sensory neurons.

Key messages

- Small heat shock proteins (small HSPs) have cytoprotective roles including chaperone-like activity, anti-apoptotic activity and preservation of cytoskeletal elements.
- The neuroprotective role of small HSPs has been studied in many disorders, including neurodegenerative disease.
- Recently, mutations in *HSPB1* (*HSP27*) and *HSPB8* (*HSP22*), two members of the small HSP superfamily, have been associated with inherited peripheral neuropathies.

HSPB1 and HSPB8 mutations in inherited peripheral neuropathies

Distal HMN, also known as hereditary distal spinal muscular atrophy (distal SMA) is a pure motor disorder of the peripheral nervous system (PNS) (26). Distal HMN is clinically characterized by progressive weakness and atrophy of the muscles in the lower limbs. Later on weakness is also present in the distal upper limbs. In a related inherited peripheral neuropathy, CMT2, sensory neurons are also involved. On clinical examination, distal HMN patients are hard to distinguish from CMT2 patients, because in CMT2 patients sensory symptoms are often absent. Therefore electrophysiological examinations are essential to confirm the diagnosis of either distal HMN, where only motor neurons are involved, or CMT2, where motor and sensory neurons are involved (27). Both disorders are clinically and genetically heterogeneous (28,29). Recently, missense mutations in *HSPB1* and *HSPB8* have been associated with distal HMN and CMT2 (Table I) (23–25). Initially two distinct missense mutations (K141N and K141E), involving the same amino acid in the α-crystallin domain of HSPB8 were found in four distal HMN type II families. Subsequently, the heterozygous amino acid change

Table I. Mutations in HSPB1 and HSPB8 and the associated phenotypes.

Small HSP	Nucleotide change	AA change	Domain	Phenotype	Reference
HSPB1	c.379C>T	R127W	α-crystallin	distal HMN	(24)
	c.404C>T	S135F	α-crystallin	distal HMN, CMT2F	(24)
	c.406C>T	R136W	α-crystallin	CMT2	(24)
	c.452C>T	T151I	α-crystallin	distal HMN	(24)
	c.545C>T	P182L	IXI/V	distal HMN	(24)
HSPB8	c.421A>G	K141E	α-crystallin	distal HMN II	(23)
	c.423G>C	K141N	α-crystallin	distal HMN II	(23)
	c.423G>T	K141N	α-crystallin	CMT2L	(25)

AA change=amino acid change; CMT2F and CMT2L=Charcot-Marie-Tooth type 2 neuropathies; distal HMN II = distal hereditary motor neuropathy type II.

(K141N) in *HSPB8* was also reported in a large Chinese CMT2 family (CMT type 2L) which was previously linked to the same chromosomal region as distal HMN type II (25,30). These observations raise two important points. First, the same *HSPB8* missense mutation (K141N) is associated with two distinct phenotypes, i.e. distal HMN and CMT2. Secondly, all reported pathogenic mutations so far are missense mutations that target the same amino acid. The conserved K141 residue in HSPB8 corresponds to R116 and R120 of the HSPB4 and HSPB5 proteins respectively (Figure 1). Interestingly, these arginine residues are mutated in patients with congenital cataract and desmin-related myopathy (21,22). Biochemical analysis of the R116C and R120G missense mutations in HSPB4 and HSPB5 shows significant changes in the secondary and tertiary structure of these small HSPs, a defective chaperone-like activity and a higher aggregate size (31). The R116C mutation in HSPB4 also reduces the protective ability in lens epithelial cell apoptosis (32). Members of the small HSP superfamily interact with each other and changes in these interactions may be a culprit mechanism in pathological conditions. The interaction between mutant HSPB4 (R116C) and wild-type HSPB1 and HSPB5 is increased, whereas the interaction with HSPB4 wild-type proteins shows little change (33). Co-immunoprecipitation studies demonstrated also that mutant HSPB8 (K141N/E) has an increased interaction with HSPB1 and thus might interfere with the activity of HSPB1 (23). These studies underline the importance of the preservation of a positively charged amino acid at that position in these small HSPs. *In vitro* studies have also shown that mutant HSPB8 promotes the formation of cytoplasmatic and perinuclear aggregates, which correlated with a significant decrease in neuronal cell viability (23). Aggregation of desmin filaments is also induced by mutant HSPB5 (R120G) (34). Accumulation of these misfolded proteins can have deleterious effects on neuronal cell function by disrupting the axonal transport and can ultimately lead to cell death (Figure 2).

Not entirely unexpected, missense mutations in HSPB1, the molecular partner of HSPB8, were also associated with distal HMN and CMT type 2F (24). Four mutations in HSPB1 target the highly conserved α-crystallin domain (R127W, S135F, R136W and T151I) and one is positioned in the C-terminal part (P182L) (Table I; Figure 1). Experimental studies with HSPB5 showed that mutations in the core α-crystallin domain decrease the chaperone function (35). The HSPB1 mutations found in distal HMN and CMT2F patients may therefore interfere with the chaperone activity. The C-terminus of several small HSPs contains a conserved IXI/V motif (Figure 1). This motif is relevant for inter-subunit interactions and oligomerization, either with regions in the α-crystallin domain or with IXI/V motifs on other monomers (36). Possibly, the C-terminal missense mutation (P182L) in HSPB1 interferes with subunit-interactions of small HSPs, which are important to maintain the oligomeric state of the protein. Furthermore, this IXI/V motif is critical in the chaperone-like activity of several bacterial small HSPs. Deletion of this motif or mutations in this motif, result in a complete loss of chaperone activity

