# Chapter 2
# Immense Cellular Implications Associated to Small Stress Proteins Expression: Impacts on Human Pathologies

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**Abstract** In addition to being potent chaperones that protect cells against the accumulation of unfolded proteins under stress conditions, mammalian small heat shock proteins (small Hsps) regulate many vital cellular processes in normal and pathological cells. Indeed, these Hsps are constitutively expressed in many tissues and show dramatic changes in their levels of expression in most human pathologies. They are characterized by a large spectrum of activities and are particularly active in protein conformational and inflammatory diseases as well as in cancer pathologies. It is now believed that the immense cellular implications of small Hsps results from their ability to interact, through particular structural changes, with many different client proteins that are subsequently modulated in their activities or half-lifes. Here, we have integrated functionally and structurally the recent data in the literature concerning the interactions of mammalian small Hsps with specific clients. Further analysis with geneMANIA software and database confirmed the incredibly large number of functions associated with these Hsps. The consequences for human pathologies as well as putative therapeutic strategies are discussed, particularly when the expression of small Hsps is harmful (as in some cancer pathologies) or when it appears beneficial for patients.

**Keywords** Mammalian small Hsps • Oligomeric complexes • Clients • Protein interactomes • Protein predictomes • Cellular implications • Pathologies

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## 2.1 Many Functions Associated with Small Hsps in Addition to Their Protective Role in Stress Condition

The last decade has been characterized by an incredible jump in the interest in the ten mammalian small Hsps. Indeed, until the turn of the century, these stress proteins were considered as exotic chaperones that did not use ATP for their activity. These "forgotten chaperones", as they were called in 2002 (Solari and Garrido 2002), are now stars among Hsps to judge by the large number of scientific and medical publications dealing with their particular behaviors and functions that fill the current literature. This renewed interest is probably linked to their constitutive expression in normal and pathological conditions as well as to the large number of unrelated functions associated with their over- or under-expression in many different cell types. Interest has also been generated by the growing number of pathological mutations in their genes that induce degenerative or myopathic diseases and by their newly described ability to be secreted.

### *2.1.1 Stress Conditions, Chaperone Activity and Anti-aggregation Properties*

Early studies dealing with HspB1 and HspB5 revealed their enhanced expression under heat shock conditions as well as their ATP-independent chaperone property (Jakob et al. 1993; Rogalla et al. 1999). It was shown that large oligomeric structures formed by small Hsps store stress-altered polypeptides in a refolding competent state that can interfere with their propensity to aggregate (Bellyei et al. 2007; Carra et al. 2005; Ehrnsperger et al. 1997, 2000; Ganea 2001; Haslbeck et al. 2005; Horwitz et al. 1992; Jakob et al. 1993; Lee et al. 1997; Markossian et al. 2009). These altered polypeptides can subsequently be refolded by the ATP-dependent Hsp70, Hsp90 and co-chaperones "foldase" machines (Buchner 1999; Bukau and Horwich 1998; Freeman and Morimoto 1996; Lee and Vierling 2000) or degraded by the CHIP-ubiquin-26S proteasome machine (McDonough and Patterson 2003). The dynamic oligomerization/phosphorylation status of small Hsps, and particularly HspB1, is an essential factor of this process (Arrigo et al. 1988; Lelj-Garolla and Mauk 2005, 2006; Paul et al. 2010; Preville et al. 1998b; Rogalla et al. 1999; Simon et al. 2013). The cytoskeleton is one of the primary targets protected by HspB1 and HspB5 in response to stress (Bellomo and Mirabelli 1992; Welch and Feramisco 1985) as well as in normal growth conditions. This property probably relies, at least in the case of HspB1, on the fact that phosphorylated small HspB1 oligomers modulate F-actin fiber growth and, indirectly, extracellular matrix organization (Dalle-Donne et al. 2001; Mounier and Arrigo 2002; Perng et al. 1999).

Under stress conditions, HspB1 and HspB5 stabilize microtubules (Hino et al. 2000; Preville et al. 1996; Xi et al. 2006). HspB5 is also very active in maintaining intermediate filaments homeostasis, particularly in muscle cells where it associates with desmin (Bennardini et al. 1992; Djabali et al. 1999). Moreover, HspB1 and HspB5 share an intriguing anti-oxidant property which appears linked to the chaperoning of several anti-oxidant enzymes, particularly G6PDH (glucose 6-phosphate dehydrogenase) (Arrigo 2001, 2007b, 2013; Arrigo et al. 2005; Firdaus et al. 2006a; Mehlen et al. 1996a; Paul and Arrigo 2000; Preville et al. 1998a, 1999; Rogalla et al. 1999; Yan et al. 2002). Consequently, damage such as protein and nucleic acid oxidation as well as lipid peroxidation is reduced and the positive effect of these Hsps towards mitochondrial $\Delta\Phi m$ increases ATP levels, which favors the activity of ATP-dependent chaperones (Mehlen et al. 1996a; Preville et al. 1999).

Only HspB1, HspB5 and HspB8 molecular chaperones are induced under stress conditions. Interestingly, constitutively expressed small Hsps, such as HspB2, HspB3, HspB4, HspB6 and HspB7, also display chaperone activities or at least anti-aggregation and pro-degradative functions (Carra et al. 2013). The anti-aggregation and anti-fibrillation properties of mammalian small Hsps are summarized in Table 2.1. Depending on the substrate, some Hsps perform these tasks better than others, suggesting that they do not all have the same chaperone-like activity. For example, HspB4 can chaperone HspB5 once in the alpha-crystallin complex (Andley 2007), while HspB3 (Asthana et al. 2012) and HspB2 exhibit significant chaperone-like activity towards specific target proteins and can attenuate the ordered amyloid fibril formation of α-synuclein (Prabhu et al. 2012). The major substrates recognized by small Hsps can be mutated polypeptides that cause degenerative or myopathic diseases (i.e. desmin, polyQ proteins, SOD, α-synuclein) or proteins that are prone to aggregate. It is also important to mention that small Hsp mutants can induce the aggregation of their substrates, such as the R120G missense mutation in HspB5 which is genetically linked to a desmin-related myopathy consequently of the aggregation of desmin (Bova et al. 1999; Vicart et al. 1998). Similarly, the P182L mutant of HspB1 leads to motor neuronopathies as a result of the formation of aggregates that sequestrate Neurofilament middle chain subunit (NF-M) and p150 Dynactin (Ackerley et al. 2005). Equally, proteins that interact with mutant small Hsps can counteract aggregation, as for example the chaperone-like effect of Bag3 towards aggregated HspB8 mutant (Hishiya et al. 2011). As a result of its interaction with Bag3, HspB8 also has the ability to trigger macroautophagy (Carra 2009; Carra et al. 2008b). This favors the elimination of aggregated polypeptides generated by heat (Nivon et al. 2009) or oxidative stress (Keller et al. 2004; Kiffin et al. 2006). Interestingly, HspB6 also appears to play a role in the Bag-3/HspB8 complex that triggers macroautophagy (Fuchs et al. 2010). Less information is available concerning HspB9 and HspB10 in spite of their ability to interact with particular polypeptides (see Table 2.2).

**Table 2.1** Small Hsps activity against aggregation or fibrillation of proteins

| Client | HspB | Resulting effects | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | By adding HspB to cells | |
| α-synuclein (wt, mutant) | HspB1 | Inhibition of fibrillation | nd | X | X | – | Nemes et al. (2004) and Outeiro et al. (2006) |
| | HspB5 | Inhibition of fibrillation | Known HspB5 sequence | X | X | – | Bruinsma et al. (2011); Outeiro et al. (2006) and Ghosh et al. (2008) |
| | HspB8 | Inhibition of fibrillation | nd | – | X | – | Bruinsma et al. (2011) |
| | HspB3/B2 | Inhibition of fibrillation | nd | – | X | – | Bruinsma et al. (2011) and Prabhu et al. (2012) |
| | HspB6 | Inhibition of fibrillation | nd | – | X | – | Bruinsma et al. (2011) |
| Aβ-amyloid (wt, mutant) | HspB8 | Inhibition of aggregation | nd | X | X | – | Wilhelmus et al. (2006a) |
| | HspB5 | Inhibition of aggregation | Known HspB5 sequence | X | X | – | Ghosh et al. (2008) and Wilhelmus et al. (2006b) |
| | HspB5 | Inhibition of fibrils elongation | nd | – | X | – | Shammas et al. (2011) |
| | HspB1 | Inhibition of aggregation | nd | X | X | – | Wilhelmus et al. (2006b) |
| | HspB6 | Inhibition of aggregation | nd | X | X | – | Wilhelmus et al. (2006b) |
| PolyQ proteins | HspB1 | Inhibition of aggregation | nd | X | X | – | Robertson et al. (2010) and Vos et al. (2010) |
| | HspB5 | Inhibition of aggregation | nd | X | X | – | Robertson et al. (2010) and Vos et al. (2010) |
| | HspB8 | Inhibition of aggregation | nd | X | X | – | Carra et al. (2008a) and Vos et al. (2010) |
| | HspB7 | Inhibition of aggregation | nd | X | X | – | Vos et al. (2010) |
| Catalase | HspB5 | Protection against inactivation | HspB5/catalase ratio: 1:2 | – | X | – | Hook and Harding (1996) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SOD1 | HspB1 | Inhibition of aggregation | Interaction with SOD1 aggregates | – | X | – | Yerbury et al. (2012) |
| | HspB5 | Inhibition of aggregation | Increased mutant SOD1 solubility | X | X | – | Shinder et al. (2001) and Yerbury et al. (2012) |
| | HspB8 | Inhibition of aggregation | Increased mutant SOD1 solubility | X | – | – | Crippa et al. (2010) |
| TDP-43 | HspB8 | Inhibition of aggregation | nd | X | – | – | Crippa et al. (2010) |
| Parkin | HspB1 | Neuronal aggregates stabilization | nd | X | – | – | Nemes et al. (2004) |
| Phosphorylated Tau | HspB1 | P-Tau degradation | nd | X | – | – | Shimura et al. (2004) |
| Desmin | HspB5 | Inhibition of aggregation | Known HspB5 sequence | – | X | – | Ghosh et al. (2007b) |
| Vimentin | HspB1 | Inhibition of aggregation | nd | X | X | – | Perng et al. (1999) |
| | HspB5 | Inhibition of aggregation | Known HspB5 sequence | X | X | – | Djabali et al. (1997, 1999) and Perng et al. (1999) |
| Tubulin | HspB5 | Inhibition of aggregation | Known HspB5 sequence | – | X | – | Ghosh et al. (2007a) and Ohto-Fujita et al. (2007) |
| Serpin | HspB5 | Inhibition of aggregation | nd | – | X | – | Devlin et al. (2003) |
| PrPc | HspB5 | Inhibition of aggregation | nd | X | X | – | Sun et al. (2005) |
| Apolipoprotein-CII | HspB5 | Inhibition of aggregation | nd | – | X | – | Hatters et al. (2001) |
| β2-microglobulin | HspB5 | Inhibition of fibrillation | Known HspB5 sequence | – | X | – | Ghosh et al. (2008) |
| Transthyretin | HspB5 | Inhibition of fibrillation | Known HspB5 sequence | – | X | – | Ghosh et al. (2008) |
| p150 dynactin | **HspB1** | Formation of aggregates | Sequestration by HspB1 P182L mutant | X | – | – | Ackerley et al. (2005) |
| NF-M | **HspB1** | Formation of aggregates | Sequestration by HspB1 P182L mutant | X | – | – | Ackerley et al. (2005) |
| Desmin | **HspB5** | Formation of aggregates | Sequestration by HspB5 R120G mutant | X | – | – | Bova et al. (1999) |
| Bag3 | **HspB5** | Inhibits aggregation of mut.HspB5 | Ile-Pro-Val regions (Bag3) | X | X | – | Hishiya et al. (2011) |

List of abbreviations: **HspB** (bold) mutant HspB, *nd* not determined, *SOD1* Copper-Zinc superoxide dismutase, *TDP-43* major disease protein in ubiquitin-positive, tau-, and alpha-synuclein-negative frontotemporal dementia, *PrPc* bovine prion protein, *NF-M* Neurofilament middle chain subunit; a protein kinase of the MLK family

