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Neuronal Diseases: Small Heat Shock Proteins Calm Your Nerves

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Mutations in HSPB1 and HSPB8, members of the small heat shock protein family, have recently been shown to cause some distal motor neuropathies. Their function in motor neurones is now under scrutiny.

Members of the small heat shock protein (sHSP) family are protein chaperones [1] that are important in the stress response of cells, particularly neurones [2]. These chaperones protect against a variety of stresses, from heat to oxidative stress and usually have anti-apoptotic activity [2,3]. One of the family members, HSPB1, can also suppress the toxic effects of the polyglutamine protein, huntingtin, and decrease the levels of reactive oxygen species produced as part of the response to this toxic protein [3]. Coincidentally, sHSPs are often upregulated in neurodegenerative diseases (see [4] and references therein) and also in motor neurone cell injury [2]. The protective activity of these proteins can be cell-type dependent because HSPB1 protects against apoptotic stimuli in neurones [2,3], but not cardiomyocytes [5]. Some sHSPs, like HSPB8 are even pro-apoptotic [6], despite being upregulated in disease [7]. Now, two recent papers in *Nature Genetics* [8,9] have identified a genetic link between HSPB1 and HSPB8 and the distal neuropathies, Charcot Marie Tooth Disease (CMT) and Hereditary Distal Motor Neuropathy (HMN), exposing an important gap in our knowledge, namely the function of these specific sHSPs in motor neurones.

The sHSPs form a large protein family [1] comprising proteins typified by a highly conserved sequence of ~90 amino acid residues in the carboxy-terminal region, called the α-crystallin domain, and by their relatively low molecular weight (~20–25 kDa). Of the 10 different human sHSPs that are currently known [1], some, like HSPB1 [8], are widely expressed, whereas others, such as HSPB8 [9], are more restricted in their expression. Both HSPB1 and HSPB8 are found in the nervous system, where they are expressed in different neuronal cell types [8,9], although both are expressed in motor neurones. HSPB8, on the other hand, is highly expressed in heart and liver with reduced levels in skeletal muscle, lung, kidney, testis and brain [9]. These observations of the sHSP expression patterns pose the question: why are motor neurones the specific target of the HSPB1 and HSPB8 mutations?

At least part of the answer lies in the diversity of the interactions and functions of sHSPs, which explains why there is not necessarily a direct correlation between sHSP levels and the tissue affected by the sHSP mutations. HSPB1 and HSPB8 operate in association with other sHSPs [7], as well as with other chaperones, such as those of the HSP70 class. HSPB8 also has kinase activity (see [7] and references therein) and potentially has a direct link to the Akt/PKB kinase pathway [10]. This complexity makes identifying a unique function for these proteins quite a difficult task, especially when one considers that potential targets can include individual proteins as well as macromolecular structures such as the cytoskeleton [11].

One important function of HSPB1 is to interact with intermediate filaments and maintain the integrity of intermediate filament networks in cells [12,13]. In this respect, HSPB1 is very much like HSPB5, another sHSP that has been linked to a protein-inclusion-based disease involving the cytoskeleton, namely desmin-related myopathy. The aggregation of intermediate filaments along with their associated sHSPs, HSPB5 and HSPB1, is a prominent histopathological feature of this disease. The R120G mutation in HSPB5 associated with desmin-related myopathy induces intermediate filament aggregation [13]. Interestingly, neurofilament aggregation in the cell body upon expression of mutant sHSP1 [8] and neurofilament mutations are also features of motor neurone disease [14]. Transfection of some of the HSPB1 mutants leads to the collapse of neurofilament networks and so the link to neurofilaments is more than just coincidental. Indeed the K141E mutation in HSPB8 is equivalent to the R120G mutation in HSPB5 [9] and, although at present HSPB8 has not been shown to interact directly with intermediate filaments, it is possible that it does associate with these filaments via HSPB1 [7]. The fact that mutations in neurofilament proteins [14] are associated with the CMT2 neuropathies and amyotrophic lateral sclerosis provides compelling genetic evidence of a key interaction between these sHSP chaperones and the intermediate filament cytoskeleton that cannot be ignored.