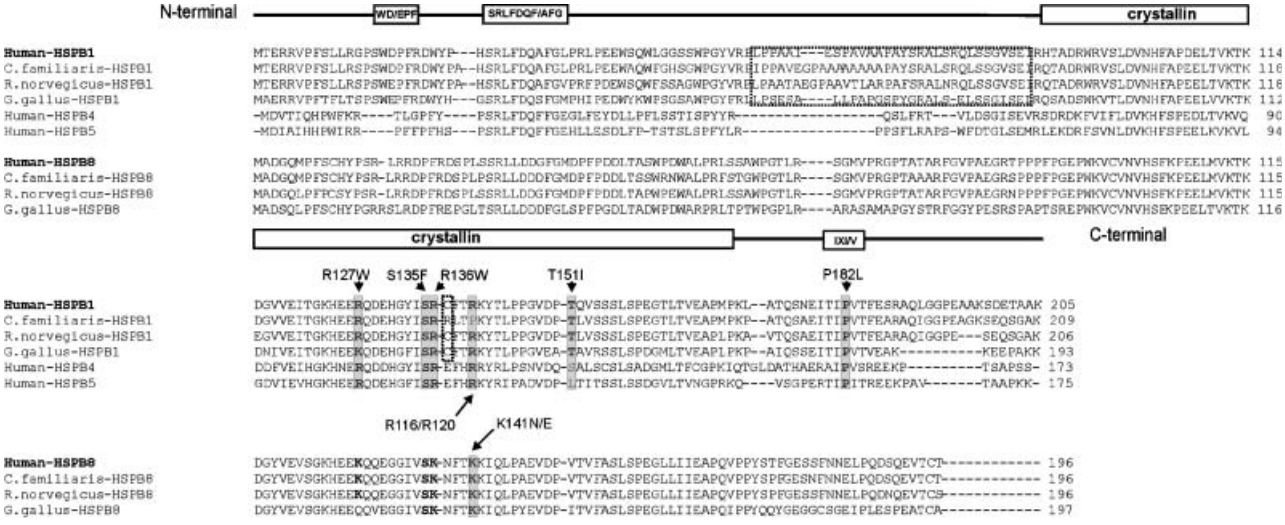

Figure 1. Structure-based sequence alignments of HSPB1 and HSPB8 orthologues and homologues HSPB4 and HSPB5. The N-terminal WD/EPF and SRLDQF/AFG domain, the α-crystallin domain and the C-terminal IXI/V domain are indicated. The peripheral neuropathy-associated mutations are boxed in grey. The cytochrome C binding motifs are indicated by a dotted line.

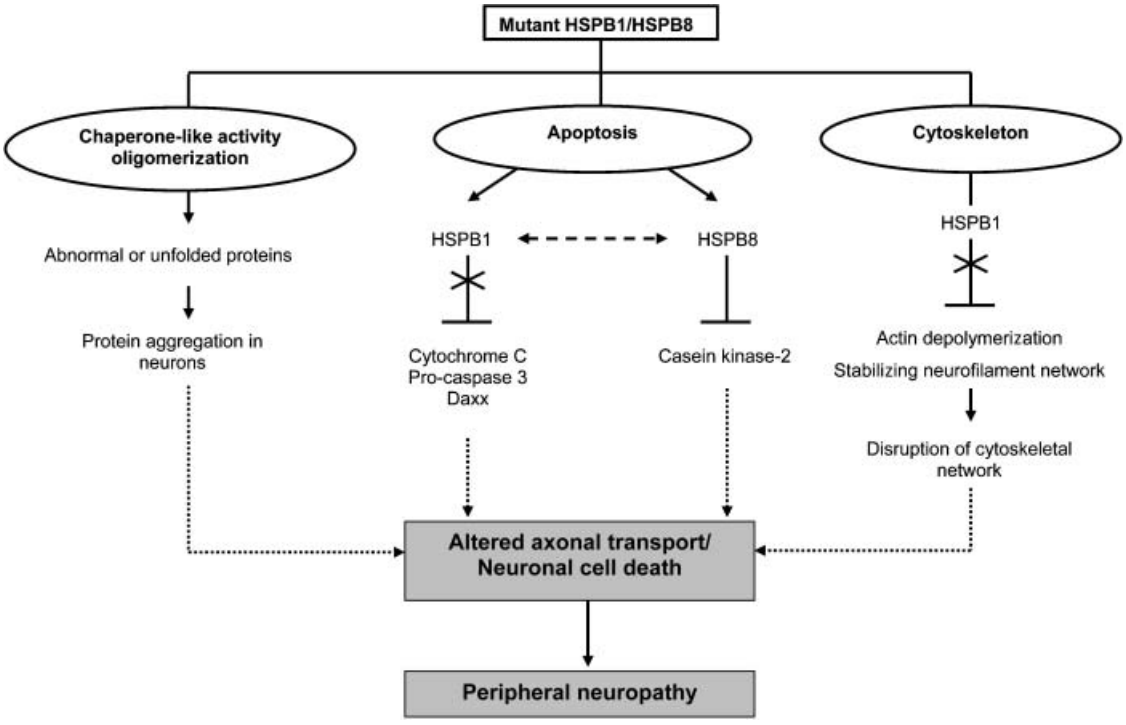

Figure 2. Suggested pleiotropic actions of mutant HSPB1 and HSPB8. Mutations in HSPB1 and HSPB8 can interfere with the chaperone-like activity, causing insoluble cellular aggregates and subsequent induction of neuronal cell death. HSPB1 and HSPB8 regulate different apoptotic pathways. Mutant HSPB1 can no longer bind cytochrome C, pro-caspase 3 and Daxx and is thus unable to prevent apoptotic cell death. Mutations in HSPB8 may result in enhanced apoptosis by inhibiting the pro-survival activity of casein kinase-2 or by negatively influencing the anti-apoptotic activity of its interacting partner, HSPB1. Furthermore, mutant HSPB1 is able to disrupt cytoskeletal functions and neurofilament assembly, resulting in neuronal cell death or altered axonal transport. Ultimately, these pathological mechanisms will cause peripheral neuropathy in humans.

