Mutations in HspB1 and hereditary neuropathies

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Abstract

Charcot-Marie-Tooth (CMT) disease is major hereditary neuropathy. CMT has been linked to mutations in a range of proteins, including the small heat shock protein HspB1. Here we review the properties of several HspB1 mutants associated with CMT. In vitro, mutations in the N-terminal domain lead to a formation of larger HspB1 oligomers when compared with the wild-type (WT) protein. These mutants are resistant to phosphorylation-induced dissociation and reveal lower chaperone-like activity than the WT on a range of model substrates. Mutations in the α -crystallin domain lead to the formation of yet larger HspB1 oligomers tending to dissociate at low protein concentration and having variable chaperone-like activity. Mutations in the conservative IPV motif within the C-terminal domain induce the formation of very large oligomers with low chaperone-like activity. Most mutants interact with a partner small heat shock protein, HspB6, in a manner different from that of the WT protein. The link between the altered physico-chemical properties and the pathological CMT phenotype is a subject of discussion. Certain HspB1 mutations appear to have an effect on cytoskeletal elements such as intermediate filaments and/or microtubules, and by this means damage the axonal transport. In addition, mutations of HspB1 can affect the metabolism in astroglia and indirectly modulate the viability of motor neurons. While the mechanisms of pathological mutations in HspB1 are likely to vary greatly across different mutations, further in vitro and in vivo studies are required for a better understanding of the CMT disease at molecular level.

Keywords Small heat shock proteins · Oligomeric structure · Mutations · Congenital diseases · Protein phosphorylation · Chaperone-like activity · Protein-protein interactions

Introduction

HspB1 belongs to the large family of small heat shock proteins detected in all phyla of living organisms including viruses, archaeobacteria, bacteria, fungi, plants, and animals (Kriehuber et al. 2010; Maaroufi and Tanguay 2013). Ten members of this family (HspB1–HspB10) are expressed in different human tissues (Fontaine et al. 2003; Kappe et al. 2003). HspB1 having an apparent molecular weight of 27 kDa and therefore earlier designated as Hsp27 is among four other small heat shock proteins (HspB1, HspB5, HspB6, HspB8) ubiquitously expressed in practically all human

tissues (Taylor and Benjamin 2005; Vos et al. 2009). The monomer of HspB1 consists of three domains: poorly ordered N-terminal domain (NTD), conservative α -crystallin domain (ACD) containing seven β -strands organized in two sheets, and short predominantly disordered C-terminal domain (CTD) (Bakthisaran et al. 2015; Baranova et al. 2011; Treweek et al. 2015) (Fig. 1a). HspB1 tends to form large oligomers consisting of more than 20 subunits. These oligomers are very labile and easily undergo reversible association/dissociation. HspB1 contains three sites (Ser18, Ser78, Ser82) located in the NTD and phosphorylated by MAPKAP kinase 2 (Rogalla et al. 1999; Stokoe et al. 1992). Phosphorylation of these sites induces dissociation of HspB1 oligomers (Jovcevski et al. 2017; Jovcevski et al. 2015; Rogalla et al. 1999). HspB1 oligomerization is also affected by the environmental conditions, such as pH (Clouser and Klevit 2017; Janowska et al. 2019). Besides being able to form homooligomers, HspB1 can interact with other small heat shock proteins (HspB5, HspB6) forming heterooligomeric complexes with distinct properties (Arrigo 2013; Bukach et al. 2009; Heirbaut et al. 2017).

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Fig. 1 **a** Schematic drawing of HspB1. NTD is marked gray, ACD including seven β -strands is marked orange, and CTD is marked green. Location of phosphorylation sites, conservative octapeptide in the NTD, and conservative tripeptide IPV in the CTD are marked red. **b** Three-dimensional structure of HspB1 ACD dimer with indication of β 7 strands which form an antiparallel interface (PDB entry 4mjh). Locations of certain point mutations associated with the Charcot-Marie-Tooth disease are marked

HspB1 is involved in many intra- and even extracellular processes (Shi et al. 2018). For instance, HspB1 is involved in carcinogenesis (Ciocca et al. 2013), in regulation of senescence and apoptosis (Calderwood and Gong 2012), and in controlling the redox state of the cell (Arrigo 2007), in the protection of cytoskeleton against different unfavorable conditions (Pivovarov et al. 2007; Wettstein et al. 2012), as well as in the prevention of irreversible aggregation of partially unfolded or misfolded proteins (Bakthisaran et al. 2015; Mymrikov et al. 2011). Since HspB1 is involved in so many different processes, any changes of its structure and properties can affect vital processes and lead to the development of diseases. One of these diseases is hereditary neuropathy.