**Table 2.2** Some of the polypeptides interacting with mammalian small Hsps

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| ***Cytoskeleton, cell adhesion, tissue integrity, epithelial to mesenchymal transition (MET)*** | | | | | | | |
| F-actin | HspB1 | Protects F-actin integrity | Small P-oligomers (HspB1) | X | – | – | Ke et al. (2011) and Mounier and Arrigo (2002) |
| | HspB5 | Protection of F-actin integrity | P-HspB5 | X | – | – | Singh et al. (2007) and Wang and Spector (1996) |
| | α-crystallin | Protection of F-actin integrity | α-crystallin/actin | X | – | – | Del Vecchio et al. (1984) and Wang and Spector (1996) |
| | HspB6 | Inhibition of stress fibers formation | nd | X | X | – | Ke et al. (2011) |
| | HspB7 | Inhibition of stress fibers formation | nd | X | X | – | Ke et al. (2011) |
| | HspB8 | Inhibition Rho GTPase/stress fibers | nd | X | X | – | Ke et al. (2011) |
| | HspB1 | Translocation to F-actin by proteasome inhibition | nd | X | – | – | Verschuure et al. (2002) |
| | HspB2 | Translocation to F-actin by proteasome inhibition | nd | X | – | – | Verschuure et al. (2002) |
| | HspB3 | Translocation to F-actin by proteasome inhibition | nd | X | – | – | Verschuure et al. (2002) |
| | HspB5 | Translocation to F-actin by proteasome inhibition | nd | X | – | – | Verschuure et al. (2002) |
| Actin | HspB5 | Chaperoning and polymerization of actin | Known HspB5 sequence | – | X | – | Ghosh et al. (2007b) |
| Tubulin | HspB1 | Chaperoning of α/β tubulin, protects microtubules | nd | X | – | – | Hino et al. (2000) and Preville et al. (1996) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | HspB5 | Microtubule assembly; inhibits tubulin aggregation | Known HspB5 sequence | – | X | – | Ghosh et al. (2007a) and Ohto-Fujita et al. (2007) |
| MAPs | HspB5 | Inhibition of microtubules aggregation | nd | X | – | – | Xi et al. (2006) |
| Vimentin IF | HspB5 | Favors and protects VIF interaction | nd | X | X | – | Perng et al. (1999) |
| Vimentin IF | HspB1 | Favors and protects VIF interaction | nd | X | X | – | Perng et al. (1999) |
| Vimentin | HspB1 | Chaperoning of vimentin | nd | X | X | – | Perng et al. (1999) |
| | HspB5 | Chaperoning of vimentin, protects IF | Known HspB5 sequence | X | X | – | Djabali et al. (1997, 1999) and Perng et al. (1999) |
| Peripherin | HspB5 | Chaperoning of peripherin, protects IF | Known HspB5 sequence | X | X | – | Djabali et al. (1997, 1999) |
| Desmin | HspB5 | Chaperoning of desmin | Known HspB5 sequence | – | X | – | Ghosh et al. (2007b) |
| GFAP IF | HspB1 | Favors GFAP filaments interaction | nd | X | X | – | Perng et al. (1999) |
| GFAP IF | HspB5 | Favors GFAP filaments interaction | nd | X | X | – | Perng et al. (1999) |
| GFAP | HspB5 | Stabilization/degradation GFAP | nd | X | – | – | Perng et al. (1999) and Tang et al. (2010) |
| Keratin | HspB1 | Chaperoning of keratin, keratin networks disassembly | nd | X | – | – | Duverger et al. (2004) and Perng et al. (1999) |
| Titin | HspB5 | Protection of integrity | i-band (titin) | X | – | – | Bullard et al. (2004) |
| Neurofilaments | HspB1 | Protection of integrity | nd | X | – | – | Bjorkdahl et al. (2008) |
| | HspB5 | Protection of integrity | nd | X | – | – | Bjorkdahl et al. (2008) |
| β-catenin | HspB1 | Cell adhesion | nd | X | – | – | Fanelli et al. (2008) |
| | HspB5 | Cell adhesion | Known HspB5 sequence | – | X | – | Ghosh et al. (2007c) |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| E-cadherin | HspB5 | Cell adhesion | nd | X | – | – | Huang et al. (2012) |
| Cadherin-16 | HspB5 | Cadherin-16-cytoskeleton connection | nd | X | – | – | Thedieck et al. (2008) |
| Snail | HspB1 | Promotes MET | nd | X | – | – | Wettstein et al. (2013) |
| DSTN | HspB8 | Destrin, actin depolymerization | nd | X | – | – | Havugimana et al. (2012) |
| Filensin | HspB5 | Chaperoning of filensin | nd | X | – | – | Muchowski et al. (1999) |
| Phakinin | HspB5 | Chaperoning of phakinin | nd | X | – | – | (Muchowski et al. 1999) |
| GRIFIN | HspB5 | ? | nd | X | – | – | (Barton et al. 2009) |
| HDAC6 | HspB1 | Stabilization of HDAC6 half-life | HspB1 500-700 kDa oligomers | X | – | – | Gibert et al. (2012a) |
| | | Stimulates microtubules deacetylation | | | | | |
| p66Shc | HspB1 | F-actin disruption | p66Shc Ser36 phosphorylation, abrogates HspB1 protective function | X | – | – | Arany et al. (2012) |
| 14-3-3gamma | HspB6 | Displaces 14-3-3 binding partners | P-HspB6 | – | X | – | Chernik et al. (2007) and Seit-Nebi and Gusev (2010) |
| TCTEL1 | HspB9 | Testis specific | nd | X | – | – | de Wit et al. (2004) |
| Sperm dense fiber | HspB10 | Linkage of sperm head to tail | nd | X | – | – | Gastmann et al. (1993) and Yang et al. (2012) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Transduction pathways, cell activation*** | | | | | | | |
| *Membrane signaling proteins* | | | | | | | |
| CD10 | HspB1 | ? | nd | X | – | – | Dall'Era et al. (2007) |
| β2-microglobulin | HspB5 | Inhibition of fibrillation known | HspB5 sequence – F, D beta strands of β2-microglobulin | X | – | – | Ghosh et al. (2008) |
| TLR4 ligand | HspB8 | TLR4 dependent dendritic cells activation | nd | – | – | X | Roelofs et al. (2006) |
| | HspB4 | TLR4 dependent dendritic cells activation | nd | – | – | X | Roelofs et al. (2006) |
| TLR3 | HspB1 | Angiogenesis activation | nd | – | – | X | Thuringer et al. (2013) |
| TLR2 | HspB5 | Macrophage activation | nd | – | – | X | van Noort et al. (2013) |
| TLR1 | HspB5 | Macrophage activation | nd | – | – | X | van Noort et al. (2013) |
| CD14 | HspB5 | Macrophage activation | nd | – | – | X | van Noort et al. (2013) |
| Plasminogen | HspB1 | Modulates plasminogen binding to ECs | nd | – | – | X | Dudani et al. (2007) |
| Angiostatin | HspB1 | Modulates angiostatin binding to ECs | nd | – | – | X | Dudani et al. (2007) |
| *Growth factors, receptors, transduction pathway factors* | | | | | | | |
| NGF-beta | HspB5 | Chaperoning of NGF-beta | Known HspB5 sequence | – | X | – | Ghosh et al. (2007c) |
| FGF-2 | HspB5 | Chaperoning of FGF-2 | Known HspB5 sequence | – | X | – | Ghosh et al. (2007c) |
| VEGF | HspB5 | Chaperoning of VEGF | Known HspB5 sequence | – | X | – | Ghosh et al. (2007c) and Kerr and Byzova (2010) |
| | HspB5 | Intracrine VEGF signaling | nd | X | – | – | Ruan et al. (2011) |
| | HspB1 | Angiogenesis activation | nd | X | – | X | Choi et al. (2014) |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| Insulin | HspB5 | Insulin stabilization | Known HspB5 sequence | – | X | – | Ghosh et al. (2007c) |
| AR | HspB1 | AR stabilization | nd | X | – | – | Zoubeidi et al. (2007) |
| Her2 | HspB1 | Her2 stabilization | nd | X | – | – | Kang et al. (2008) |
| ERβ | HspB1 | Estrogen signaling | P-HspB1 | X | – | – | Al-Madhoun et al. (2007) |
| 14-3-3zeta | HspB6 | Modulates 14-3-3 interactome affinity | P-HspB6 | – | X | – | Sluchanko et al. (2011) |
| 14-3-3gamma | HspB6 | Displaces 14-3-3 binding partners | P-HspB6 | – | X | – | Chernik et al. (2007) and Seit-Nebi and Gusev (2010) |
| Beta-arrestin2 | HSPB1 | Essential for TRAIL-triggered Src-Akt/ERK survival signaling | P-HspB1 (ser78/82) | X | – | – | Qi et al. (2014) |
| Src | HspB1 | Essential for TRAIL-triggered Src-Akt/ERK survival signaling | P-HspB1 (ser78/82) | X | – | – | Qi et al. (2014) |
| DAXX | HspB1 | Blocks DAXX-mediated apoptosis | nd | X | – | – | Charette and Landry (2000) |
| TRAF6 | HspB1 | TRAF6 ubiquitination, IKKβ activation | P-HspB1 (ser78/82) | X | – | – | Wu et al. (2009) |
| *Protein kinases, phosphatases* | | | | | | | |
| PI3K | HspB6 | Inhibits PI3K activity and Akt pathway | nd | X | – | – | Matsushima-Nishiwaki et al. (2013) |
| PTEN | HspB1 | Stabilization of PTEN levels | nd | X | – | – | Cayado-Gutierrez et al. (2012) |
| Akt, P38, MK2 | HspB1 | Akt activation, scaffold MK2 to Akt | aa117-128 (Akt) | X | – | – | Rane et al. (2001) and Wu et al. (2007) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| PKCΔ | HspB1 | Inhibits HspB1 activity | PKCΔ V5 region (7 a.a.) | X | – | – | Kim et al. (2007) and Lee and Lee (2010) |
| RhoA, PKCα | HspB1 | Muscle contraction | P-HspB1 | X | – | – | Patil et al. (2004) |
| Phk | HspB1 | ? | Small HspB1 oligomers | X | – | – | Chebotareva et al. (2010) |
| p90Rsk | HspB1 | HspB1 phosphorylation | nd | X | X | – | Zoubeidi et al. (2010) |
| PRKD1 | HspB1 | HspB1 phosphorylation | HspB1 P-serine 82 | X | – | – | Doppler et al. (2005) |
| PPM1A | HspB1 | ? | nd | X | – | – | Wang et al. (2011) |
| Calponin, PKC | HspB1 | Contraction of smooth muscles | nd | X | – | – | Patil et al. (2004) |
| IKKβ | HspB5 | Stimulates IKKβ kinase activity | nd | X | – | – | Adhikari et al. (2011) |
| IKKα | HspB1 | Suppresses NF-κB activation (SAP) | P-HspB1 | X | – | – | Kammanadiminti and Chadee (2006) |
| IKKβ | HspB1 | Suppresses NF-κB activation (SAP) | P-HspB1 | X | – | – | Kammanadiminti and Chadee (2006) |
| DMPK | HspB2 | Activates DMPK | Oligomers of HspB2 | – | X | – | Prabhu et al. (2012) |
| ASK1 | HspB1 | Inhibited by HspB1-DAXX interaction | nd | X | – | – | Charette and Landry (2000) |
| ***Apoptotic, autophagic and redox factors, aging*** | | | | | | | |
| Caspase-3 | HspB1 | Pro-caspase-3 stabilization | 150-200 kDa HspB1 oligomers | X | – | – | Gibert et al. (2012a) and Pandey et al. (2000) |
| | HspB5 | Negative regulation of activity | nd | X | – | – | Hu et al. (2012) |
| | HspB4 | Negative regulation of activity | nd | X | – | – | Hu et al. (2012) |
| Cytochrome c | HspB1 | Inhibits cytochrome c apoptotic activity | a.a. 51-88 of HspB1 | X | – | – | Bruey et al. (2000a) |
| Bcl-xs | HspB5 | Inhibits translocation mitochondria | nd | X | X | – | Mao et al. (2004) |
| | HspB4 | Inhibits translocation mitochondria | nd | X | X | – | Mao et al. (2004) |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| Bax | HspB5 | Inhibits translocation mitochondria | nd | X | X | – | Hu et al. (2012) and Mao et al. (2004) |
| | HspB4 | Inhibits translocation mitochondria | nd | X | X | – | Hu et al. (2012) and Mao et al. (2004) |
| p53 | HspB5 | Inhibits translocation mitochondria | nd | X | – | – | Liu et al. (2007) |
| | HspB5 | Inhibits p53 mediated apoptosis | DNA-binding domain of p53 | X | – | – | Watanabe et al. (2009) |
| GranzymeA | HspB1 | GranzymeA stimulation | Mono/dimers of HspB1 | X | – | – | Beresford et al. (1998) |
| DAXX | HspB1 | Inhibits Fas apoptosis | Small HspB1oligomers | X | – | – | Charette and Landry (2000) |
| PEA-15 | HspB1 | Inhibits Fas apoptosis | nd | X | – | – | Hayashi et al. (2012) |
| CIAPIN1 | HspB8 | ? | nd | X | – | – | Havugimana et al. (2012) |
| XIAP | HspB5 | Overrides XIAP activity | nd | X | – | – | Lee et al. (2012) |
| HDAC1 | HspB5 | Enhances apoptosis of RPE cells | nd | X | – | – | Noh et al. (2008) |
| 14-3-3gamma | HspB6 | Displaces 14-3-3 binding partners | P-HspB6 | – | X | – | Chernik et al. (2007) and Seit-Nebi and Gusev (2010) |
| 14-3-3zeta | HspB6 | Modulates 14-3-3 interactome affinity | P-HspB6 | – | X | – | Sluchanko et al. (2011) |
| P66Shc | HspB1 | F-actin disruption, apoptosis | nd | X | – | – | Arany et al. (2012) |
| G6PDH | HspB1 | Stimulation of activity | Small HspB1 P-oligomers | X | – | – | Arrigo (2013), Cosentino et al. (2011) and Preville et al. (1999) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bag3 | HspB8 | Co-chaperone | β4, β8 hydrophobic grooves (HspB8)<br>IPV (Ile-Pro-Val) motifs (Bag3) | – | X | – | Carra et al. (2008b) and Fuchs et al. (2010) |
| | HspB6 | Co-chaperone | β4, β8 hydrophobic grooves (HspB6)<br>IPV (Ile-Pro-Val) motifs (Bag3) | – | X | – | Fuchs et al. (2010) |
| ***Transcription/translation, gene expression*** | | | | | | | |
| *Transcription factors* | | | | | | | |
| STAT-2 | HspB1 | STAT-2 stabilization | 200–600 kDa oligomers of HspB1 | X | – | – | Gibert et al. (2012a) |
| STAT-3 | HspB1 | STAT-3 stabilization | nd | X | – | – | Rocchi et al. (2005) |
| P-Stat-3 | HspB1 | P-STAT-3 stabilization | nd | X | – | – | Gibert et al. (2012a) |
| HSF-1 | HspB1 | HSF sumoylation | Large HspB1oligomers | X | – | – | Brunet Simioni et al. (2009) |
| GATA-1 | HspB1 | GATA-1 degradation | P-HspB1 | X | – | – | de Thonel et al. (2010) |
| Snail | HspB1 | Snail stabilization | nd | X | – | – | Wettstein et al. (2013) |
| p53 | HspB1 | ? | nd | X | – | – | Sun et al. (2007) |
| *Activators of transcription factors* | | | | | | | |
| IKKα | HspB1 | Suppression of NF-κB activation (SAP) | P-HspB1 | X | – | – | Kammanadiminti and Chadee (2006) |
| IKKβ | HspB1 | Suppression of NF-κB activation (SAP) | P-HspB1 | X | – | – | Kammanadiminti and Chadee (2006) |
| IKKβ | HspB5 | Activation of NF-κB, cell adhesion | nd | X | – | – | Adhikari et al. (2011) and Dieterich et al. (2013) |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| *Spliceosome assembly, pre-mRNA processing* | | | | | | | |
| SAM68 | HspB8 | Inhibits SAM68 activity | a.a. 62-133 of HspB8 | X | X | – | Badri et al. (2006) |
| Ddx20 | HspB8 | Ribonucleoprotein processing | nd | X | – | – | Sun et al. (2010) |
| EFTUD2 | HspB1 | Spliceosome modulation | nd | X | – | – | Hegele et al. (2012) |
| SC35 | HspB7 | Localized to SC35 splicing speckles | nd | X | – | – | Vos et al. (2009) |
| | HspB5 | HspB5-HDAC1 localized to SC35 | nd | X | – | – | Noh et al. (2008) |
| *Translation initiation factors* | | | | | | | |
| eIF4G | HspB1 | Inhibition translation during HS | nd | X | – | – | Cuesta et al. (2000) |
| eIF4E | HspB1 | Tumor cell survival | nd | X | – | – | Andrieu et al. (2010) |
| *mRNA half-life* | | | | | | | |
| AUF1 | HspB1 | AUF1 degradation | P-HspB1 | X | – | – | Knapinska et al. (2011) and Sinsimer et al. (2008) |
| *Ribosomes* | | | | | | | |
| p90Rsk | HspB1 | HspB1 phosphorylation | nd | X | – | – | Zoubeidi et al. (2010) |
| ***Regulators of protein degradation – ubiquitination by sHsps-E3*** | | | | | | | |
| Smad/Smurf2 | HspB1 | HspB1 ubiquitination/ degradation | nd | X | – | – | Sun et al. (2011 |
| TIF1gamma | HspB5 | Disruption of Smad4 ubiquitination | nd | X | – | – | Bellaye et al. (2014) |
| p27kip1 | HspB1 | Favors p27kip1 ubiquitination/ degradation | nd | X | – | – | Parcellier et al. (2006) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Ubiquitin | HspB1 | Protein degradation | nd | X | – | – | Parcellier et al. (2003) |
| HDM2 | HspB1 | HDM2 stabilization | nd | X | – | – | O'Callaghan-Sunol et al. (2007) |
| Fbx4 | HspB5 | Cyclin D1 ubiquitination/ degradation | nd | X | X | – | Lin et al. (2006) |
| TRAF6 | HspB1 | TRAF6 ubiquitination | P-HspB1 | X | – | – | Wu et al. (2009) |
| C8/α7 proteasome subunits | HspB5 | Proteasome assembly/ degradation of HspB5 bound proteins | nd | X | – | – | Boelens et al. (2001) |
| ***Protein sumoylation*** | | | | | | | |
| Ubc9 (UBE2I) | HspB1 | Modulation of HSF-1 activity by sumoylation | nd | X | – | – | Ahner et al. (2012) and Brunet Simioni et al. (2009) |
| F508del CFTR | HspB1 | Stimulates the degradation of Ubc9-HspB1 sumoylated F508del CFTR | nd | X | – | – | Ahner et al. (2012) |
| ***Protein deacetylation*** | | | | | | | |
| KDAC8 | HspB1 | Acetylation of HspB1 | nd | X | – | – | Chen et al. (2013) |
| KDAC8 | HspB6 | Acetylation of HspB6 | nd | X | – | – | Chen et al. (2013) |
| HDAC6 | HspB1 | Microtubules deacetylation | HspB1 500–700 kDa oligomers | X | – | – | Gibert et al. (2012a) |
| HDAC1 | HspB5 | Stimulates apoptosis of RPE cells | nd | X | – | – | Noh et al. (2008) |
| ***Proteins of the complement, acute phase proteins and coagulation factors*** | | | | | | | |
| Coagulation factors V, X; Complement C1qA, 1qB, 1qC, C1s, C1r, C5, C3, C2, C6, C7, C8, C9; Phosphatidylinositol-glycan-specific phospholipase D; vitamin K-dependent protein S cartilage acidic protein 1; mannosyl-oligosaccharide 1,2-alpha-mannosidase 1A; serpin A10 protein Z-dependent protease inhibitor; Insulin-like growth factor-binding protein; phenylcysteine oxidase 1; carboxypeptidase B2 and N subunit 2; thrombosporin; ficolin-3; platelet factor 4; glutathione peroxidase | | | | | | | |
| | HspB5 | Modulation of inflammation | nd | – | – | X | Rothbard et al. (2012) |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| ***Plasma proteins*** | | | | | | | |
| Factor XIII | HspB1 | Platelet FXIII regulation | P-HspB1<br>a.a. 484–503 of Factor XIII | X | – | – | Zhu et al. (1994) |
| ***Protein transport*** | | | | | | | |
| XPORT | HspB1 | Transport of TRP and Rh1 | nd | X | – | – | Rosenbaum et al. (2011) |
| SMN | HspB5 | SMN nuclear import and assembly | P-HspB5 | X | – | – | den Engelsman et al. (2005) |
| ***Cell cycle, tumor suppressors*** | | | | | | | |
| Cyclin D1 | HspB5 | Ubiquitination by HspB5- FBX4 | nd | X | X | – | Lin et al. (2006) |
| Myc | HspB1 | ? | nd | X | – | – | Agrawal et al. (2010) and Koch et al. (2007) |
| p53 | HspB1 | ? | nd | X | – | – | Sun et al. (2007) |
| | HspB5 | Inhibition translocation mitochondria | nd | X | – | – | Liu et al. (2007) |
| | HspB5 | Inhibition of p53 mediated apoptosis | DNA-binding domain of p53 | X | – | – | Watanabe et al. (2009) |
| ***Base excision repair enzymes*** | | | | | | | |
| UDG | HspB1 | ? | nd | X | X | – | Mendez et al. (2000) |
| HAP1 | HspB1 | ? | nd | X | X | – | Mendez et al. (2000) |
| ***Senescence*** | | | | | | | |
| HDM2 | HspB1 | Inhibition of p53 induced senescence *via* HDM2 stabilization | nd | X | – | – | O'Callaghan-Sunol et al. (2007) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Nucleases*** | | | | | | | |
| Rpp20 (POP7) | HspB1 | Enhances RNase P activity | nd | X | – | – | Jiang and Altman (2001) |
| ***Golgi proteins*** | | | | | | | |
| GM130 | HspB5 | Golgi vesicles | nd | X | – | – | Gangalum and Bhat (2009) |
| ***Lens proteins*** | | | | | | | |
| HspB4 | HspB5 | Chaperoning | Hetero-oligomeric complex | X | X | – | Groenen et al. (1994) and Skouri-Panet et al. (2012) |
| HspB5 | HspB4 | Chaperoning | Hetero-oligomeric complex | – | X | – | Skouri-Panet et al. (2012) |
| | HspB1 | Chaperoning | Hetero-oligomeric complex | – | X | – | Skouri-Panet et al. (2012) |
| betaB2-crystallin | HspB5 | Chaperoning | Hetero-oligomeric complex | X | – | – | Fu and Liang (2002) |
| gammaC-crystallin | HspB5 | Chaperoning | Hetero-oligomeric complex | X | – | – | Fu and Liang (2002) |
| MIP26/AQP0 | HspB4 | Modulation of refractive index | nd | X | – | – | Liu and Liang (2008) |
| ***sHsps co-chaperones, negative regulators*** | | | | | | | |
| p66Shc | HspB1 | Negative regulator of HspB1 | Requires p66Shc Ser36 phosphorylation | X | – | – | Arany et al. (2012) |
| PASS1 | HspB1 | Negative regulator of HspB1 | a.a. 108–208 of PASS1 | X | X | – | Liu et al. (2000) |
| Hic-5 (ARA55) | HspB1 | Negative regulator of HspB1 | C-terminal domain of HspB1 | X | – | – | Jia et al. (2001) |
| | | | hic-5 LIM domains | | | | |