and the truncated protein was unable to form oligomers (37). In addition, a site-directed mutation of P160 in HSPB5, which corresponds to P182 in HSPB1, also influences the chaperone function (35). These effects may contribute to the reduced cell viability of neuronal cells seen in transient transfection studies with mutated HSPB1 (24).

Cellular and cytoprotective properties of HSPB1 and HSPB8

Under normal conditions HSPB1, as most small HSPs, forms large oligomers consisting of about 24 subunits. These oligomeric complexes are formed by dimeric building blocks composed of small HSP monomers. The well conserved N-terminal SRLFDQFFG motif determines the oligomeric size of small HSPs and contributes to their structural stability (Figure 1) (38). A unique cysteine residue in Hspb1 (C141) regulates the formation of dimers (39). Hspb1 with a mutation at this cysteine residue showed decreased ability to multimerize, and was less efficient in inhibiting staurosporine-induced apoptosis (39). Interestingly, the cysteine residue, corresponding to C137 in HSPB1, is located next to the amino acids S135 and R136, which are mutated in individuals with a motor and sensory neuropathy (24). Mutations located near this functionally important cysteine residue, possibly interfere with oligomerization. The oligomeric structure of HSPB8 is not clear. An *in vitro* study showed that Hspb8 exists as a monomer in contrast to other small HSPs (40), however in other studies, HSPB8 predominantly formed dimers (15,41) and high molecular mass complexes in the heart (42). The oligomerization of mammalian small HSPs is regulated by phosphorylation (1). Mitogen activating proteins (MAP), MAPKAP kinase 2 and 3, which are activated by p38 MAP kinase, are the main kinases involved in the phosphorylation of small HSPs. Stress-induced phosphorylation and oligomerization may thus play an important role in the biological activities of small HSPs (43,44).

We will now summarize the three main cytoprotective functions of small HSPs: 1) the chaperone-like activity, 2) the capacity to interfere with cellular death pathways and 3) the role in stabilizing the cytoskeleton (Figure 2).

HSPB1 and HSPB8 act as molecular chaperones

Small HSPs contribute to protection of the cell against stress by their chaperone-like activity, i.e. their capacity to bind non-native proteins. *In vitro* studies have demonstrated that mammalian small HSPs recognize misfolded proteins and prevent intracellular protein aggregation by keeping them in a soluble phase (45). The *in vitro* molecular chaperone activity of HSPB1 and HSPB8 has been extensively documented (34,40,41,44,46). They prevented aggresome formation in cells expressing mutant HSPB5 (R120G) by forming complexes with misfolded HSPB5 (34). Interestingly, in contrast to HSPB1, HSPB8 did not remain associated with the mutant HSPB5 complexes. This suggests that HSPB8 is involved in protein folding, and therefore can be considered as a true chaperoning molecule (34).

Several studies demonstrated an important, but not sufficient role of the α-crystallin domain in the chaperone-activity of small HSPs (35), since additional parts of the protein are required, such as the N-terminal proline-phenylalanine-rich region containing a WD/EPF (single letter amino acid code) motif in HSPB1 and the C-terminal region (Figure 1) (47,48). The chaperone-like activity of small HSPs, as documented in *in vitro* studies, may of course be highly relevant for their role in human disorders. However this activity still needs to be confirmed by *in vivo* studies (45).

HSPB1 and HSPB8 act as modulators of apoptosis

The protective effect of small HSPs is also related to their interference with apoptotic pathways. HSPB1 prevents the formation of apoptosomes and prevents cell death triggered by various stimuli. HSPB1 maintains the redox status and mitochondrial stability in the cell and prevents apoptosis due to reactive oxygen species (44,49). It also inhibits apoptosis by direct binding to molecular components of cell death pathways. HSPB1 binds to both cytochrome C, when it is released from the mitochondria to the cytosol, and to pro-caspase-3, thus preventing the activation of the caspase cascade (Figure 2) (50,51). The cysteine residue at position 137, as well as the N-terminal region, seem to be essential for binding to cytochrome C (Figure 1) (50). HSPB1 also affects the caspase-independent apoptotic pathway, by its interference in the translocation of Daxx to the membrane and its interaction with Fas (52). Daxx is a nuclear protein that translocates to the membrane during Fas-mediated caspase-independent apoptosis.

Another function of HSPB1 related to cell death, is its role in proteasome-mediated degradation of selected proteins. HSPB1 is able to interact with specific components of the proteasome and to bind ubiquitin, thus facilitating protein degradation (53). The interaction between HSPB1 and ubiquitin explains the co-localization with ubiquitinated proteins in cytoplasmatic inclusions that characterize some neurodegenerative diseases.