Charcot-Marie-Tooth disease and HspB1

Currently hereditary neuropathies are classified as Charcot-Marie-Tooth disease (CMT) if motor and sensory systems are both affected, as hereditary sensory and autonomic neuropathy if sensory deficit and/or autonomic dysfunction prevail, and as distal hereditary neuropathy (dHMN) if the motor deficit is predominant (Weis et al. 2017). Charcot-Marie-Tooth disease is one of the most common inherited neuropathies affecting 1 in 2500 (Bucci et al. 2012; Skre 1974). There are many types and subtypes of CMT (Saporta et al. 2011), forming three main groups depending on the observed nerve conduction. Demyelinating CMT1 is characterized by low upper limb nerve conduction velocity (less than 38 m/s). In this case myelin sheath is damaged. Axonal CMT2 features a higher nerve conduction velocity (more than 38 m/s). In this case the axon itself undergoes degeneration. Finally, intermediate CMT (ICMT) is characterized by intermediate nerve conduction velocity (in the range of 25–45 m/s). The specific diagnosis of CMT1 is seen more often than CMT2 probably due to the fact that the majority of causative genes of CMT2 remain still undetermined (Foley et al. 2012). Different forms of

distal hereditary motor neuropathy (dHMN) are similar to CMT, and therefore, it is difficult to strictly distinguish between these diseases clinically. At present more than 45 (Reilly et al. 2011) or even approximately 100 genes have been linked to CMT-like phenotypes (Yoshimura et al. 2018). In the case of CMT1 that accounts for about 60% of all CMT cases, the main affected genes are those encoding peripheral myelin protein 22 (*PMP22*), myelin protein zero (*MPZ*), and gap junction protein β 1 (*GJB1*) (Foley et al. 2012; Murphy et al. 2012; Yoshimura et al. 2018). In the case of CMT2 that accounts for about 20% of all CMT cases, the main affected genes are those encoding mitofusin 2 (*MFN2*), gap junction protein β 1 (*GJB1*), HspB1 (*HSPB1*), and myelin protein zero (*MPZ*) (Murphy et al. 2012; Yoshimura et al. 2018). About 7–9% of CMT2 are associated with different HspB1 mutations (Murphy et al. 2012; Sivera et al. 2013; Yoshimura et al. 2018). The onset of CMT or dHMN associated with HspB1 mutations is usually recorded in elderly patients (51–60 years old) (Yoshimura et al. 2018); however, in some cases, the onset was seen in young patients as well (Benndorf et al. 2014). At present about 30 mutations of HspB1 associated with CMT and/or dHMN are described (Table 1), and this number is constantly growing (Echaniz-Laguna et al. 2017; Heilman et al. 2017). These mutations are spread over the whole HspB1 structure and are detected both in the N-terminal (NTD), α -crystallin (ACD), and C-terminal (CTD) domains. The properties of HspB1 mutants associated with CMT and/or dHMN will be described elsewhere.