The neurofilament cytoskeleton is highly dependent upon the efficient transport of filament subunits [15]. These transport processes might also call upon another cytoskeletal role of HSPB1, namely the modulation of actin-based networks [12]. To us it would seem highly likely that the reported HSPB1 mutations will either directly or indirectly disrupt cytoskeletal function and affect neurofilament transport. The reported disruption of the neurofilament networks in cells transfected with HSPB1 mutants is good supporting evidence [8]. One of the downstream consequences of such a disruption could then be mitochondrial dysfunction [14,16], which will propagate apoptotic pathways and of course this would be exacerbated by the loss of sHSP activity, seeing as most are known inhibitors of apoptosis, with the exception of HSPB8 which can be proapoptotic [6].

To complete our unravelling of the present conundra, it is worth noting that the most recent literature now links protein inclusion formation to ageing via sHSPs in *Caenorhabditis elegans* ([17] and references therein).

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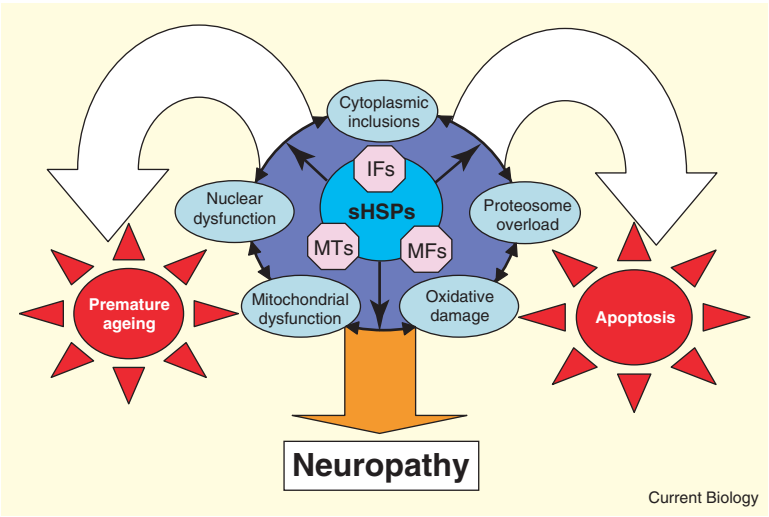

Figure 1. The relationships between sHSPs, elements of the cytoskeleton and various cellular processes.

So far, sHSP mutations are known to affect intermediate filament (IF) function, causing the aggregation of these filaments into cytoplasmic inclusions typical of the peripheral neuropathies recently described [8,9]. sHSPS also interact with microfilaments (MFs) and microtubules (MTs), but disruption of this interaction has not yet been linked to any human diseases. Loss of sHSP function contributes to increased oxidative damage in the cell and also to increased proteosomal load. Mutations in intermediate filament proteins, and possibly also in sHSPs, can cause mitochondrial and nuclear dysfunction. Oxidative damage, proteosomal inhibition, mitochondrial and nuclear dysfunction can all trigger apoptosis, which in motor neurones induces the described neuropathies as a result of the HSPB1 and HSPB8 mutations. Chronic exposure of cells to oxidative stress also induces premature ageing by accelerating the onset of senescence.

Given the fact that patients with CMT neuropathies clinically present only later in life [8], the aggregates may have indirect and delayed effects rather than immediate consequences for normal tissue function. The sHSP mutations may have invoked a chronic rather than an acute process with accumulated damage perhaps as a result of oxidative stress in these motor neurones that eventually culminates in their early death. In proliferating cells, this type of stress can induce premature ageing, but the differentiated phenotype of the neurone does not allow such an outcome and the steady loss of motor neurones ensues instead. It is worth mentioning at this point that mutations in A-type lamins, encoded by a progeria-linked gene [18], also cause CMT2 [19]. At a time when sHSPs have been linked to extended lifespan [17], hyperproliferation via p53 stability [20] and the inhibition of apoptosis [2], it is clear that we need to consider carefully the full potential of sHSP function in neurones as we unravel the pathogenic process in these tissues (Figure 1).

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