In contrast to the anti-apoptotic activity of most small HSPs, a pro-apoptotic activity was assigned to HSPB8 when forced to express in tumor cell lines (54). HSPB8 triggered apoptosis is caspase- and p38 MAP kinase-dependent and cell-type specific since not all cell-types overexpressing HSPB8 showed an enhanced apoptosis (54). This is well documented in the heart were HSPB8 plays a dual role; at low doses it induces myocardial hypertrophy through the activation of the Akt or protein kinase B (Akt/PKB) pathway, while at high doses it has a pro-apoptotic activity via protein kinase-dependent mechanisms (55). HSPB8 also interacts with casein kinase-2 (CK-2), which has anti-apoptotic effects. It is speculated that HSPB8 promotes apoptosis by inhibition of the CK-2 activity in cardiac myocytes (55). Since HSPB8 interacts with HSPB1 and other small HSPs, mutant HSPB8 may induce apoptosis by disturbing the anti-apoptotic effect of other small HSPs (23,42). Although the apoptotic activity of HSPB8 in motor neurons is unknown, both HSPB8 and HSPB1 associate with the Akt/PKB kinase pathway, a major signaling pathway in non-neuronal and neuronal cells for growth and survival (56,57). This pathway is also activated in spinal motor neurons after nerve injury, wherein HSPB1 plays a role in promoting cell survival (56).

HSPB1 and HSPB8 act as protectors of the cytoskeleton

A cytoprotective function of HSPB1 is also to regulate cytoskeletal dynamics. The cytoskeleton maintains the shape of the cell by modulating the spatial arrangement and dynamics of its structural elements, consisting of microtubules, intermediate filaments and actin microfilaments. During stress, the integrity of this cytoskeletal network is disrupted by disorganization and aggregation of the filament system. Abnormalities in cytoskeletal organization and aggregates are frequent histopathological hallmarks in neurodegenerative disorders. HSPB1 and microfilaments interact to prevent disruption of the cytoskeleton during exposure to stress stimuli (2). Although the molecular mechanism by which HSPB1 stabilizes the actin network is not fully understood, an inhibition of actin polymerization

was demonstrated (58). The depolymerization of actin is regulated by the structural organization of HSPB1 and its p38 MAP kinase-mediated phosphorylation (59). Unphosphorylated HSPB1 monomers effectively block polymerization, whereas phosphorylated monomers and unphosphorylated oligomers are not able to prevent actin polymerization (58). The interaction between HSPB1 and actin, and the related depolymerization effect were assigned to amino acid sequences at the N-terminus of HSPB1 (60).

Besides the interaction with actin microfilaments, HSPB1 also associates with intermediate filaments. *In vitro* studies show that this interaction controls filament-filament interactions, which are important for cell survival by preventing intermediate filament aggregation (61). The fact that mutant HSPB1 (S135F) results in disruption of the cellular neurofilament (NF-L) network, provides evidence for an essential role of HSPB1 in regulating this dynamic network (24). Furthermore, the R120G mutation in HSPB5, is also associated with impaired neurofilament assembly, resulting in pathological aggregates of desmin filaments (34). These findings underscore the functional relationship between small HSPs and the cytoskeleton, and link several neuronal diseases with intermediate filaments. Interestingly, mutations in the neurofilament light chain gene (*NEFL*) cause another variant of CMT2 (CMT2E) (62) and mutations in the peripherin gene (*PRPH*) are associated with ALS (63). At present, HSPB8 has not been shown to interact directly with cytoskeletal elements, however since small HSPs operate in association with each other, it is possible that HSPB8 does interact with microfilaments and intermediate filaments via its molecular partner HSPB1. The missense mutations in HSPB1 and HSPB8 in distal HMN and CMT2 could therefore interfere either directly or indirectly with cytoskeletal functions and affect axonal transport in motor and sensory neurons (Figure 2). Of note is that mutations in dynamin 2 (DNM2) and dynactin (DCTN1), proteins that are involved in axonal transport, result in peripheral neuropathy (64,65).

Neuroprotective role of HSPB1

HSPB1 is expressed in the PNS and might be important for axonal outgrowth, since it is induced in Schwann cells and regenerated axons (66,67). Furthermore, expression of HSPB1 is upregulated after damage to adult peripheral sensory and motor neurons (66,68,69). In addition, exogenous delivery of HSPB1 is able to rescue injured sensory, sympathetic and motor neurons *in vitro* and *in vivo* (69,70). This neuroprotective activity of HSPB1 is crucial, because *in vivo* antisense RNA studies showed that HSPB1 is necessary to inhibit neuronal cell death (69). As for other cellular functions of HSPB1, the cell survival-promoting activity seems to be regulated by phosphorylation (69). It has been shown that p38 MAP kinase, which regulates the phosphorylation of HSPB1, plays a fundamental role in the PNS by regulating cell shape and gene expression associated with Schwann cell myelination (71).

Increased neuronal cell survival by HSPB1 is not limited to the PNS because several studies indicate its upregulation and protective action also in the central nervous system. In hippocampal and retinal ganglion neurons, Hspb1 is also upregulated after injury (72,73). Exogenous HSPB1 protects against neuronal loss in an *in vivo* model of epilepsy, in a cellular model of Huntington disease, and in an *in vitro* mammalian cell model of α-synuclein-neurotoxicity (49,74,75). Furthermore, the neuroprotective effect of increased HSPB1 levels was demonstrated in transgenic HSPB1 mice, which had reduced kainate-induced seizures and hippocampal cell death (76).