Physico-chemical properties of HspB1 associated with motor neuropathies

At least 8 mutations are located in the N-terminal domain of HspB1. Two frame-shifting mutations (L58AfsX105 and A61RfsX100) lead to accumulation of truncated forms of HspB1 that are probably rapidly eliminated by cellular

Table 1 Mutations in HspB1 associated with motor neuron degeneration

Mutation	Location	Disease/inheritance of the phenotype	References
P7R	NTD	CMT2	(Fortunato et al. 2017; Luigetti et al. 2010)
G34R	NTD	dHMN	(Capponi et al. 2011)
P39L	NTD	dHMN/CMT2 (AD)	(Capponi et al. 2011; Houlden et al. 2008)
E41K	NTD	dHMN (AD)	(Capponi et al. 2011)
G53D	NTD	dHMN (AR)	(Echaniz-Laguna et al. 2017)
L58fs*105	NTD	dHMN (AD)	(Echaniz-Laguna et al. 2017)
A61fs*100	NTD	dHMN	(Echaniz-Laguna et al. 2017)
G84R	NTD	dHMN/CMT2 (AD)	(Houlden et al. 2008; James et al. 2008)
L99M	ACD (β 3)	dHMN/CMT2 (AR)	(Houlden et al. 2008)
R127W/R127L	ACD (β 5)	dHMN (AD)	(Dierick et al. 2008; Evgrafov et al. 2004; Tang et al. 2005; Ylikallio et al. 2015)
Q128R	ACD (β 5)	dHMN (AD)	(Echaniz-Laguna et al. 2017)
D129E	ACD (β 5)	dHMN	(Lewis-Smith et al. 2016)
S135F/S135C	ACD (β 7)	dHMN/CMT2 (AD)	(Benedetti et al. 2010; Evgrafov et al. 2004; Houlden et al. 2008; Oberstadt et al. 2016)
R136W/R136L	ACD (β 7)	CMT2 (AD)	(Capponi et al. 2011; Evgrafov et al. 2004; Stancanelli et al. 2015)
T139M	ACD (β 7)	CMT2F (AD)	(Amornvit et al. 2017)
R140G	ACD (β 7)	dHMN/CMT2 (AD)	(Houlden et al. 2008)
K141Q	ACD (β 7)	dHMN (AD)	(Ikeda et al. 2009)
T151I	ACD	dHMN (AD)	(Dierick et al. 2008; Evgrafov et al. 2004)
P158fs*200	ACD (β 8)	Severe peripheral neuropathy (AD)	(Capponi et al. 2011; Mandich et al. 2010)
T164A	ACD (β 9)	CMT2 (AD)	(Lin et al. 2011)
M169fs*2	ACD	dHMN	(Ylikallio et al. 2015)
Q175*	CTD	dHMN/CMT2	(Rossor et al. 2012)
T180I	CTD	CMT2 sporadic	(Luigetti et al. 2010)
P182L/P182S	CTD	dHMN (AD)	(Dierick et al. 2008; Evgrafov et al. 2004; Kijima et al. 2005)
S187L	CTD	dHMN de novo	(Echaniz-Laguna et al. 2017)
R188W	CTD	CMT2	(Capponi et al. 2011)
Q190H	CTD	ALS (sporadic)	(Capponi et al. 2016)
A204fs*6	CTD	ALS (sporadic)	(Capponi et al. 2016)

ACD, α -crystallin domain; ALS, amyotrophic lateral sclerosis; AD, autosomal dominant; AR, autosomal recessive; CMT2, Charcot-Marie-Tooth disease of second type; CTD, C-terminal domain; dHMN, distal hereditary motor neuropathy; NTD, N-terminal domain

proteases. Six other are point mutations with replacing of only one amino acid residue. Three closely located mutations G34R, P39L, and E41K are found in highly conservative region detected in the primary structure of HspB1, HspB2, HspB4, HspB5, and HspB6 (Table 1) (Capponi et al. 2011; Echaniz-Laguna et al. 2017; Muranova et al. 2015). All these mutations were accompanied by decreased thermal stability and increased resistance to limited chymotrypsinolysis of HspB1. All mutants form homooligomers with the size larger than that of the wild-type protein (Muranova et al. 2015). This

finding agrees with suggestion that the N-terminal domain of small heat shock proteins plays a crucial role in formation of large oligomers of HspB1 (Jehle et al. 2011). As already mentioned, HspB1 is phosphorylated at three sites (Ser15, Ser78, Ser82) located in the N-terminal domain, and this phosphorylation is accompanied by partial or complete dissociation of large oligomers of the wild-type HspB1 (Rogalla et al. 1999). G34R, P39L, and E41K mutants were phosphorylated with the rate comparable with that of the wild-type protein; however, phosphorylation of these mutants induced only marginal