(continued)

**Table 2.2** (continued)

| Client | HspB | Consequences | Interacting structure/sequence | Observations made | | | References |
|---|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | by adding HspB to cells | |
| Bag3 | HspB8 | Co-chaperone | β4, β8 hydrophobic grooves (HspB8) | – | X | – | Carra et al. (2008b) and Fuchs et al. (2010) |
| | | | IPV (Ile-Pro-Val) motifs (Bag3) | | | | |
| | HspB6 | Co-chaperone | β4, β8 hydrophobic grooves (HspB6) IPV (Ile-Pro-Val) motifs (Bag3) | – | X | – | Fuchs et al. (2010) |
| ***Viral proteins*** | | | | | | | |
| NS5A (Hepatitis C) | HspB1 | ? | a.a. 1-122 of HspB1 | X | – | – | Choi et al. (2004) |
| | | | a.a. 1-181 of NS5A | | | | |
| EBNA5 (EBV) | HspB1 | ? | ? | X | – | – | Forsman et al. (2008) |
| ***Some signaling pathways modulated by small Hsps but the targeted proteins are unknown*** | | | | | | | |
| ? | HspB1 | | (de)phosphorylation cascade controlling the activity of the splicing regulator SRp38 | Marin-Vinader et al. (2006) | | | |
| ? | HspB1 | | Pro-inflammatory cell signalling and gene expression, may act upstream of TAK1 | Alford et al. (2007) | | | |

| | | | |
|---|---|---|---|
| ? | HspB4 | Modulation of PKCalpha, RAF/MEK/ERK and AKT signaling pathways preventing UVA-induced apoptosis | Liu et al. (2004) |
| ? | HspB5 | Modulation of PKCalpha, RAF/MEK/ERK and AKT signaling pathways preventing UVA-induced apoptosis | Liu et al. (2004) |
| ? | HspB5 | Inhibition of RAS activation inducing p53 dependent apoptosis | Li et al. (2005) |
| ? | HspB8 | TAK1-dependent death pathways | Smith et al. (2012) |

The polypeptides listed here have been demonstrated to interact in vivo and/or in vitro with the different small Hsps. The interactions detected using yeast 2-hybrid approaches that were not confirmed by immunoprecipitation or pull-down experiments were not considered. List of abbreviations: nd: not determined; P-: phosphorylated; a.a.: amino acids; 200–400 kDa: oligomers with native size of 200–400 kDa. HspB1: also known as Hsp27, Hsp28 and Hsp25 in murine; HspB2: also known as MKBP; HspB4: alphaA-crystallin; HspB5: alphaB-crystalline; α-crystallin: HspB4:HspB5 complex (3:1); HspB6: also known as Hsp20; HspB7: also known as cvHsp; HspB8: also known as Hsp22; VIF: Vimentin intermediate filaments; GFAP: Glial fibrillary acidic protein; F508del CFT: deletion F508 of CFT responsible for most cystic fibrosis pathologies; CD10: 100 kDa transmembrane metallo-endopeptidase; VEGF: vascular endothelial growth factor; FGF-2: Fibroblast growth factor 2; NGF-beta: Nerve growth factor beta; Her2: Human Epidermal Growth Factor Receptor-2; AR: androgen receptor; ERβ: estrogen receptor β; Hic-5 (ARA55): androgen receptor associated protein 55, a focal adhesion protein and steroid receptor co-activator; TRP: transient receptor potential channels; XPORT: exit protein of TRP and Rh1; Rh1: rhodopsin; HDAC6: histone deacetylase 6; KDAC8: lysine deacetylase 8; TRAF6: tumor necrosis factor receptor-associated factor 6; DAXX: death domain-associated protein 6; p90rsk: p90 ribosomal S6 kinase; IF: intermediate filaments;

(continued)