It is assumed that loss of constitutive HSPB1 correlates with and promotes neurodegeneration. In the 'paralyse mouse mutant', a model of early onset SMA, the expression level of Hspb1 is decreased compared with control mice (77). Furthermore, in a cellular model for spinocerebellar ataxia type 3, expressing mutant ataxin-3, HSPB1 was downregulated, suggesting that loss of HSPB1 has a pathologic effect (78). HSPB1 expression is also differentially and temporally regulated in mouse models of ALS. Recently, decreased Hspb1 expression was demonstrated several weeks prior to onset of motor neuron death in Cu/Zn superoxide dismutase-1 (SOD1) mutant mice (79). Other studies demonstrated that expression of Hspb1 was gradually upregulated in spinal cords of early and late symptomatic SOD1 mutant mice relative to non-symptomatic mutant mice (20,80).

Until now, the exact mechanism of protection against neuronal cell death is unclear, but the cytoprotective roles of HSPB1 including chaperone-like activity, anti-apoptotic activity and preservation of cytoskeletal stability, may all contribute to neuroprotection. The survival-promoting effect may be associated with the anti-apoptotic actions of HSPB1. More recently, a direct anti-apoptotic activity of HSPB1 was shown in a cellular model of Parkinson disease. In this model overexpression of HSPB1 delays the release of cytochrome C, reduces caspase activity and prevents apoptosis (81).

Until now there is no direct evidence that HSPB8 is also neuroprotective. Since HSPB8 acts as a cytoprotective component in non-neuronal cell lines (34), it is plausible that it has the ability to protect neurons from cell death. The specific mechanisms underlying this neuroprotection are still to be defined. It will be important to characterize the mechanisms by which HSPB1 and HSPB8 contribute to neuronal survival, because this may lead to the identification of new strategies to prevent neuronal death.

Indeed, compounds inducing HSPs may be useful as a potential treatment for human neurodegenerative diseases. Recently a co-inducer of HSP expression, arimoclomol, significantly delayed disease progression in a mouse model of ALS, even when administered after the disease onset (82). Furthermore, geldanamycin, a naturally occurring anticancer drug, induces HSP expression in different neuronal cell types and suppresses huntingtin protein aggregation in a cellular model of Huntington disease by modulating the expression of HSP40, HSP70 and HSP90 (83). Also immunophilin-ligands, which are used in transplantation to suppress allograft such as FK506, rapidly induce the expression of HSP70 and HSPB1. The administration of FK506 dramatically reduces signs of neuropathy and markedly protects against axonal loss during acrylamide induced neuropathy (84). Major drawbacks of these components are the considerable side effects, which limit their application in humans (85).

Concluding remarks

The identification of mutations in small HSPs associated with human motor and sensory neuropathies, indicates their pivotal role in the PNS. The diversity of interactions and cytoprotective functions of small HSPs make identifying the underlying pathologic mechanism of these neuromuscular disorders difficult. The mutations could interfere with the chaperone-like activity or oligomerization property of these small HSPs and may be responsible for the aggregates found in cultured cells expressing the mutant proteins (Figure 2) (23,34). Another possibility to explain the induction of neuronal cell death by mutant small HSPs, is their capacity to modulate apoptotic pathways, either directly or indirectly (Figure 2). Therefore, the HSPB1 mutations observed in patients with inherited neuropathies could interfere with binding of cytochrome C (24,50). In this respect, mutations in these small HSPs could also influence the pro-survival activity of HSPB1 or the pro-apoptotic activity of HSPB8. Furthermore, the disruption of neurofilament assembly in cells expressing mutant HSPB1 is an indication that this small HSP has a role in the maintenance of cytoskeletal dynamics and axonal transport, which could also be relevant for the pathologic mechanism in these inherited peripheral neuropathies. The length of sensory and motor axons make these cell-types highly susceptible to defects in axonal transport. The identification of the complete molecular and functional components of small HSPs in motor and sensory neurons will allow understanding why these neurons are specifically affected by mutations in HSPB1 and HSPB8, and will subsequently be useful to develop therapeutic approaches in the future.

If mutations in HSPB1 and HSPB8 result in a loss-of-function phenotype, components that upregulate HSP-expression will be useful for hereditary peripheral neuropathies. However, if mutant small HSPs act as toxic components that induce neuronal cell death, an upregulation of these mutant proteins could make the pathology even worse. The observed mutant phenotype could also be a complex combination of loss-of-function and dominant-negative effects. Therefore, the ideal therapeutic strategy would be to selectively inhibit the expression of the mutant protein and maintain the expression of wild-type proteins. Hypothetically, since small HSPs are structurally closely related, development of safe therapeutic approaches with the aim to upregulate HSPB1 in patients with mutant HSPB8 and HSPB8 in patients with mutant HSPB1 may improve the neuronal loss observed in distal HMN and CMT2.

Acknowledgements

ID is supported by a PhD fellowship of the Institute for Science and Technology (IWT), and JI is supported by a postdoctoral fellowship of the Fund for Scientific Research (FWO-Flanders), Belgium.

References

1. Gusev NB, Bogatcheva NV, Marston SB. Structure and properties of small heat shock proteins (sHsp) and their interaction with cytoskeleton proteins. Biochemistry (Mosc). 2002;67:511–9.
2. Mounier N, Arrigo AP. Actin cytoskeleton and small heat shock proteins: how do they interact? Cell Stress Chaperones. 2002;7:167–76.
3. Arrigo AP. In search of the molecular mechanism by which small stress proteins counteract apoptosis during cellular differentiation. J Cell Biochem. 2005;94:241–6.
4. Mehlen P, Mehlen A, Godet J, Arrigo AP. hsp27 as a switch between differentiation and apoptosis in murine embryonic stem cells. J Biol Chem. 1997;272:31657–65.

5. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem Biophys Res Commun. 2003;304:505–12.
6. Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. Genomics. 2002;79:464–70.
7. Kappe G, Franck E, Verschuure P, Boelens WC, Leunissen JA, De Jong WW. The human genome encodes 10 alpha-crystallin-related small heat shock proteins: HspB1-10. Cell Stress Chaperones. 2003;8:53–61.
8. Franck E, Madsen O, van Rheede T, Ricard G, Huynen MA, De Jong WW. Evolutionary diversity of vertebrate small heat shock proteins. J Mol Evol. 2004;59:792–805.
9. Mörner CT. Untersuchung der proteinsubstanzen in den leichtbrechenden medien des auges. Hoppe Seylers Z Physiol Chem. 1894;18:61–106.
10. Ingolia TD, Craig EA. Four small Drosophila heat shock proteins are related to each other and to mammalian alpha-crystallin. Proc Natl Acad Sci U S A. 1982;79:2360–4.
11. Dubin RA, Wawrousek EF, Piatigorsky J. Expression of the murine alpha B-crystallin gene is not restricted to the lens. Mol Cell Biol. 1989;9:1083–91.
12. Hickey E, Brandon SE, Potter R, Stein G, Stein J, Weber LA. Sequence and organization of genes encoding the human 27 kDa heat shock protein. Nucleic Acids Res. 1986;14:4127–45.
13. Charpentier AH, Bednarek AK, Daniel RL, Hawkins KA, Laflin KJ, Gaddis S, et al. Effects of estrogen on global gene expression: identification of novel targets of estrogen action. Cancer Res. 2000;60:5977–83.
14. Smith CC, Yu YX, Kulka M, Aurelian L. A novel human gene similar to the protein kinase (PK) coding domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (ICP10) codes for a serine-threonine PK and is expressed in melanoma cells. J Biol Chem. 2000; 275:25690–9.
15. Benndorf R, Sun XK, Gilmont RR, Biedermann KJ, Molloy MP, Goodmurphy CW, et al. HSP22, a new member of the small heat shock protein superfamily, interacts with mimic of phosphorylated HSP27 ((3D)HSP27). J Biol Chem. 2001;276:26753–61.
16. Kappe G, Verschuure P, Philipsen RL, Staalduinen AA, Van de BP, Boelens WC, et al. Characterization of two novel human small heat shock proteins: protein kinase-related HspB8 and testis-specific HspB9. Biochim Biophys Acta. 2001;1520:1–6.
17. Shimura H, Miura-Shimura Y, Kosik KS. Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival. J Biol Chem. 2004;279:17957–62.
18. Renkawek K, Stege GJ, Bosman GJ. Dementia, gliosis and expression of the small heat shock proteins hsp27 and alpha B-crystallin in Parkinson's disease. Neuroreport. 1999; 10:2273–6.
19. Head MW, Corbin E, Goldman JE. Overexpression and abnormal modification of the stress proteins alpha B-crystallin and HSP27 in Alexander disease. Am J Pathol. 1993;143:1743–53.
20. Vleminckx V, Van Damme P, Goffin K, Delye H, Van Den BL, Robberecht W. Upregulation of HSP27 in a transgenic model of ALS. J Neuropathol Exp Neurol. 2002;61:968–74.
21. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet. 1998;7:471–4.
22. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet. 1998;20:92–5.
23. Irobi J, Van Impe K, Seeman P, Jordanova A, Dierick I, Verpoorten N, et al. Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. Nat Genet. 2004;36:597–601.
24. Evgrafov OV, Mersiyanova IV, Irobi J, Van den Bosch L, Dierick I, Schagina O, et al. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. Nat Genet. 2004;36: 602–6.
25. Tang BS, Zhao GH, Luo W, Xia K, Cai F, Pan Q, et al. Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. Hum Genet. 2005;116:222–4.
26. Emery AEH. Review: The nosology of the spinal muscular atrophies. J Med Genet. 1971;8:481–95.
27. Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. Brain. 1980;103:259–80.
28. Irobi J, De Jonghe P, Timmerman V. Molecular genetics of distal hereditary motor neuropathies. Hum Mol Genet. 2004;13:195–202.
29. Shy ME. Charcot-Marie-Tooth disease: an update. Curr Opin Neurol. 2004;17:579–85.
30. Tang BS, Luo W, Xia K, Xiao JF, Jiang H, Shen L, et al. A new locus for autosomal dominant Charcot-Marie-Tooth disease type 2 (CMT2L) maps to chromosome 12q24. Hum Genet. 2004;114:527–33.
31. Bera S, Thampi P, Cho WJ, Abraham EC. A positive charge preservation at position 116 of alpha A-crystallin is critical for its structural and functional integrity. Biochemistry. 2002;41:12421–6.
32. Andley UP, Patel HC, Xi JH. The R116C mutation in alpha A-crystallin diminishes its protective ability against stress-induced lens epithelial cell apoptosis. J Biol Chem. 2002;277:10178–86.
33. Fu L, Liang JJ. Alteration of protein-protein interactions of congenital cataract crystallin mutants. Invest Ophthalmol Vis Sci. 2003;44:1155–9.
34. Zobel ATC, Loranger A, Marceau N, Theriault JR, Lambert H, Landry J. Distinct chaperone mechanisms can delay the formation of aggresomes by the myopathy-causing R120G alpha B-crystallin mutant. Hum Mol Genet. 2003;12:1609–20.
35. Muchowski PJ, Wu GJ, Liang JJ, Adman ET, Clark JI. Site-directed mutations within the core "alpha-crystallin" domain of the small heat-shock protein, human alphaB-crystallin, decrease molecular chaperone functions. J Mol Biol. 1999;289:397–411.
36. Pasta SY, Raman B, Ramakrishna T, Rao C. The IXI/V motif in the C-terminal extension of alpha-crystallins: alternative interactions and oligomeric assemblies. Mol Vis. 2004;10:655–62.
37. Studer S, Obrist M, Lentze N, Narberhaus F. A critical motif for oligomerization and chaperone activity of bacterial alpha-heat shock proteins. Eur J Biochem. 2002;269:3578–86.
38. Pasta SY, Raman B, Ramakrishna T, Rao C. Role of the conserved SRLFDQFFG region of alpha-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange, and chaperone-like activity. J Biol Chem. 2003;278:51159–66.