changes in their quaternary structure. For instance, phosphorylation of P39L mutant up to 2 mol of phosphate per mole of protein had no any effect on its quaternary structure thus blocking phosphorylation-dependent regulation of HspB1 activity (Muranova et al. 2015). Moreover, three analyzed mutants formed heterooligomeric complexes with another small heat shock protein HspB6; however, the rate of subunits exchange was significantly lower than in the case of the wild-type protein and only one (instead of two characteristic for the wild-type HspB1) type of complexes was formed. The chaperone-like activity measured in vitro with three different model protein substrates was lower (G34R, E41K mutants) or similar (P39L mutant) with that of the wild-type HspB1 (Muranova et al. 2015). Thus, point mutations G34R, P39L, and E41K located in the very N-terminal part of HspB1 affect stability, phosphorylation-dependent regulation of quaternary structure, and interaction with protein partners (HspB6) and model protein substrates.

Two other mutations are located at the border of the NTD (G84R) or in the very beginning of ACD (L99M) (Table 1) (Houlden et al. 2008; James et al. 2008). Both these sites are highly conservative and are preserved in HspB1 of different species such as *Danio rerio*, *Xenopus laevis*, *Gallus gallus*, *Bos taurus*, *Rattus norvegicus*, and *Homo sapiens* (Nefedova et al. 2013b). Mutations at these sites induce destabilization of quaternary structure, and at low concentration, oligomers of G84R and L99M mutants tend to dissociate, whereas at high concentration, these mutants form unstable oligomers with the size larger than the corresponding oligomers of the wild-type protein (Nefedova et al. 2013b). Due to destabilization of quaternary structure, phosphorylation results in rapid dissociation of large oligomers of these mutants even at low level of phosphorylation. Also, both mutations decreased interaction of HspB1 with its partner HspB6 and as a rule possessed lower chaperone-like activity in vitro if lysozyme and myosin subfragment-1 were used as model substrates (Nefedova et al. 2013b). It was supposed that replacement of small glycine by large charged arginine in G84R mutant can restrict mobility of NTD and by this means affect HspB1 oligomerization. At the same time L99 is located in close vicinity to the hydrophobic core of the α -crystallin domain. The replacement of leucine by methionine can destabilize the intersubunit interaction (Nefedova et al. 2013b).

The largest part of HspB1 mutations associated with CMT or dHMN is located in the conservative α -crystallin domain (ACD) (Table 1, Fig. 1b). Of note, replacement of nearly every residue in the range 127–141 is associated with different forms of distal neuropathy. This is probably due to the fact that many residues within the β 5 strand, the loop connecting β 5 and β 7 strands, and the β 7 strand in particular are directly involved in the formation of the HspB1 dimer (Baranova et al. 2011; Clark et al. 2011; Laganowsky et al. 2010). Most recently, it was shown that local unfolding of β 7 strand affects

the equilibrium between dimers and monomers, which in turn affects the chaperone-like activity of HspB1 (Alderson et al. 2019).

Residues R127 located in the β 5 strand as well as S135 and R136 located in the β 7 strand are highly conserved in different human small heat shock proteins (Weeks et al. 2018). Mutations R127W, S135F, and R136W decrease thermal stability of HspB1 measured by intrinsic Trp fluorescence and temperature-dependent light scattering, and this effect is especially pronounced in the case of R127W mutant. Mutations R127W and S135F destabilize the quaternary structure of HspB1, and therefore at low concentration, oligomers of these mutants dissociate forming dimers or tetramers. However, at high concentration or in the presence of crowding agents, all analyzed mutants (R127W, S135F, and R136W) form large oligomers with the size larger than that of the wild-type HspB1 (Weeks et al. 2018). All mutants undergo phosphorylation by MAPKAP kinase 2. At low level of phosphorylation, oligomers of R127W and S135F mutants easily undergo dissociation and in this respect are similar to G84R and L99M mutants. At the same time, oligomers of R136W mutant are rather stable and undergo dissociation only at high level of phosphorylation. Unstable oligomers of R127W and S135F easily exchange subunits with another small heat shock protein HspB6 both at low and high temperature and form heterooligomers different from those formed by the wild-type protein. At the same time, R136W mutant was unable to form heterooligomers with HspB6 (Weeks et al. 2018). All analyzed mutants possessed lower chaperone-like activity than the wild-type protein when myosin subfragment-1 or lysozyme was used as a model protein substrate, whereas they demonstrated higher chaperone-like activity when insulin was used as a model substrate.