**Table 2.2** (continued)

Factor XIII: transglutaminase, platelet Factor XIII; PhK: rabbit skeletal muscle phosphorylase kinase; MK2: MAPK-activated protein kinase-2; P38: P38 MAP Kinase; PKCΔ: protein kinase CΔ; Akt: also known as protein kinase B (PKB); TAK1: TGF-β activated kinase 1; p27kip1: cyclin-dependent kinase inhibitor p27kip1; DMPK: myotonic dystrophy protein kinase; PTEN: phosphatase and TENsin homolog; PEA-15: astrocytic phosphoprotein PEA-15; HDM2: human double minute 2; Bax: Bcl-2-associated X protein; Bag3: Bcl-2 associated athanogene 3; CIAPIN1: Anamorsin, a cytokine-induced inhibitor of apoptosis; ASK1: Apoptosis signaling kinase 1; Ubc6: ubiquitin conjugating enzyme E2 6; Ubc9: SUMO conjugating enzyme E2 9; GFAP: glial fibrillary acidic protein; GATA-1: globin transcription factor 1; HSF-1: heat shock factor 1; NF-κB: nuclear factor kappaB; IKKα and IKKβ: I kappa B kinase alpha and beta; SAP: soluble amebic proteins that suppress NF-κB activation through the binding of HspB1 to IKKα and IKKβ; STAT-2 and -3: signal transducer and activator of transcription 2 and 3; Fbx4: Fbox only protein 4; eIF4E, eukaryotic translation initiation factor 4E; eIF4G: eukaryotic translation initiation factor 4G; Smad-Smurf2: Smad ubiquitination regulatory factor 2, E3 ubiquitin protein ligase 2; TIF1gamma: ubiquitious nuclear protein Transcriptional Intermediary Factor 1gamma; Snail: zinc finger protein that binds and inhibits E-cadherin promoter to induce EMT (Epithelial-to-Mesenchymal Transition); SC35: serine/arginine-rich splicing factor 2; PASS1: protein associated with small stress proteins 1; Ddx20: DEAD box protein Ddx20 (gemin3, DP103); DSTN: Destrin or actin depolymerizing factor or ADF; G6PDH: glucose 6-phosphate dehydrogenase; p66Shc: 66 kDa isoform of ShcA (Src homology 2 domain containing transforming protein 2); SAM68: c-Src kinase during mitosis. MAPs: Microtubule-associated proteins; GRIFIN: galectin-related interfiber protein; SMN: survival motor neuron protein; SOD1: Cu/Zn-superoxide dismutase; UDG: uracil DNA glycosylase; HAP1: human AP endonuclease; TCTEL1: a light chain component of cytoplasmic and flagellar dynein; EFTUD2: U5-116KD, Snu114, Snrp116, elongation factor Tu GTP-binding domain-containing protein 2; PRKD1: Serine/threonine-protein kinase D1; PPM1A: protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform; ECs: endothelial cells; MIP26/AQP0: major lens fiber membrane protein; RPE: retinal pigment epithelial cells

### *2.1.2 Enormous Cellular Implications Associated with Constitutively Expressed Small Hsps*

Mammalian small Hsps are expressed in the absence of apparent stress in specific tissues of developing and adult organisms as well as in pathological conditions (Arrigo 2012b; Bhat and Nagineni 1989; Gernold et al. 1993; Huang et al. 2007; Klemenz et al. 1993; Mymrikov et al. 2011; Quraishe et al. 2008; Srinivasan et al. 1992; Tanguay et al. 1993). For example, HspB1 and HspB6 are highly abundant in muscles. However, the overall tissue distribution of these two proteins is different since HspB6 is specific to muscles (Seit-Nebi and Gusev 2010) while HspB1 is expressed in almost all tissues. Similarly, HspB5, which forms with HspB4 the lens alpha-crystallin complex is also expressed in the heart, skeletal muscle fibers, brain and kidney while HspB4 is also present in pancreas. In contrast, HspB9 and HspB10 are restricted to testis expression (de Wit et al. 2004; Yang et al. 2012). Other important points concern the expression of these proteins in pathological conditions as well as the drastic effects (neuropathies, myopathies, cardiomyopathies, cataracts) induced by some of their mutations (i.e. mutations in HspB1, HspB3, HspB4, HspB5, HspB6 and HspB8) (Benndorf et al. 2014; Kwok et al. 2011; Mymrikov et al. 2011; Vicart et al. 1998). So, what is the function of these Hsps in specific tissues? (see Sect. 2.1.2.1).

#### 2.1.2.1 Small Hsps Client Concept

The recent literature is quite abundant in descriptions of new functions associated with constitutively expressed small Hsps. Moreover, each small Hsp appears to have its own panel of activities (Fig. 2.1). An intriguing point is the unrelated nature of those activities distributed in almost all essential cellular pathways or activities, from cytoskeleton homeostasis to signal transduction pathways, gene expression and cell death (see Fig. 2.1). To understand why so many activities are associated with small Hsps, we must first explain their particular structural organization. Indeed, these proteins share, as a result of their crystallin homology, complex oligomeric structures that allow for the formation of dynamic homo and hetero-oligomeric structures (from 50 to >700 kDa, depending on the small Hsps) (Arrigo 2007a; 2011, Arrigo et al. 1988; Basha et al. 2011; Garrido 2002; Simon et al. 2013). Moreover, phosphorylation plays a key role in the case of HspB1, HspB5 and HspB4. These Hsps bear several serine sites phosphorylated by specific kinases, including stress and MAP kinases. Another key parameter is the cellular environment that modulates, in a dynamic and reversible way, the oligomeric organization and phosphorylation of some of these proteins, such as HspB1 (Arrigo et al. 1988; Arrigo 2000, 2007b, 2011; Arrigo and Gibert 2012; Bruey et al. 2000b; Mehlen and Arrigo 1994; Mehlen et al. 1997a; Paul et al. 2010). This suggests an intracellular sensor activity associated with small Hsps that can record changes in cellular environment. For example, HspB1 reorganizes differently its phosphorylation and oligomerization status in cells exposed to different apoptotic inducers (Paul et al. 2010). What could this mean? Since HspB1 is an anti-apoptotic protein its structural changes could instruct the cell

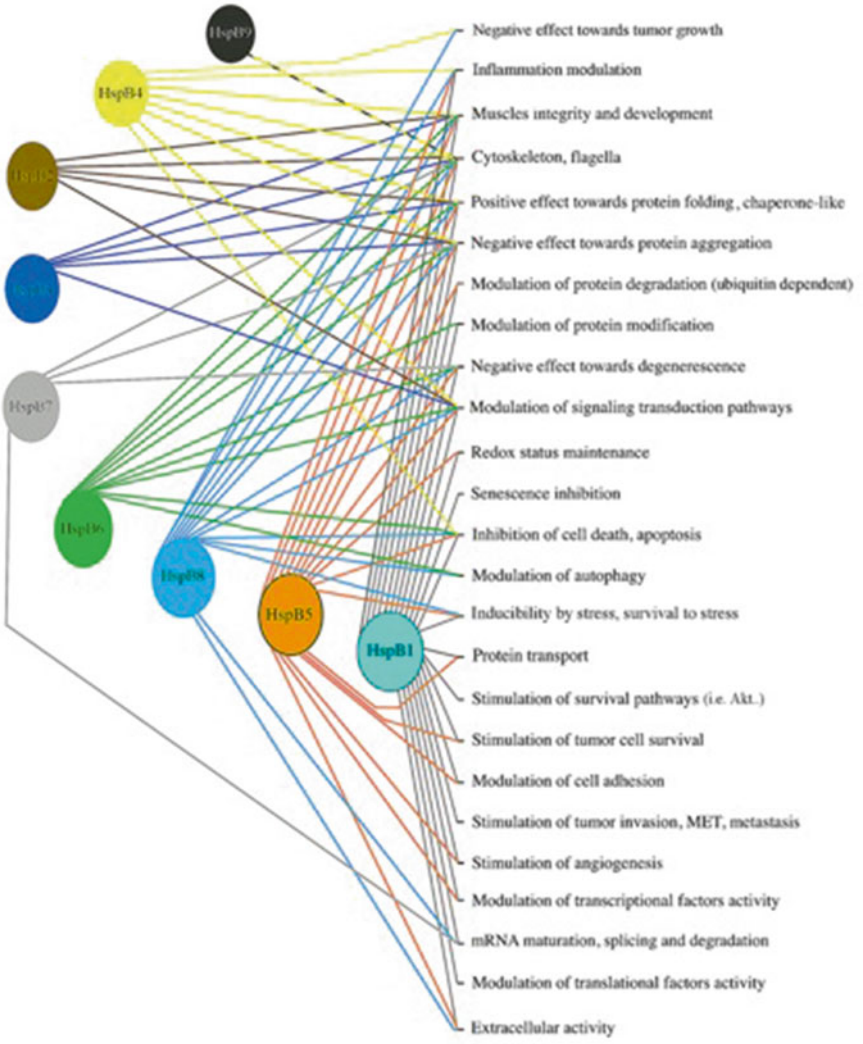


**Fig. 2.1** Large spectrum of cellular activities associated with mammalian small Hsps. The functional activities of the different members of the small Hsps family are presented in a cartoon where each Hsp is characterized by a specific color

to choose the best strategy to counteract the effects of a particular apoptotic inducer. How can this be done? Do small Hsps have multiple enzymatic activities because of their complex oligomeric organization, and are they thus pleotropic polypeptides, or are they acting via chaperone-like activities towards other polypeptides? Recently published reports revealed that the novel activities of small Hsps often correlate with their ability to interact with different polypeptides. Hence, could the apparent pleotropic effects of small Hsps be indirect and, as previously described for Hsp90

(Georgakis and Younes 2005; Neckers et al. 1999), result from the modulation of the activity and/or half-life of many clients? (list of Hsp90 clients: http://www.picard.ch/downloads). To clarify this point, we analyzed three polypeptides pro-caspase-3, HDAC6 and STAT-2 interacting with HspB1 in HeLa cells and discovered that their half-life was greatly enhanced by interacting with HspB1 (Gibert et al. 2012a), which confirmed that, in the same cell, HspB1 can recognize different protein clients. The updated list of the major proteins interacting with mammalian small Hsps and the cellular consequences mediated by these interactions is presented in Table 2.2, see also (Arrigo 2013; Arrigo and Gibert 2012, 2013; Ciocca et al 2013). Clients are listed according to their activity in major cellular functions, such as transduction pathways, apoptosis, protein degradation, translation, transcription, cytoskeletal organization and homeostasis or cell adhesion. When available, information is given about the structural organization of small Hsps or their corresponding clients involved in the interactions. The little information already available confirms the important role played by the oligomerization and phosphorylation patterns of small Hsps. Several consequences can result from small Hsps/clients interactions, such as modulation of half-life, enzymatic activity, structural organization or modification of the client. For example, some clients interact with HspB1 to increase their half-life and thus avoid their rapid proteolytic degradation (Her2 oncogene, pro-caspase 3, HDM2, the histone deacetylase HDAC6, Androgen Receptor AR and the transcription factors STAT-2 and STAT-3) while the opposite effect occurs for the rapidly degraded PTEN polypeptide when it is bound to HspB1. The transcription factor HSF1 is sumoylated as a result of its interaction with HspB1 coupled to the Ubc-9 like sumoylating enzyme UBE21. Moreover, some cellular effects mediated by small Hsps are well known but the targeted proteins are still not defined. One striking example is the modulation of the TAK-1 inflammation pathway by HspB8 (see Table 2.2).

Two major questions arise from these observations: (i) what are the cellular consequences induced by the interaction of small Hsps to so many protein targets and (ii) how do small Hsps recognize client protein targets?

(i) Concerning the first question one can easily conclude by analyzing Table 2.2 that small Hsps modulate the maturation and activity of a wide range of client proteins including regulators of the life and death of the cell and signal transducer polypeptides, such as kinases and transcription factors. Therefore, by regulating a large repertoire of cellular functions small Hsps have a huge importance on normal biology, disease and evolutionary processes. Hence, as does Hsp90 (McClellan et al. 2007; Moulick et al. 2011; Taipale et al. 2010), these Hsps appear as global regulators of cell systems through their chaperone/client interactome systems. However, it is difficult to obtain a realistic view of the global cellular consequences generated by small Hsps interactomes. To meet this challenge we have performed protein interaction networks analysis using the geneMANIA software and database (Warde-Farley et al. 2010) (http://www.genemania.org/). This web interface shows the relationships between gene products and predicts their functional association in biological processes, pathways or diseases. Such data can help elucidate cellular pathways, create functional links between gene products and diseases, and can enable investigators to extract significantly more

information about the cellular impact generated by the expression of small Hsps than by relying solely on primary literature (Table 2.2). However, care must be taken when using these data since some interactions are only predicted. An example presented in Fig. 2.2 illustrates the proteins interacting with HspB1, HspB5, HspB6 and HspB8. Only 100 proteins interacting with the four Hsps are analyzed, so some clients mentioned in Table 2.2 are not listed while new ones are mentioned. Nevertheless, this analysis further confirms that small Hsps interact with a wide spectrum of polypeptides and consequently modulate many different cellular pathways, as for example those dealing with protein kinases, gene expression, cell adhesion and migration, cell death, catabolic processes, responses to stimulation, confirming their broad implications in cell biology.