39. Diaz-Latoud C, Buache E, Javouhey E, Arrigo AP. Substitution of the unique cysteine residue of murine hsp25 interferes with the protective activity of this stress protein through inhibition of dimer formation. Antioxid Redox Signal. 2005;7:436–45.
40. Chowdary TK, Raman B, Ramakrishna T, Rao CM. Mammalian Hsp22 is a heat-inducible small heat-shock protein with chaperone-like activity. Biochem J. 2004;381:379–87.
41. Kim MV, Seit-Nebi AS, Marston SB, Gusev NB. Some properties of human small heat shock protein Hsp22 (H11 or HspB8). Biochem Biophys Res Commun. 2004;315:796–801.
42. Sun X, Fontaine JM, Rest JS, Shelden EA, Welsh MJ, Benndorf R. Interaction of human HSP22 (HSPB8) with other small heat shock proteins. J Biol Chem. 2004;279:2394–402.
43. Giese KC, Vierling E. Changes in oligomerization are essential for the chaperone activity of a small heat shock protein in vivo and in vitro. J Biol Chem. 2002;277:46310–8.
44. Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, et al. Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. J Biol Chem. 1999;274: 18947–56.
45. Haslbeck M, Buchner J. Chaperone function of sHsps. Prog Mol Subcell Biol. 2002;28:37–59.
46. Jakob U, Gaestel M, Engel K, Buchner J. Small heat shock proteins are molecular chaperones. J Biol Chem. 1993;268:1517–20.
47. Theriault JR, Lambert H, Chavez-Zobel AT, Charest G, Lavigne P, Landry J. Essential role of the NH2-terminal WD/EPF motif in the phosphorylation-activated protective function of mammalian Hsp27. J Biol Chem. 2004; 279:23463–71.
48. Lindner RA, Carver JA, Ehrnsperger M, Buchner J, Esposito G, Behlke J, et al. Mouse Hsp25, a small shock protein. The role of its C-terminal extension in oligomerization and chaperone action. Eur J Biochem. 2000; 267:1923–32.
49. Wyttenbach A, Sauvageot O, Carmichael J, Diaz-Latoud C, Arrigo AP, Rubinsztein DC. Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. Hum Mol Genet. 2002;11:1137–51.
50. Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, et al. Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol. 2000;2:645–52.
51. Pandey P, Farber R, Nakazawa A, Kumar S, Bharti A, Nalin C, et al. Hsp27 functions as a negative regulator of cytochrome c-dependent activation of procaspase-3. Oncogene. 2000;19:1975–81.
52. Charette SJ, Lavoie JN, Lambert H, Landry J. Inhibition of Daxx-mediated apoptosis by heat shock protein 27. Mol Cell Biol. 2000;20:7602–12.
53. Parcellier A, Schmitt E, Gurbuxani S, Seigneurin-Berny D, Pance A, Chantome A, et al. HSP27 is a ubiquitin-binding protein involved in I-kappaBalpha proteasomal degradation. Mol Cell Biol. 2003;23:5790–802.
54. Gober MD, Smith CC, Ueda K, Toretsky JA, Aurelian L. Forced expression of the h11 heat shock protein can be regulated by DNA methylation and trigger apoptosis in human cells. J Biol Chem. 2003;278:37600–9.
55. Hase M, Depre C, Vatner SF, Sadoshima J. H11 has dose-dependent and dual hypertrophic and proapoptotic functions in cardiac myocytes. Biochem J. 2005;388:475–83.
56. Murashov AK, Ul H, I, Hill C, Park E, Smith M, Wang X, et al. Crosstalk between p38, Hsp25 and Akt in spinal motor neurons after sciatic nerve injury. Brain Res Mol Brain Res. 2001;93:199–208.
57. Depre C, Hase M, Gaussin V, Zajac A, Wang L, Hittinger L, et al. H11 kinase is a novel mediator of myocardial hypertrophy in vivo. Circ Res. 2002;91:1007–14.
58. Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, Lutsch G. Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. J Biol Chem. 1994;269:20780–4.
59. Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, Landry J. Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. J Cell Sci. 1997;110(Pt 3):357–68.
60. Wieske M, Benndorf R, Behlke J, Dolling R, Grelle G, Bielka H, et al. Defined sequence segments of the small heat shock proteins HSP25 and alphaB-crystallin inhibit actin polymerization. Eur J Biochem. 2001;268:2083–90.
61. Perng MD, Cairns L, van den IJssel P, Prescott A, Hutcheson AM, Quinlan RA. Intermediate filament interactions can be altered by HSP27 and alpha B-crystallin. J Cell Sci. 1999;112:2099–112.
62. Mersiyanova IV, Perepelov AV, Polyakov AV, Sitnikov VF, Dadali EL, Oparin RB, et al. A new variant of Charcot-Marie-Tooth disease type 2 (CMT2E) is probably the result of a mutation in the neurofilament light gene. Am J Hum Genet. 2000;67:37–46.
63. Gros-Louis F, Lariviere R, Gowing G, Laurent S, Camu W, Bouchard JP, et al. A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis. J Biol Chem. 2004;279:45951–6.
64. Züchner S, Noureddine M, Kennerson M, Verhoeven K, Claeys K, De Jonghe P, et al. Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. Nat Genet. 2005;37:289–94.
65. Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, Mann E, et al. Mutant dynactin in motor neuron disease. Nat Genet. 2003;33:455–6.
66. Costigan M, Mannion RJ, Kendall G, Lewis SE, Campagna JA, Coggeshall RE, et al. Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. J Neurosci. 1998;18:5891–900.
67. Hirata K, He J, Hirakawa Y, Liu W, Wang S, Kawabuchi M. HSP27 is markedly induced in Schwann cell columns and associated regenerating axons. Glia. 2003;42:1–11.
68. Lewis SE, Mannion RJ, White FA, Coggeshall RE, Beggs S, Costigan M, et al. A role for HSP27 in sensory neuron survival. J Neurosci. 1999;19:8945–53.
69. Benn SC, Perrelet D, Kato AC, Scholz J, Decosterd I, Mannion RJ, et al. Hsp27 upregulation and phosphorylation is required for injured sensory and motor neuron survival. Neuron. 2002;36:45–56.
70. Wagstaff MJ, Collaco-Moraes Y, Smith J, de Belleroche JS, Coffin RS, Latchman DS. Protection of neuronal cells from apoptosis by Hsp27 delivered with a herpes simplex virus-based vector. J Biol Chem. 1999;274:5061–9.
71. Fragoso G, Robertson J, Athlan E, Tam E, Almazan G, Mushynski WE. Inhibition of p38 mitogen-activated protein kinase interferes with cell shape changes and gene expression associated with Schwann cell myelination. Exp Neurol. 2003;183:34–46.