Two other mutants of HspB1 located in the ACD and associated with distal hereditary neuropathies are R140G and K141Q (see Table 1, Fig. 1b). These mutants have lower thermal stability than the wild-type protein, and in this respect, they are similar to R127W, S135F, and R136W mutants carrying replacement in ACD. At low protein concentration, oligomers of both K141Q and especially R140G mutants tend to dissociate, whereas at high concentration, both these mutants form oligomers with the size larger than in the case of the wild-type HspB1 (Nefedova et al. 2013a). These properties are also similar to those described for R127W and S135F mutants. Both mutants were able to form heterooligomeric complexes with HspB6; however, the probability of chemical crosslinking of C46S/E116C double mutant of HspB6 (containing single Cys in position homologous to that of Cys137 of HspB1) and R140G mutant of HspB1 was lower than in the case of the wild-type protein. In vitro chaperone-like activity of both mutants was lower than that of the wild-type protein, and this difference was especially pronounced in the case of R140G mutant (Nefedova et al. 2013a). Both K141 and

especially R140 play a crucial role in stabilization of intersubunit contact in HspB1 since K141 forms salt bridge with E126, whereas R140 forms salt bridge with D129 of the neighbor subunit (Nefedova et al. 2013a). It is worthwhile mentioning that R116 of HspB4, R120 of HspB5, and K141 of HspB8 located in position homologous to that of R140 of HspB1 probably play a similar role in stabilization of intersubunit contacts. Mutation of these residues is associated with cataract in the case of HspB4 (Clark et al. 2011; Hansen et al. 2007), cataract, myopathy, and cardiomyopathy in the case of HspB5 (Chavez Zobel et al. 2003; Vicart et al. 1998) and dHMN in the case of HspB8 (Echaniz-Laguna et al. 2017; Irobi et al. 2010).

Another mutation of HspB1 associated with dHMN/CMT is located at the end of ACD (Lin et al. 2011). In this case, conservative Thr residue in position 164 is replaced by an alanine. This mutation also destabilizes the quaternary structure of HspB1, and therefore, this mutant has comparatively low thermal stability, and at low protein concentrations, oligomers of T164A mutant dissociate forming dimers or tetramers (Chalova et al. 2014). In vitro this mutant had chaperone-like activity comparable with that of the wild-type protein.

Hence, practically all mutations of HspB1 associated with dHMN/CMT and carrying mutations in crystallin domain have lower thermal stability. As a rule (except of R136W), homooligomers of these mutants tend to dissociate at low protein concentration forming small oligomers; however, at high concentration or in the presence of crowding agents, these mutants form large oligomers with the size that is usually larger than that of the wild-type HspB1. Measured in vitro chaperone-like activity of these mutants is usually lower than that of the wild-type HspB1; however, with certain model protein substrate chaperone-like activity of these mutants can be equal or even larger than that of the wild-type protein. Mutations in ACD affect interaction of HspB1 with protein partner HspB6 modulating either the rate of subunit exchange or the nature of heterooligomeric complexes formed.