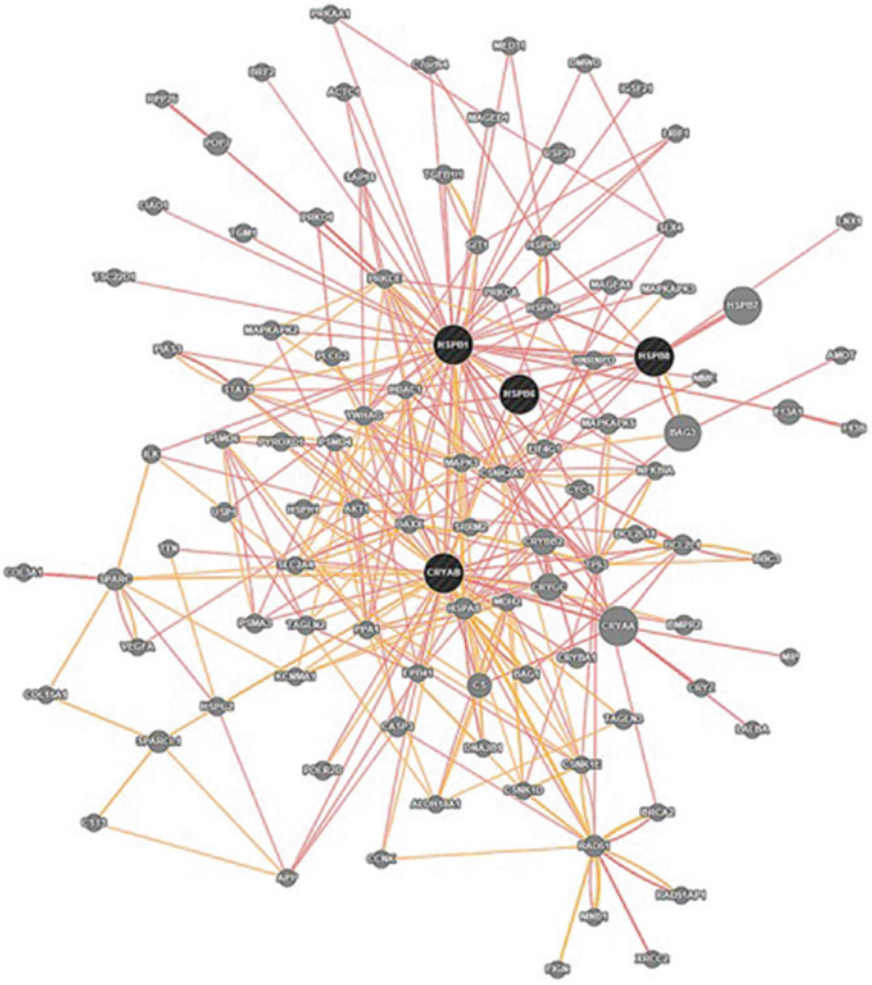


**Fig. 2.2** Human HspB1, HspB5, HspB6 and HspB8 protein interactomes and predictomes as proposed by GeneMANIA software and database including BioGRID and PathwayCommons.

**Fig. 2.2** (continued) Analyzed Hsps are indicated in *black* while interacting proteins are in *grey*. Physical interactions (*red lines*) and predicted (*orange lines*) ones were analyzed. The software was set to analyze up to hundred gene products and at most hundred related attributes. Automatically selected weighting method. Predicted interactions could be for instance, two proteins known to interact in another organism, such as S. cerevisiae. Abbreviations: *CRYAB* HspB5, *CRYAA* HspB4, *HSPA8* heat shock 70 kDa protein 8, *HSPH1* heat shock 105 kDa/110 kDa protein 1, *DNAJB1* DnaJ (Hsp40) homolog, subfamily B, member 1, *CRYGC* crystallin, gamma C, *CRYBB2* crystallin, beta B2, *CRYZ* crystallin, zeta (quinone reductase), *F13A1* coagulation factor XIII, A1 polypeptide, *BAG3* BCL2-associated athanogene 3, *CS* citrate synthase, *POP7* processing of precursor 7, ribonuclease P/MRP subunit (S. cerevisiae), *STAT-3* signal transducer and activator of transcription 3 (acute-phase response factor), *SPARCL1* SPARC-like 1 (hevin), *RAD51* RAD51 homolog (S. cerevisiae), *SPARC* secreted protein, acidic, cysteine-rich (osteonectin), *USP38* ubiquitin specific peptidase 38, *BCL2L1* BCL2-like 1, *MAPKAPK5* mitogen-activated protein kinase-activated protein kinase 5, *CRYBA1* crystallin, beta A1, *TAGLN3* transgelin 3, *CASP3* caspase 3, apoptosis-related cysteine peptidase, *BMPR2* bone morphogenetic protein receptor, type II (serine/threonine kinase), *CYCS* cytochrome c, somatic, *MAPKAPK2* mitogen-activated protein kinase-activated protein kinase 2, *TGFB1I1* transforming growth factor beta 1 induced transcript 1, *YWHAG* tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide, *PSMA3* proteasome subunit, alpha type, 3, *MAPKAPK3* mitogen-activated protein kinase-activated protein kinase 3, *POLR2D* polymerase (RNA) II (DNA directed) polypeptide D, *TAGLN2* transgelin 2, *PLCG2* phospholipase C, gamma 2 (phosphatidylinositol-specific), *PYROXD1* pyridine nucleotide-disulphide oxidoreductase domain 1, *TGM1* transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase), *USP1* ubiquitin specific peptidase 1, *EIF4G1* eukaryotic translation initiation factor 4 gamma, 1, *HNRNPD* heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37 kDa), *PRKCE* protein kinase C, epsilon, *HSPG2* heparan sulfate proteoglycan 2, *PRKAA1* protein kinase, AMP-activated, alpha 1 catalytic subunit, *DMWD* dystrophia myotonica, WD repeat containing, *PRKD1* protein kinase D1, *ILK* integrin-linked kinase; *MAGED1* melanoma antigen family D, 1, *SAP18* Sin3A-associated protein, 18 kDa, *GIT1* G protein-coupled receptor kinase interacting ArfGAP 1, *MAPK3* mitogen-activated protein kinase 3, *MAGEA6* melanoma antigen family A, 6, *BRF2* BRF2, subunit of RNA polymerase III transcription initiation factor, BRF1-like, *CCNK* cyclin K, *IGSF21* immunoglobin superfamily, member 21, *MME* membrane metallo-endopeptidase, *PSMD4* proteasome 26S subunit, non-ATPase, 4, *PSMD6* proteasome 26S subunit, non-ATPase, 6, *TTN* titin, *CIAO1* cytosolic iron-sulfur protein assembly 1, *DAXX* death-domain associated protein, *EPB41* erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked), *PPA1* pyrophosphatase (inorganic) 1, *ACTC1* actin, alpha, cardiac muscle 1. *AKT1* v-akt murine thymoma viral oncogene homolog 1, *KCNMA1* potassium large conductance calcium-activated channel, subfamily M, alpha member 1, *LNX1* ligand of numb-protein X, *MED31* mediator complex subunit 31, *C7orf64* chromosome 7 open reading frame 64, *NFKBIA* nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, *SLC2A4* solute carrier family 2 (facilitated glucose transporter) member 4, *TP53* tumor protein p53, *TSC22D1* TSC22 domain family, member 1, *ALDH18A1* aldehyde dehydrogenase 18 family, member A1, *AMOT* angiomotin, *APP* amyloid beta (A4) precursor protein, *BAG1* BCL2-associated athanogene, *BBC3* BCL2 binding component 3, *BCL2L11* BCL2-like 11 (apoptosis facilitator), *BRCA2* breast cancer 2, early onset, *COL15A1* collagen, type XV, alpha 1, *COL3A1* collagen, type III, alpha 1, *CSNK1D* casein kinase 1, delta, *CSNK1E* casein kinase 1,epsilon, *CSNK2A1* casein kinase 2, alpha 1 polypeptide, *CST3* cystatin C, *F13B* coagulation factor XIII, B polypeptide, *FIGN* fidgetin, *HDAC1* histone deacetylase 1, *LALBA* alpha-lactalbumin, *LRIF1* ligand dependent nuclear receptor interacting factor 1, *MDH2* malate dehydrogenase 2, NAD (mitochondrial), *MIP* major intrinsic protein of lens fiber, *MND1* meiotic nuclear divisions 1 homolog (S. cerevisiae), *PIAS3* protein inhibitor of activated STAT-3, *PRKCA* protein kinase C, alpha RAD51AP1: RAD51 associated protein 1, *RPP25* ribonuclease P/MRP 25 kDa subunit, *SLX4* SLX4 structure-specific endonuclease subunit homolog (S. cerevisiae), *SRRM2* serine/arginine repetitive matrix 2, *VEGFA* vascular endothelial growth factor A

(ii) As for the second question, we believe that small Hsps act as does Hsp90 to recognize clients by taking advantage of a variety of conformational states to interact with co-chaperones and clients (Hessling et al. 2009; Mickler et al. 2009). Compared to other mammalian small Hsps, HspB1 has the most dynamic phospho-oligomeric organization, a property that could explain its ability to recognize a large number of protein clients probably through the rapid generation of interacting platforms (Arrigo and Gibert 2012, 2013; Ciocca et al. 2013; Gibert et al. 2011, 2012a; Paul et al. 2010). Consequently, HspB1 dynamic interactome may allow cells to respond quickly and mount the most effective response to a particular condition. However, an unanswered question is how small Hsps generate specific interacting platforms to act on client repertoire. At least in the case of HspB1, the phenomenon may depend on the complex patterns of MAPKAPK2,3-dependent phosphorylation of three serines sites located in the N-terminal domain of HspB1 (Arrigo and Gibert 2012, 2013; Paul et al. 2010; Rouse et al. 1994; Simon et al. 2013; Stokoe et al. 1992). Our recent observations favor this hypothesis since in growing HeLa cells pro-caspase-3 interacts mainly with the serine 15 phosphorylated small oligomers of HspB1 while HDAC6 is recovered at the level of the large serine 82 phosphorylated oligomers. In contrast, STAT-2 binds to the medium and large sized HspB1 oligomers (Arrigo and Gibert 2013; Gibert et al. 2012a). Thus, in growing HeLa cells, the specific phospho-oligomeric organization of HspB1 consists of signaling structures that recognize and bind at least three different polypeptides and subsequently modulate their half-life. This observation confirms the hypothesis that the dynamic structural plasticity of small Hsps structure can lead to at least 300 different stoichiometries that favor the recognition of many particular target proteins (Stengel et al. 2010).

An increased complexity arises by taking into account another fundamental property of small Hsps. Once they are expressed in the same cells, they have the ability to interact with each other and form multiple combinatorial oligomeric structures (Table 2.3, see also Arrigo 2013; Bukach et al. 2009; den Engelsman et al. 2009 #3479; Saha and Das 2004; Simon et al. 2007; Zantema et al. 1992). Since interaction between two small Hsps mutually affects the structure and chaperone activity of both partners (Aquilina et al. 2013; Bukach et al. 2009; den Engelsman et al. 2009; Gibert et al. 2013; Mymrikov et al. 2012; Simon et al. 2013; Skouri-Panet et al. 2012), it cannot be excluded that the chimeric oligomers can recognize novel client proteins and/or are unable to bind those interacting with parental small Hsps. Moreover, not all sHsps interact equally efficiently with each other in vitro (Mymrikov et al. 2012). In that respect, the molecular ratio between small Hsp partners is often different (e.g. 3:1 in the case of HspB4:HspB5 and HspB2:HspB3 complexes). In vivo, the phenomenon is probably even more complex since modifications, such as phosphorylation, which depend on the type of cell considered and its physiology are of prime importance (Paul et al. 2010; Simon et al. 2013). For example, in cells expressing an equimolar ratio of HspB1 and HspB5, only 90 % of

**Table 2.3** Chimeric hetero-oligomeric HspB complexes

| Client | HspB | Resulting effects | Interacting structure/sequence | Observations made | | References |
|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | |
| HspB1 | HspB5 | HspB1 chaperoning | 400–800 kDa hetero-oligomers | X | X | Fu and Liang (2003) and Simon et al. (2013) |
| | HspB6 | ? | Hetero-oligomers formation | – | X | Bukach et al. (2009) and Mymrikov et al. (2012) |
| | HspB8 | ? | Hetero-oligomers formation | X | X | Sun et al. (2004) |
| HspB2 | HspB3 | Role in myogenic differentiation | 150 kDa oligomers (B2:B3 ratio 3:1) | X | X | den Engelsman et al. (2009) and Sugiyama et al. (2000) |
| HspB3 | HspB2 | Role in myogenic differentiation | 150 kDa oligomers (B2:B3 ratio 3:1) | X | X | den Engelsman et al. (2009) and Sugiyama et al. (2000) |
| | HspB8 | ? | Hetero-oligomers formation | X | X | Fontaine et al. (2005) |
| HspB4 | HspB5 | HspB4 chaperoning | Alpha-crystallin (B4:B5 ratio: 3:1) | X | X | Groenen et al. (1994), Skouri-Panet et al. (2012), Sreelakshmi and Sharma (2006), and Srinivas et al. (2008) |
| HspB5 | HspB1 | HspB5 chaperoning | 400–800 kDa hetero-oligomers | X | X | Fu and Liang (2003) and Simon et al. (2013) |
| | HspB6 | ? | Hetero-oligomers formation | – | X | Bukach et al. (2009) and Mymrikov et al. (2012) |
| HspB6 | HspB1 | ? | Hetero-oligomers formation | – | X | Mymrikov et al. (2012) |
| | HspB8 | ? | Hetero-oligomers formation | X | X | Fontaine et al. (2005) |
| HspB7 | HspB8 | ? | Hetero-oligomers formation | X | X | Sun et al. (2004) |
| HspB8 | HspB1 | ? | Hetero-oligomers formation | X | X | Sun et al. (2004) |
| | HspB2 | ? | Hetero-oligomers formation | X | X | Sun et al. (2004) |
| | HspB3 | ? | Hetero-oligomers formation | X | X | Fontaine et al. (2005) |
| | HspB6 | ? | Hetero-oligomers formation | X | X | Fontaine et al. (2005) |
| | HspB7 | ? | Hetero-oligomers formation | X | X | Sun et al. (2004) |
| | HspB5 | ? | Hetero-oligomers formation | X | X | Fontaine et al. (2005) and Mymrikov et al. (2012) |
| HspB9 | – | – | – | – | – | – |
| HspB10 | – | – | – | – | – | – |