72. Krueger-Naug AM, Emsley JG, Myers TL, Currie RW, Clarke DB. Injury to retinal ganglion cells induces expression of the small heat shock protein Hsp27 in the rat visual system. Neuroscience. 2002;110:653–65.
73. Valentim LM, Geyer AB, Tavares A, Cimarosti H, Worm PV, Rodnight R, et al. Effects of global cerebral ischemia and preconditioning on heat shock protein 27 immunocontent and phosphorylation in rat hippocampus. Neuroscience. 2001;107:43–9.
74. Kalwy SA, Akbar MT, Coffin RS, de Belleroche J, Latchman DS. Heat shock protein 27 delivered via a herpes simplex virus vector can protect neurons of the hippocampus against kainic-acid-induced cell loss. Brain Res Mol Brain Res. 2003;111:91–103.
75. Zourlidou A, Payne S, Latchman DS. HSP27 but not HSP70 has a potent protective effect against alpha-synuclein-induced cell death in mammalian neuronal cells. J Neurochem. 2004;88:1439–48.
76. Akbar TM, Lundberg AM, Liu K, Vidyadaran S, Wells KE, Dolatshad H, et al. The neuroprotective effects of heat shock protein 27 overexpression in transgenic animals against kainate-induced seizures and hippocampal cell death. J Biol Chem. 2003;278:19956–65.
77. Pieri I, Cifuentes-Diaz C, Oudinet JP, Blondet B, Rieger F, Gonin S, et al. Modulation of HSP25 expression during anterior horn motor neuron degeneration in the paralyse mouse mutant. J Neurosci Res. 2001;65: 247–53.
78. Wen FC, Li YH, Tsai HF, Lin CH, Li C, Liu CS, et al. Down-regulation of heat shock protein 27 in neuronal cells and non-neuronal cells expressing mutant ataxin-3. FEBS Lett. 2003;546:307–14.
79. Maatkamp A, Vlug A, Haasdijk E, Troost D, French PJ, Jaarsma D. Decrease of Hsp25 protein expression precedes degeneration of motoneurons in ALS-SOD1 mice. Eur J Neurosci. 2004;20:14–28.
80. Batulan Z, Shinder GA, Minotti S, He BP, Doroudchi MM, Nalbantoglu J, et al. High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. J Neurosci. 2003;23:5789–98.
81. Gorman AM, Szegezdi E, Quigney DJ, Samali A. Hsp27 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis in PC12 cells. Biochem Biophys Res Commun. 2005;327:801–10.
82. Kieran D, Kalmar B, Dick JR, Riddoch-Contreras J, Burnstock G, Greensmith L. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med. 2004;10:402–5.
83. Sittler A, Lurz R, Lueder G, Priller J, Lehrach H, Hayer-Hartl MK, et al. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. Hum Mol Genet. 2001;10:1307–15.
84. Gold BG, Voda J, Yu X, Gordon H. The immunosuppressant FK506 elicits a neuronal heat shock response and protects against acrylamide neuropathy. Exp Neurol. 2004;187:160–70.
85. Klettner A. The induction of heat shock proteins as a potential strategy to treat neurodegenerative disorders. Drug News Perspect. 2004;17:299–306.