Certain HspB1 mutations associated with dHMN/CMT are located in the C-terminal domain. Two of these mutants (P182S and R188W) had lower thermal stability, whereas the third mutant T180I was slightly more stable than the wild-type HspB1 (Chalova et al. 2014). T180I mutant formed stable oligomers with the size comparable with that of the wild-type protein, whereas R188W mutant formed slightly larger stable oligomers. Unusual properties were demonstrated by P182S mutant. This protein formed very large oligomers (aggregates) with apparent molecular weight of more than 1000 kDa. Addition of the wild-type HspB1 resulted in formation of mixed oligomers having smaller apparent molecular weight and higher thermal stability than isolated P182S mutant (Chalova et al. 2014). T180I mutant had in vitro chaperone-like activity comparable with that of the wild-

type HspB1, whereas both P182S and R188W mutants demonstrated lower chaperone-like activity than the wild-type HspB1 with all analyzed model protein substrates. The C-terminal domain of HspB1 contains conservative IPV tripeptide that is involved in the interaction with hydrophobic groove formed by $\beta 4$ and $\beta 8$ strands of ACD. This hydrophobic fragment of the C-terminal domain can remain unbound or form contacts with the same monomer or with the hydrophobic groove formed by monomer belonging to the neighbor dimer inside of HspB1 oligomer (Delbecq et al. 2012; Hochberg and Benesch 2014) thus stabilizing interdimer interaction. P182 is in the center of this key tripeptide, and therefore, its mutations can dramatically affect quaternary structure of HspB1 and induce aggregation of P182S mutant. The C-terminal domain of small heat shock proteins contains the sites responsible for interaction with protein substrates (Lindner et al. 2000; Treweek et al. 2007); therefore, mutation R188W can affect chaperone-like activity of HspB1.

In summary, we can conclude that mutants of HspB1 associated with motor neuron degeneration have some common features (Table 2). Firstly, practically all these mutants are less stable to thermal-induced unfolding and heat-induced aggregation. Secondly, mutations associated with dHMN/CMT induce changes in the quaternary structure of HspB1. As a rule, oligomers formed by mutants are larger than corresponding oligomers formed by the wild-type protein. These large oligomers either are very stable and do not undergo dissociation (in the case of mutations located in the NTD) or easily dissociate upon dilution (in the case of mutations located in the ACD). Thirdly, phosphorylation-induced regulation of HspB1 quaternary structure is disturbed in practically all analyzed mutants. Mutants carrying mutations in the N-terminal domain are resistant to phosphorylation-induced oligomer dissociation, whereas mutants carrying mutations in ACD (except of R136W mutant) more readily dissociate upon phosphorylation than the wild-type Hsp27 (Table 2). Fourthly, all analyzed mutants interact with partner protein HspB6 in the manner different from that of the wild-type HspB1. And finally, as a rule, chaperone-like activity measured in vitro with different model protein substrates was lower for mutants than for the wild-type HspB1. Thus, mutations analyzed induce significant changes in HspB1 properties (Table 2) and the question arises how all these changes induce motor neuron degeneration.

Probable molecular mechanisms underlying the effect of HspB1 mutation on distal motor neuropathies

As already mentioned, all analyzed mutations induce significant changes in the properties of HspB1 probably affecting its physiologically important activities. HspB1 is ubiquitously

Table 2 Some properties of HspB1 mutants

HspB1 species	Oligomeric mass at 1–3 mg/ml	Oligomeric mass at 0.2 mg/ml	Effect of heating	Effect of phosphorylation	Chaperone-like activity
WT	530–600 kDa	530–600 kDa	Aggregation at high temperature	Dissociation to dimers with >0.5 mol phosphate per mole	Normal
NTD mutants	600–740 kDa	530–600 kDa	Aggregation at lower temperature	Oligomers are preserved even with >1 mol phosphate per mole	Usually lower than WT
ACD mutants (except for R136W)	830–950 kDa	60–65 and 520–520 kDa	Aggregation at lower temperature	Dissociation to dimers with <0.5 mol phosphate per mole	Variable
CTD mutants P182S	530–600 kDa > 1000 kDa	530–600 kDa > 1000 kDa	Aggregation at lower temperature	ND	Usually lower than WT

expressed in practically all human tissues (Taylor and Benjamin 2005; Vos et al. 2009). This means that mutated forms of HspB1 will be expressed in practically all organs and defects characteristic for these mutants should demonstrate their deleterious effects in practically all human tissues. At the same time, mutations of HspB1 affect only motor neurons inducing different forms of distal neuropathy and probably certain forms of myopathy (Lewis-Smith et al. 2016). The question arises why motor neurons predominantly suffer from HspB1 mutations.