Listed here are the interactions between small Hsps as demonstrated in vitro or in vivo using conventional biochemical approaches when these proteins are expressed in same cell

**Table 2.4** HspB chimeric hetero-oligomeric complexes: biological consequences and targeted polypeptides

| Client | HspB complex | Resulting effects | Interacting structure/sequence | Observations made | | References |
|---|---|---|---|---|---|---|
| | | | | In cells | In vitro | |
| ? | HspB2/ HspB3 | Muscle development | ? | X | – | den Engelsman et al. (2009) and Sugiyama et al. (2000) |
| Bag3 | HspB8/ HspB6 | Chaperone Bag3 activity | β4, β8 hydrophobic grooves (HspB8/B6) | X | X | Carra et al. (2008b) and Fuchs et al. (2010) |
| | | | IPV (Ile-Pro-Val) motifs (Bag3) | | | |
| G6PDH | HspB1/ HspB5 | Stimulation of activity | Interaction with hyperphosphorylated HspB1, no interaction with HspB5 | X | – | Arrigo (2013) |

Listed here are the clients that interact with small Hsps consequently of the formation of hetero-oligomeric Hsps complexes

HspB1 forms chimeric molecules with HspB5. This enhances the phosphorylation of the remaining 10 % of non interacting HspB1 which can now recognize a new client, G6PDH, and can stimulate its detoxicant enzymatic activity (Table 2.4) (Arrigo 2013). Unfortunately, no clear data are yet available concerning the protein targets recognized by chimeric small Hsps (Table 2.4), as for example in the case of HspB2:HspB3 complex involved in the development of muscle cells. Similarly, it is not known whether Bag3, which interacts with HspB8 and HspB6, can bind to HspB8:HspB6 complex to modulate autophagy. Another important consequence of the above mentioned property of small Hsps is the dominant effect of a mutated small Hsp that can dramatically spread between other interacting members of the family (Diaz-Latoud et al. 2005; Fontaine et al. 2006; Simon et al. 2013). These pathological interactions can also lead to the accumulation of cytoplasmic protein aggregates linked to diseases.

## 2.2 Examples Illustrating the Broad Spectrum of Positive or Negative Roles of Small Hsps in Human Pathologies

Nowadays, the medical literature is filled with reports explaining that the level of expression of small Hsps is highly modulated, as they are often upregulated in pathological conditions such as protein conformational disorders (neurodegenerative diseases, myopathies, cataracts), inflammatory diseases and cancers. Many functions were attributed to HspB1 and HspB5 and, probably due to their more recent discovery, less frequently to the other small Hsps. As mentioned above, these

proteins probably act by interacting with pathology specific clients. Based on earlier observations, we proposed that the upregulation of these proteins had a negative effect (for the patient) in cancer pathologies while it was positive in the case of degenerative diseases (Arrigo and Simon 2010; Arrigo 2005; Arrigo et al. 2007). The most recent studies have complicated this hypothesis since, as described below (Sect. 2.2.3), one small Hsp can be beneficial in one type of cancer and harmful in another. In fact, from a patient point of view, the major effects mediated by these interactions will depend on the friendly or hostile nature of the interacting clients. Thus, more work is needed to increase our knowledge of the pathology-dependent clients that interact with small Hsps, and future therapeutic interventions will have to be carefully planned to avoid dramatic off-target effects for patients.

## *2.2.1 Degenerative Diseases*

### 2.2.1.1 Protective Role of Small Hsps

Elevated levels of Hsps, such as HspB1, HspB5 and high molecular weight Hsps, are observed in cells with altered protein folding homeostasis as a result of the expression of proteins prone to aggregate or fibrillate (see Table 2.1). Hence, high levels of these Hsps are observed in cortical Lewy bodies, Alzheimer's disease plaques containing β-amyloid peptide, granules of neurones expressing polyQ mutants of Huntingtin polypeptide, Rosenthal fibers of Alexander disease, Creutzfeldt-Jakob altered neurons, neurofibrillary tangles, α-synuclein deposit associated with Parkinson's disease, SOD1 aggregates in amyotrophic lateral sclerosis, myopathy-associated inclusion body such as muscle cells expressing mutated desmin as well as in neurones from cerebral ischemia or heart cells altered by myocardial infarction or atrial fibrillation (Bruinsma et al. 2011; Brundel et al. 2008; Goldfarb et al. 2004; Muchowski 2002; Muchowski and Wacker 2005; Renkawek et al. 1994; Wyttenbach 2004; Yerbury et al. 2012). In these cells, HspB1 and HspB5 trigger a beneficial protection by reducing the formation of pathological protein aggregates (Eaton et al. 2000; Efthymiou et al. 2004; Latchman 2005; Lewis et al. 1999). Protective activity has recently been reported for other small Hsps, such as HspB2, HspB3, HspB6, HspB7, HspB8 (Bruinsma et al. 2011; Brundel et al. 2008; Carra et al. 2005, 2008a; Ke et al. 2011; Vos et al. 2010). However, these Hsps are effective in their own way in counteracting protein aggregation or fibrillation. For example HspB7, which, unlike HspB1, does not improve the refolding of heat-denatured polypeptides, is nevertheless the most efficient small Hsp in suppressing polyQ aggregation and polyQ-induced cellular toxicity (Vos et al. 2010). Taken together these observations lead to the conclusion that small Hsps are beneficial proteins that interfere with pathological processes leading to neurodegenerative, myopathic, cardiomyopathic, cataract and retinal diseases (Andley 2007; Firdaus et al. 2006a; Lee et al. 2006; Outeiro et al. 2006; Perrin et al. 2007; Wilhelmus et al. 2006a, b; Wyttenbach et al. 2002). This conclusion was further supported by

mutations which inhibit the chaperone activity of HspB1, HspB3, HspB4, HspB5, HspB6 and HspB8 and provoke pathological diseases, such as amyotrophic lateral sclerosis (ALS), axonal Charcot-Marie-Tooth disease, inherited peripheral and motor neuropathies, myofibrillar myopathies, cardiomyopathies and cataracts (Ackerley et al. 2005; Benndorf et al. 2014; Bova et al. 1999; Datskevich et al. 2012; Dierick et al. 2007; Elicker and Hutson 2007; Evgrafov et al. 2004; Kijima et al. 2005; Vicart et al. 1998). However, depending on the clients that are recognized by these Hsps, the consequences of their mutations will vary, with HspB1, HspB3, and HspB8 causing motor neuropathies, while HspB5 induces particular myopathies called αB-crystallinopathies (Benndorf et al. 2014).

#### 2.2.1.2 Oxidative Stress Generated by Aggregated Polypeptides

In addition to their anti-aggregation and fibrillation properties the fact that at least HspB1 and HspB5 can act as anti-oxidant molecules (Arrigo 1998, 2013; Arrigo et al. 2005; Chen et al. 2006; Firdaus et al. 2006a, b; Mehlen et al. 1996a; Wyttenbach et al. 2002) is of prime importance as it can counteracts some of the harmful effects induced by aggregated polypeptides. Indeed, a disregulated intracellular redox leading to permanent oxidative conditions is a common feature observed in many degenerative diseases and in cells bearing aggregated polypeptides (Bharath et al. 2002; Browne et al. 1999; Choi et al. 2005; Firdaus et al. 2006b; Fox et al. 2007; Halliwell 2001; Jenner and Olanow 1996; Tabner et al. 2001; Turnbull et al. 2003). This phenomenon is a consequence of Huntingtin, β-amyloid and α-synuclein being metal homeostasis modulating or direct iron/copper binding polypeptides (Hilditch-Maguire et al. 2000; Huang et al. 2004). Hydroxyl radical over-production through the metal-mediated alteration of the hydroxyl radical generating Fenton reaction is thus a common feature of cells containing these aggregated polypeptides (Halliwell and Gutteridge 1984; Sayre et al. 2000 #1935; Shoham and Youdim 2000). Hydroxyl radicals stimulate protein aggregation and interfere with proteasome function (Firdaus et al. 2006a, b; Janue et al. 2007; Liu et al. 2006; Wyttenbach et al. 2002). These observations lead to the conclusion that some small Hsps, as HspB5 (Bjorkdahl et al. 2008; Ousman et al. 2007), could be considered as therapeutic agents to treat degenerative diseases.

### *2.2.2 Inflammation*

HspB1 is essential for both IL-1 and TNF-induced pro-inflammatory signaling pathways leading to the expression of pro-inflammatory genes, such as cyclooxygenase-2, IL-6, and IL-8 (Alford et al. 2007). Increased cyclooxygenase-2 and IL-6 expression appears to occur through the stabilisation of their respective mRNAs as a result of the enhanced activation of the kinase downstream of p38 MAPK, MK2 by HspB1. The client(s) targeted by HspB1 to perform this task are still unknown,

but may reside at the level or more upstream of the pivotal kinase TAK1. This study also shows that in this context many signaling events depend on HspB1, such as downstream signalling by p38 MAPK, JNK and their activators (MKK-3, -4, -6, -7) and IKKβ. In that respect, it is worth noting that HspB1 can interact with the activating kinases IKKα and IKKβ of the transcription factor NF-κB (Dodd et al. 2009). Another role has been proposed for HspB1 through its association with the AUF1- and signal transduction-regulated complex, ASTRC, that regulates mRNA degradation machinery. This could lead to a mechanism that combines proinflammatory cytokine induction with monocyte adhesion and motility (Sinsimer et al. 2008). HspB5 also plays several roles in inflammation. The first one describes HspB5 as a new regulator of leukocyte recruitment, through its ability to enhance NF-κB pro-inflammatory signaling pathways and the expression of endothelial adhesion molecule during endothelial activation (Dieterich et al. 2013). No putative client has yet been described to support this activity. The second activity concerns a role for HspB5 as an extracellular protein (see Sect. 2.2.4) and deals with its ability, when added to the plasma of patients suffering of multiple sclerosis, rheumatoid arthritis, and amyloidosis as well of mice with experimental allergic encephalomyelitis, to interact with some relative apparent selectivity with at least 70 different pro-inflammatory mediators (acute phase proteins, members of the complement cascade, and coagulation factors) (Rothbard et al. 2012) (see Table 2.2). Of great interest, the presence of exogenous HspB5 decreased inflammation as a result of a reduced concentration of these mediators. Using a similar approach, another study points to the activation of an immune-regulatory macrophage response and inhibition of lung inflammation using HspB5-loaded microparticles (van Noort et al. 2013). These observations, as well as that of Kurnellas et al. (2012), confirm that exogenous HspB5 could be used as an anti-inflammation therapeutic agent. HspB1 and HspB5 also have beneficial protective roles against inflammation since their anti-oxidant properties may favor their interference with tumor necrosis factor (TNFα) signaling pathways, as observed in the case of asthma (Alford et al. 2007; Kammanadiminti and Chadee 2006; Mehlen et al. 1995; Merendino et al. 2002). Taken together, these observations suggest crucial, but different, roles for HspB1 and HspB5 in inflammatory processes.

### *2.2.3 Cancers*

Multiple molecular alterations are key characteristics of most cancer cells. However, an overall view of the major proteins involved in oncogenic signaling pathways is currently beyond reach. In that respect, small Hsps are among the proteins whose expression is altered in cancer cells. It is now well recognized that they have key roles in cancer biology as a result of their interaction with specific clients that modulate tumor development through their activity at the level of apoptosis, mitotic signaling pathways, angiogenesis, cell escape and survival, senescence, epithelial-to-mesenchymal transition (EMT) and metastasis (Arrigo and Gibert 2014).

In recent years, the major small Hsps reported to play important roles in cancer pathologies were HspB1 and HspB5 (Arrigo 2007a; Arrigo and Simon 2010; Arrigo and Gibert 2014; Arrigo et al. 2007; Calderwood et al. 2006; Ciocca and Calderwood 2005). Recent observations now include HspB4, HspB6 and HspB8 as well as the intriguing dual pro- and anti-tumorigenic properties of some small Hsps.

#### 2.2.3.1 Pro-tumorigenic Effects of Small Hsps

Elevated levels of expression of HspB1 and HspB5 were the first indicators of the putative role of small Hsps in some cancer cells. It was first discovered that a high level of expression of these proteins protects against apoptotic death (Mehlen et al. 1996b) and is pro-tumorigenic (Garrido et al. 1998). Recent studies have analyzed their mode of action favoring tumor development.

##### Protection Against Cell Death, Apoptosis

Protection against apoptotic cell death by HspB1 was discovered in 1996 (Mehlen et al. 1996b, 1997b; Samali and Cotter 1996). This property suggested that the high level of expression of HspB1 observed in many cancer cells could promote carcinogenesis, tumor maintenance and dissemination, an assumption demonstrated two years later (Garrido et al. 1998). HspB1 anti-apoptotic property is a consequence of its interaction with many client proteins in the initiation and execution phases of apoptosis (Arrigo 2012a; Arrigo and Gibert 2014; Ciocca et al. 2013). In fact, based on the signal transduction-dependent dynamic reorganization of its phosphorylation and oligomerization status (Paul et al. 2010; Rogalla et al. 1999), HspB1 can interact with the more appropriate clients to counteract apoptotic processes. This leads to the hypothesis that HspB1 has multiple strategies to counteract inducer-specific intrinsic and extrinsic apoptosis (Arrigo 2011; Paul et al. 2010). For example, by acting towards F-actin and t-Bid translocation, HspB1 reduces cytochrome c (Paul et al. 2002) and Smac-diablo (Chauhan et al. 2003) release from mitochondria. In addition, it also decreases apoptosome and caspase-9 activation by a direct interaction with cytosolic cytochrome c (Bruey et al. 2000a; Garrido et al. 1999). A surprising effect occurs at the level of procaspase-3 whose activation is negatively regulated by phosphorylated small oligomers of HspB1 (Arrigo and Gibert 2013; Gibert et al. 2012a; Pandey et al. 2000). In the meantime, HspB1 increases procaspase-3 half-life by down-regulating its degradation by the ubiquitin-proteasome machinery (Gibert et al. 2012a). Among the death receptor pathways that are under the control of HspB1 are Fas, TNFα and TRAIL (Mehlen et al. 1995, 1996b; Zhuang et al. 2009). In the Fas signal transduction mechanism, phosphorylated dimers of HspB1 abolished the link between activated Fas receptor and apoptotic signaling kinase1 (Ask1) by interacting with DAXX (Charette et al. 2000). The protection against TNFα mediated transduction death signal is less well documented. Nevertheless, HspB1 may protect cells directly through the classical apoptotic machinery and/or its ability to interfere with the oxidative stress generated by this inflammatory

cytokine (Mehlen et al. 1995, 1996a). In contrast (see below section "Stimulation of cell survival pathways, senescence"), the inhibitory effect of HspB1 against TRAIL induced death does not appear to occur at the level of the apoptotic machinery but rather through the stimulation a cell survival mechanism (Qi et al. 2014).

HspB5 and HspB4 have also been reported as anti-apoptotic proteins (Andley et al. 2000; Kamradt et al. 2005) and several reports mention their action towards tumorigenicity (Arrigo 2007a; Chen et al. 2012; Kase et al. 2009; Mahon et al. 1987; Rigas et al. 2009). Their anti-apoptotic modes of action differ from that of HspB1, however. Indeed, in addition to their action towards caspase-3, these Hsps negatively regulate members of the Bcl-2 family, Bcl-$X_L$, Bcl-XS and Bax, as well as cytoplasmic p53 by interfering with their redistribution into mitochondria in apoptotic conditions (Hu et al. 2012; Liu et al. 2007; Mao et al. 2004). HspB5 was also shown to modulate p53 level (Watanabe et al. 2009). Moreover, both HspB4 and HspB5 can prevent apoptosis through interactions with clients involved in regulating signaling Raf/MEK/ERK and PKCαlpha pathways (Liu et al. 2004). Moreover, HspB5 modulates the activity of XIAP, an endogenous inhibitor of caspases (Lee et al. 2012), and inhibits RAS activation responsive to the calcium-activated Raf/MEK/ERK signaling pathway mediated p53-dependent apoptosis (Li et al. 2005). HspB5 expression can also be correlated with pERK1/2 expression (van de Schootbrugge et al. 2013b). However, it is important to note that these particular properties are usually tissue specific; for example, in pancreatic cancer cells HspB4 has a surprising opposite effect and acts as a negative regulator of carcinogenesis (Deng et al. 2010) (see below section "Anti-tumorigenic Effects"). HspB5 also protects retinal pigment epithelial cells through its association with HDAC1 on SC35 speckles (Noh et al. 2008), which suggests that HspB5 knockout could be beneficial to vitreoretinopathy therapy.

It is also interesting to note that 14-3-3 polypeptide is a client of phosphorylated HspB6. Hence, this Hsp can compete with the large number of regulator proteins interacting with 14-3-3 and indirectly modulate many cellular processes, such as those involved in actin cytoskeleton reorganization or Bad mediated apoptosis (Chernik et al. 2007; Seit-Nebi and Gusev 2010; Sluchanko et al. 2011; Zha et al. 1997).

### Stimulation of Cell Survival Pathways, Senescence

HspB1 still appears as being the major small Hsp involved in the stimulation of cell survival pathways through its interaction with specific clients. Among those pathways, the Akt signaling cascade is a major one which includes key factors such as Akt, PI3K, PTEN, mitogen-activated protein kinase kinase-3,6, BAD and Forkhead transcription factors. In cancer cells, high expression levels of HspB1 result in its interaction with Akt and PTEN. HspB1 action towards Akt kinase activity and the stimulation of the degradation of the phosphatase PTEN stimulate the PI3K/Akt signaling pathway and thus enhance the survival of these pathological cells (Cayado-Gutierrez et al. 2012; Rane et al. 2003; Wu et al. 2007). An interesting survival pathway also modulated by HspB1 is the PEA-15 molecular switch linking cell proliferation to Fas-induced apoptosis. In that regard, the interaction of HspB1 with PEA-15 inhibits

Fas-induced apoptosis and promotes cell survival and proliferation (Hayashi et al. 2012). Another example concerns the Src-Akt/ERK pro-survival signaling transduction triggered by TRAIL death receptor. Analysis of the molecular mechanism revealed that phosphorylated HspB1 activates the pathway by interacting with Src and by scaffolding protein beta-arrestin2 (Qi et al. 2014). The signaling complex made of phospho-HspB1/beta-arrestin2/Src appears therefore to be responsible for activating the TRAIL-triggered Src-Akt/ERK pro-survival pathway. HspB1 also appears to act in signaling pathways promoting survival of gliomas, but the molecular mechanism is not yet known (Golembieski et al. 2008; McClung et al. 2012).

In addition to improving cell survival, HspB1 has a p53 dependent negative action towards the oncogene-induced senescence (OIS) pathway which normally blocks cancer progression (O'Callaghan-Sunol et al. 2007). Indeed, HspB1 depletion usually induces a senescent-like phenotype in cancer cells. Among the morphological changes that were observed one can note a drastic reduction in the mitotic index through induction of p21waf expression (O'Callaghan-Sunol et al. 2007) and a particular cellular multi-nucleation which appears to be the result of the degradation of HDAC6 (Gibert et al. 2012a), an HspB1 client acting as a powerful contributor to oncogenic pathways activation (Lee et al. 2008). HDAC6 is proteolytically stabilized by HspB1 serine 82 phosphorylated oligomers (Arrigo and Gibert 2013; Gibert et al. 2012a). Among the other clients and/or pathways effective in supporting the negative effect of HspB1 towards senescence are the p53 stabilizator HDM2, an ubiquitin ligase (E3) that targets p53 for degradation (O'Callaghan-Sunol et al. 2007; Yang et al. 2005) and the PI3K/AKT induced OIS (Ghosh et al. 2013).

### Cell Escape, Epithelial-to-Mesenchymal Transition (EMT), Metastasis

In addition to counteracting cell death and promoting cell survival pathways, HspB1 and HspB5 have been shown to bear tumorigenic (Garrido et al. 1998, 2006) and pro-metastatic (Bausero et al. 2006; Lemieux et al. 1997; Nagaraja et al. 2012b) properties. In that regard, several clients interacting with these proteins have been identified (Arrigo and Gibert 2014) that are particularly active at the level of the cytoskeleton and extracellular matrix (Arrigo and Gibert 2013; Gibert et al. 2012a; Lavoie et al. 1993; Mounier and Arrigo 2002; Perng et al. 1999; Wettstein et al. 2012; Xi et al. 2006). For example, in cancer cells, HspB1 is necessary for F-actin mediated cytokinesis and interferes with the accumulation of giant polynucleated cells (Gibert et al. 2012a). Another important client interacting with both HspB1 and HspB5 is β-catenin (Fanelli et al. 2008; Ghosh et al. 2007c) and the resulting effect is a modulation of cadherin-catenin cell adhesion proteins (Fanelli et al. 2008). At least in the case of HspB1, the interaction plays a crucial role in promoting tumor growth. Among the other clients of HspB1, one can cite several metalloproteinases (Bausero et al. 2006; Xu et al. 2006) as well as SPARC (secreted protein, acidic and rich in cysteine), a polypeptide that plays an important role in cell adhesion and migration (Golembieski et al. 2008; McClung et al. 2012; Schultz et al. 2012). In several cancer pathologies, HspB5 also promotes cell migration and

invasion. For example, HspB5 induces the EGF- and anchorage-independent growth of human breast basal-like tumors through the constitutive activation of the MAPK kinase/ERK (MEK/ERK) pathway and transforms immortalized human mammary epithelial cells in invasive mammary carcinomas that have the same aspect as basal-like breast tumors (Gruvberger-Saal and Parsons 2006; Moyano et al. 2006). At least in the kidney, HspB5 can participate in maintaining tissue integrity by interacting with Ksp-cadherin-16 and promoting its connection to the cytoskeleton (Thedieck et al. 2008).

HspB1 is still the major small Hsp that stimulates metastasis (Bausero et al. 2004, 2006; Gibert et al. 2012b; Nagaraja et al. 2012a, b). Epithelial-to-mesenchymal transition (EMT) is the major parameter controlling metastasis that appears under the control of HspB1 (Shiota et al. 2013; Wei et al. 2011). Indeed, HspB1 modulates the expression of pro-metastatic genes (Nagaraja et al. 2012b), such as those dependent on STAT3/Twist signaling by enhancing the binding of the transcription factor STAT3 to the promoter of the Twist gene (Shiota et al. 2013). This transcriptional event generates two hallmarks of EMT: N-cadherin up-regulation and E-cadherin downregulation. It is therefore possible that the interaction of HspB1 with phosphorylated and activated STAT3 could be one of the key events regulating this phenomenon (Gibert et al. 2012a). HspB1 also binds to and stabilizes the transcription factor Snail, and consequently induces EMT features (Wettstein et al. 2013). The phenomenon probably occurs via a Snail-induced transcriptional blockage of E-cadherin gene expression (Batlle et al. 2000). E-cadherin downregulation is necessary to trigger epithelial-to-mesenchymal transition and acquisition of metastatic potential at late stages of epithelial tumour progression. Concerning HspB5, a recent study mentions that its expression is associated with distant metastases formation in head and neck squamous cell carcinoma, a link that might relate to the chaperone function of HspB5 in mediating folding and secretion of VEGF and stimulating cell migration (van de Schootbrugge et al. 2013a). Thus, among the different small Hsps, at least HspB1 and HspB5 are considered as potent stimulators of tumor progression. However, we should be cautious before coming to a general conclusion on this topic, since, as indicated below (Sect. 2.2.3.2), in some tumors these Hsps have been recently shown to have an anti-tumor activity that counteracts tumor development.

### Angiogenesis

Do small Hsps participate in the process triggering the excessive formation of blood vessels that irrigate cancer cells? Until recently, no answer could be given to this question since no data supported such a pro-angiogenic hypothesis. However, recent game-changing reports have clearly demonstrated that small Hsps indeed play a role in this process. First, it was shown that, in addition to their intracellular distribution, small Hsps can also be localized in plasma membrane and can be exported in the extracellular milieu (Chowdary et al. 2006; Rayner et al. 2008; Tsvetkova et al. 2002), a phenomenon that correlates with tumor growth and metastasis formation (Bausero et al. 2004). In addition to a possible

immunological role for small Hsps, a first observation was that recombinant HspB1 added to the growth medium has a pro-angiogenic effect mediated by Toll-like receptor 3 (TLR3) at the surface of human microvascular endothelial cells (HMECs). The interaction stimulates NF-κB dependent vascular endothelial growth factor (VEGF) gene transcription and promotes secretion of VEGF-activating VEGF receptor type 2 and angiogenesis (Thuringer et al. 2013). Indeed, the production by endothelial cells of intracellular autocrine (intracrine) VEGF is critical for vasculature homeostasis. A more recent study showed that HspB1 is directly released from endothelial cells (ECs) and confirmed that it modulates angiogenesis via direct interaction with VEGF. However, these authors also showed that HspB1 can be cleaved by MMP9 (Matrix MetalloProteinase 9) and recovered as anti-angiogenic fragments which interfere with VEGF-induced ECs activation and tumor progression (Choi et al. 2014). Thus, it appears that the effect mediated by extracellular HspB1 in cancer pathologies may depend on the efficiency of its cleavage by MMP9. However, the first study used recombinant HspB1 added to culture medium, so that the cleavage activity of endogenous MMP9 could have been overwhelmed by an excess of HspB1 and thus a pro-angiogenic effect was observed. Thus, in vivo, HspB1 released from cells appears as an anti-angiogenic polypeptide. This is also supported by the fact that MMP inhibitors have failed in clinical trials, probably through their efficient knock out of HspB1 fragmentation.