Motor neurons differ from other cells having very long (about 1 m) axons. Different components synthesized in soma should be transported to axon's terminal, whereas other organelles (lysosomes, autophagosomes, endosomes) should be transported from axon terminal back to soma (Holzbaur and Scherer 2011). Axonal transport is also of great importance for bringing mitochondria to the places with high energy consumption often located far from cell body. Therefore, axonal transport is of special importance for motor neurons, and any damages of intracellular traffic often result in neuron death and dHMN/CMN onset (Bucci et al. 2012; Gentil and Cooper 2012; Pareyson et al. 2015). Therefore, one can suppose that HspB1 mutations somehow affect axonal stability or axonal transport. It was shown that HspB1 is able to interact and by this means stabilize microtubules (Almeida-Souza et al. 2011a). It was supposed that mutations in ACD (R127W, S135F) induce dissociation of HspB1 oligomers and formation of small oligomers (or even monomers) possessing higher affinity to different protein substrates and among them to tubulin (Almeida-Souza et al. 2010; d'Ydewalle et al. 2011). Indeed, these mutations destabilize large HspB1 oligomers that tend to dissociate at low protein concentration and form very large oligomers at high protein concentration or under crowding conditions (Weeks et al. 2018). Depending on the nature of model protein substrates, these mutants demonstrate either lower or higher chaperone-like activity than the wild-type HspB1 (Weeks et al. 2018). As shown earlier, they can tightly interact with tubulin and by this means stabilize microtubules (Almeida-Souza et al. 2011a).

On the cell level, expression of S135C mutant of HspB1 is accompanied by the increase of density of microfilaments in axon (Benedetti et al. 2010). There is dynamic equilibrium in the rate of tubulin polymerization and depolymerization. Therefore, on early stages of disease, interaction of HspB1 mutants with tubulin will induce overstabilization of microtubules, and in order to overcome this unfavorable effect on late stages of disease, the cell somehow increases activity of histone deacetylase 6 (HDAC6) that deacetylates tubulin and by this means induces microtubule depolymerization (Almeida-Souza et al. 2011b; d'Ydewalle et al. 2011) (Fig. 2). In this respect, it is worthwhile to mention that inhibitors of histone deacetylase 6 (HDAC6) were recommended for pharmacological therapy of axonal CMT associated with HspB1 mutations (Benoy et al. 2017; d'Ydewalle et al. 2011). Recently published investigation detected significantly slower speed of retrograde mitochondria transportation in motor neurons expressing S135F, P39L, or R140G mutants of HspB1 (Kalmar et al. 2017). At the same time, anterograde mitochondria transportation was not significantly affected and this leads to imbalance in transport of mitochondria in two directions. In addition it was shown that mitochondrial membrane potential and activity of complex I of mitochondria was decreased in motor neurons expressing HspB1 mutants. All these events can lead to mitochondria redox imbalance and cause low level of oxidative stress that can somehow affect mitochondrial axonal transport (Kalmar et al. 2017).

Neurofilaments which belong to the class of intermediate filaments also play an important role in the maintenance axonal transport. Specifically, a range of mutations of neurofilament light chain (NFL) are associated with autosomal dominant CMT2 (Bucci et al. 2012). Expression of P182L mutant of HspB1 in the primary neuronal cells leads to accumulation of intracellular aggregates and disruption of neurofilament assembly (Ackerley et al. 2006). This effect is expected since this mutant tends to form large aggregates even in isolated state (Chalova et al. 2014). S135F mutant of HspB1 induced progressive degeneration of motor neurons with disruption of neurofilament network and aggregation of neurofilament

Fig. 2 **a** Proposed mechanisms of CMT pathogenesis induced by HspB1 mutations. HspB1 mutations affect the cytoskeleton of neurons. Specifically, such mutations can induce overstabilization of microtubules. These changes are followed by tubulin deacetylation and microtubule depolymerization during later stages of disease. In addition mutations of HspB1 can induce the disruption of neurofilament network and/or affect neurofilament phosphorylation and anterograde transportation of neurofilaments. **b** Mutations of HspB1 can affect astroglia metabolism and induce the production of reactive oxygen substances leading to axon degeneration. See text for details

proteins, whereas the wild-type HspB1 prevented aggregation of mutated NFL associated with CMT (Zhai et al. 2007). In addition it was shown that R127W, S135F, and P182L mutants of HspB1 increase Cdk-5 phosphorylation of neurofilaments and affect the binding of neurofilaments to kinesin thus reducing their anterograde transportation and proper cytoskeleton organization (Holmgren et al. 2013) (Fig. 2). Our attempts to detect the exact effect of mutations on the HspB1 interaction with the NFL were unsuccessful (Nefedova et al. 2017). Both the wild-type HspB1 and all analyzed mutants equally well regulated the process of polymerization of isolated NFL. It is possible that the effect of HspB1 mutation can be observed only in the case of neurofilaments composed of all three components (light, medium and heavy chains) or that this effect is indirect and depends on the presence of protein kinases and phosphorylation of certain component of neurofilaments.

Mutations of HspB1 affected not only motor neuron but also other cells. For instance, fibroblasts obtained from patients with CMT2 and expressing either frame-shifting mutant M169fs*2 or point mutant R127L had lower heat shock

tolerance and increased cellular sensitivity to protein misfolding induced by culturing in the presence of canavanin (arginine analog inducing protein misfolding) (Fig. 2). This effect was especially pronounced in the case of HspB1 with truncated C-terminus (M169fs*2) (Ylikallio et al. 2015).

Interesting results were obtained by producing transgenic mice expressing mutant forms of human HspB1. Unfortunately results obtained by this technique were not completely consistent. In one case transgenic mice expressing R136W mutant of human HspB1 demonstrated symptoms of age-related motor neuropathy and axonal pathology of peripheral nerve with impaired neurofilament cytoskeleton. However, all these symptoms were subclinical and were not completely identical to those detected in CMT (Srivastava et al. 2012). In another case expression of S135F mutant of human HspB1 in mouse model was accompanied by reduced locomotion, elevated level of phosphorylated neurofilament, and reduced level of tubulin acetylation (Lee et al. 2015). All these symptoms were identical to those characteristic for CMT, and no additional phenotype besides motor neuronal defects were observed. Interpretation of results obtained on

cellular level or on transgenic animals is rather complicate. Special experiments were performed in an astrocyte-motor neuron co-culture model system (Heilman et al. 2017). In these experiments, astrocytes expressing mutants superoxide dismutase (SOD1(G93A)) induced motor neuron death. Expression of wild-type HspB1 in astrocytes increased motor neuron survival, whereas expression of G84R or R136W mutants of HspB1 in astrocytes had no effect on survival of motor neurons (Heilman et al. 2017). The authors conclude that expression of HspB1 mutants can somehow modulate interaction of astrocytes and motor neurons and by this means induce neuropathy (Fig. 2). Recently published data indicate that expression of CMT mutants of HspB1 impairs autophagy by affecting interaction with autophagy receptor SQSTM/p62 and impairing phagophore formation (Haidar et al. 2019).

Conclusions

Mutations associated with dHMN/CMT induce significant changes in the structure and properties of HspB1. They affect thermal stability, quaternary structure, phosphorylation-induced changes in oligomeric structure, and interaction with partners and substrate proteins. Although these mutations differently affect HspB1 properties, all of them induce similar clinical symptoms. These effects as well as detailed pathomechanisms responsible for neuropathies associated with HspB1 mutations remain enigmatic. According to one plausible hypothesis, HspB1 mutations affect cytoskeleton and axonal transport disturbing transport of different cargoes along the axon. Alternatively, HspB1 mutations could decrease thermal stability and increase cellular sensitivity to protein misfolding. Finally, HspB1 mutations could indirectly affect motor neuron survival by modulating astrocyte activity. Explanation of the molecular mechanisms underlying the different neuropathies associated with HspB1 mutations will require joint efforts of biologists and clinicians.

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Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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