Another small Hsp involved in angiogenesis is HspB5 since it is crucial for endothelial cell survival and is up regulated during vessel morphogenesis. For example, tumor vessels in HspB5 (–/–) mice showed signs of caspase-3 activation and apoptosis and tumors grown in such mice were significantly less vascularized than wild-type tumors and displayed increased areas of apoptosis/necrosis (Dimberg et al. 2008). Recently, it was shown that HspB5 is a VEGF chaperone that protects this growth factor against proteolytic degradation (Kerr and Byzova 2010; Ruan et al. 2011). HspB5 appears therefore strongly involved in the pathway maintaining intracrine VEGF signaling that sustains aberrant tumor angiogenesis (Dimberg et al. 2008; Ruan et al. 2011).

## Gene Expression

The control by HspB1 of several crucial transcription factors (among them Snail, STAT3, NF-κB and HSF1) can have dramatic consequences particularly towards apoptosis inhibition and EMT promotion. HSF1 (heat shock factor 1), the transcription factor responsible for Hsps expression, has also been shown to play a crucial role in tumorogenesis (Mendillo et al. 2012). HSF1 is SUMO-2/3 modified by HspB1-Ubc9 complex (Brunet Simioni et al. 2009). This modification does not affect HSF1 DNA-binding ability but blocks its transactivation function suggesting that it could act, together with NuRD factors, as a transcriptional inhibitor that

represses genes that oppose metastasis. Other hypotheses suggest that it could modulate energy metabolism or permit the development of polyploidy in cancer cells (Calderwood 2012; Mendillo et al. 2012).

HspB1, HspB7 and HspB8 can also favor the expression of pro-tumorigenic proteins though the control of mRNAs. Indeed, some clients of these Hsps regulate mRNA splicing, such as SAM68, Ddx20, EFTUD2 and SC35 (Badri et al. 2006; Hegele et al. 2012; Sun et al. 2010; Vos et al. 2009), while others play a role in translational initiation (eIF4G) (Andrieu et al. 2010) or mRNA stability (AUF1) (Sinsimer et al. 2008).

#### 2.2.3.2 Anti-tumorigenic Effects

In contrast to the classical view described above favoring a pro-tumorigenic activity for HspB1 and HspB5, recent observations indicate that, in some cancer types, HspB1, HspB5 and HspB4 polypeptides display intriguing tumor suppressive activities. Moreover, recent studies dealing with HspB8 and HspB6 clearly show that these polypeptides promote tumor growth resistance and decrease cell survival.

##### Tumor Suppressive Role of HspB1

As mentioned above, HspB1 released from endothelial cells (ECs) regulates angiogenesis by interacting with VEGF (vascular endothelial growth factor). However, new observations have revealed that MMP9 (matrix metalloproteinase 9) can cleave HspB1 and release anti-angiogenic fragments that inhibit lung and liver tumor progression of B16F10 melanoma cells and lung tumor progression of CT26 colon carcinoma cells. The failure of MMP inhibitors in clinical trials could then be explained by their ability to decrease HspB1 fragmentation leading to pro-tumorigenic effects (Choi et al. 2014).

##### Tumor Suppressive Role of HspB5

In the case of nasopharyngeal carcinoma (NPC), an intriguing observation was that HspB5 downregulation is significantly associated with the progression of NPC while its overexpression interferes with NPC progression-associated phenotypes such as loss of cell adhesion, invasion, interaction with the tumor microenvironment, invasive protrusion formation and expression of epithelial-mesenchymal transition-associated markers. Molecular analysis revealed that HspB5 suppresses NPC progression by interacting with the cadherin/catenin adherens junction. This indirectly decreases the levels of expression of critical downstream targets such as cyclin-D1 and c-myc (Huang et al. 2012)

## HspB4

The role of HspB4 in tumorigenesis appears rather equivocal (Deng et al. 2010). Indeed, depending of the tumor type the level of this protein is either up- or downregulated. In normal conditions, HspB4 is mainly expressed in the lens and is also detectable in the pancreas. Consequently, many of the lens tumor cells display high levels of HspB4 expression, such as those from retinoblastoma and eyelids with sebaceous carcinoma (Kase et al. 2009; Mahon et al. 1987; Rigas et al. 2009). In these cells, HspB4, like HspB5, can promote tumorigenesis since it bears an anti-apoptotic activity (Andley et al. 2000; Ciocca and Calderwood 2005) whose major property is to negatively regulate the pro-apoptotic members of the Bcl-2 family and caspase-3 (Hu et al. 2012). Contrasting with these observations, the moderate level of expression of HspB4 observed in normal human pancreas samples appears significantly reduced in many cases of pancreatic carcinoma of different types. Unfortunately, to date, the mechanism controlling HspB4 down-regulation in pancreatic carcinoma cells is not known. Another interesting point, as demonstrated by genetically forced expression of this protein, concerns the fact that, in the pancreas, HspB4 can act as a negative regulator that blocks cell transformation and retards cell migration (Deng et al. 2010). However, the mechanism by which HspB4 performs this pancreatic task is not yet solved. It may occur through a modulation of ERK MAP kinase activity regulating AP-1 expression and activity to halt cell transformation and retard cell migration (Chen et al. 2012; Deng et al. 2010). Thus, in spite of some common properties towards apoptosis, cell proliferation and tumor metastasis more work is needed to unravel the particular role of HspB4 in pancreatic carcinogenesis.

## HspB8

It has been recently shown that in a large fraction of melanoma tumors, which are aggressive and drug-resistant cancers, HspB8 gene is silenced through aberrant DNA methylation. This phenomenon modulates Aza-C (5-Aza-2′-deoxycytidine) treatment efficiency (Smith et al. 2011). The anti-tumor property of HspB8 was then identified by experiments aimed at restoring its expression. Indeed, putting HspB8 back in cells inhibited tumor growth and induced the death of genetically diverse melanoma lines as a result of the activation of TAK1 (TGF-β activated kinase 1)-dependent death pathways (Li et al. 2007; Smith et al. 2012). Among the TAK1 putative down-stream pathways that could be involved is the inflammasome independent activation of caspase-1 resulting from the upregulation of ASC (apoptosis-associated speck-like protein containing a CARD). Apoptosis could then be caused by caspase-1-mediated cleavage of Beclin-1, a polypeptide upregulated in melanoma tumors as a result of mTOR (mammalian target of rapamycin) phosphorylation.

HspB6

Recent findings have shown that, in human hepatocellular carcinoma (HCC), HspB6 expression levels are inversely correlated with the progression of HCC. The negative effect mediated by HspB6 appears to result from its interaction with PI3K (phosphoinositide 3-kinase, an upstream kinase of Akt). This interaction suppresses PI3K activity, inhibits the AKT survival pathway and subsequently decreases HCC survival and growth (Matsushima-Nishiwaki et al. 2013).

Therapeutic Thoughts About Tumor Suppressive Small Hsps in Cancer

The examples presented above clearly indicate that, in some cancer cells, small Hsps can be associated with anti-tumorigenic activity. Hence, it is intriguing to note that cancer cells can devise strategies to improve their growth and dissemination by down-regulating the expression of these polypeptides. This may open up new therapeutic options aimed at restoring or up regulating the expression or activity of these proteins. However, restoring the specific expression of transcriptionally silenced genes is quite difficult. Moreover, as in the case of HspB8, the approach can be limited by the genetic diversity of the tumors. A better way to improve therapeutic strategies would be to mimic chemically the activation performed by small Hsps, as for example towards the TAK1 pathway in the case of melanoma. Similarly, restoring HspB4 or HspB5 level of expression, up-regulating HspB6 activity towards PI3K or stimulating HspB1 cleavage by MMP9 could be a challenge. In the meantime a better understanding of the role of HspB4 towards ERK MAP kinase activity and AP-1 expression as well as of HspB6 inhibitory interaction with PI3K may help in the discovery of new drugs effective against pancreatic and hepatic cells carcinogenesis.

### *2.2.4 Extracellular Roles of Small Hsps*

Recently, a major discovery was that HspB1, HspB5 and HspB8 can localize in plasma membrane and be secreted in spite of their major intracellular localization (Chowdary et al. 2006; Rayner et al. 2008; Sreekumar et al. 2010; Tsvetkova et al. 2002). Thus, what could be the functions of these proteins at the cell surface or in the extracellular milieu? Do these circulating proteins share some of the properties of circulating Hsp70 (De Maio 2011)? For example, are they associated with immunogenic peptides which trigger an immune response (Delneste et al. 2002), or are they pro-immunosuppressive polypeptides (Chalmin et al. 2010). Are they involved in anti-inflammation, alarmone or other pathways by interacting with specific cellular receptors? Recent observations suggest that circulating HspB1 is not associated with immunogenic peptides but could have immunoregulatory activity. For

example, circulating HspB5 stimulates macrophages through its ability to recognize CD14, TLR1 and TLR2 (Toll-like receptor 1 and 2) at their surface (van Noort et al. 2013). Similarly, HspB8 and HspB4 recognize TLR4 and induce dendritic cells activation (Roelofs et al. 2006). HspB1 was also found to activate NF-κB in macrophages (Salari et al. 2012). In addition, this protein recognizes several cell surface polypeptides such as CD10 (Dall'Era et al. 2007), Plasminogen, Angiostatin (Dudani et al. 2007) and TLR3 (Thuringer et al. 2013). In 4T1 breast adenocarcinoma cells, HspB1 cell surface expression appears correlated with tumor growth and metastasis formation (Bausero et al. 2004, 2006). Moreover, the angiogenic property of HspB1 is regulated by the cleavage efficiency of MMP9 (Choi et al. 2014; Thuringer et al. 2013) (see also Sect. Angiogenesis).

A key aspect of circulating small Hsps is that they can be either beneficial or harmful to patients suffering from different pathologies. In that regard they behave like intracellular small Hsps. For example, a major positive effect of circulating HspB1 is its impressive atheroprotective effect (Rayner et al. 2008; Salari et al. 2012). On the other hand, secreted HspB1 correlates with vascular complications in type 1 diabetic patients (Gruden et al. 2008) and is not a positive signal in cancers. Consequently, major care will have to be taken in case of therapeutic approaches targeting circulating Hsps. More studies are urgently needed to evaluate the multiple roles played by these extracellular proteins in normal and pathological physiological conditions.

### *2.2.5 Conclusions*

As described here, small Hsps have immense cellular implications as a result of their interaction with many specific client polypeptides whose number is growing exponentially. Their ability to bind polypeptides and modulate their folding is a property that was originally discovered in heat shock treated cells where HspB1 was shown to interact with aberrantly folded polypeptides to prevent their aggregation. It is now well known that small Hsps can modulate folding or induce modifications in interacting clients. They also have the crucial ability to positively or negatively modulate their half-lifes. Taken together, these observations show that small Hsps can have a drastic influence on the level of expression as well as on the activity of interacting clients. Consequently, these Hsps indirectly appear to have a huge number of functions that allow cells to rest, grow or better adapt to changes in their physiology or pathological status. Moreover, by targeting specific clients, small Hsps can be protective and beneficial against cell degeneration. They can also have a disastrous effect by causing some cancer cells to proliferate and create metastasis.

The proteomic analysis presented here confirms our feeling that small Hsps, as Hsp90 (McClellan et al. 2007; Moulick et al. 2011; Taipale et al. 2010), are global regulators of cell systems that exert marked effects on normal biology and diseases through their chaperone/client interactome systems. Hence, we are now facing

problems that are even more complex than those encountered by researchers working with Hsp90. The first of these illustrates the complexity associated with small Hsps and deals with the chimeric structures that can form between two small Hsps. These structures appear to have lost the properties associated with parental homo-oligomers, but do they have specific interactomes or are they inert? The second problem is common to small Hsps and Hsp90: what is the structural dynamic that acts on a diverse client repertoire in defined cellular conditions? In the case of HspB1, phosphorylation and oligomerization appear as key factors that dynamically react and provide a recognition platform for specific clients (Arrigo and Gibert 2013; Paul et al. 2010), however nothing is known about the molecular signaling mechanisms involved in this process. Thus, more in-depth structural work, signaling studies as well as analysis of the organization of small Hsps in living cells are necessary to unravel the problem of how these chaperones recognize client polypeptides. The third problem deals with therapeutic strategies aimed at modulating the level or activity of these chaperones. In the case of Hsp90, drugs interfering with its chaperone activity and broad interaction with clients have been clinically tested. Their modest effects and unsuspected side effects resulted in lack of FDA recognition (Whitesell et al. 2012). More specific drugs targeting only a subset of Hsp90-clients may prove more useful (Moulick et al. 2011). Similarly, the use of genetic techniques to invalidate the expression of small Hsps appears efficient (Gibert et al. 2012b; Wettstein et al. 2013) but in the long term they could be disappointing because of the complete disruption of small Hsps protein interactomes. Drugs or genetic techniques altering the structure of small Hsps can lead to interesting results (Gibert et al. 2011; Heinrich et al. 2011) but will require in-depth analysis of their effects on small Hsps interactomes. More work is needed to build comprehensive dynamic interactomes of small Hsps in specific pathologies. This will be necessary in characterizing both the good and pathological clients recognized by these Hsps. The discovery of new drugs or genetic techniques that preserve their interaction with the good clients and destroy those with the ugly ones will probably have a bright future.

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