

REVIEW ARTICLE

The small heat shock proteins and their role in human disease

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Small heat shock proteins (sHSPs) function as molecular chaperones, preventing stress induced aggregation of partially denatured proteins and promoting their return to native conformations when favorable conditions pertain. Sequence similarity between sHSPs resides predominately in an internal stretch of residues termed the α -crystallin domain, a region usually flanked by two extensions. The poorly conserved N-terminal extension influences oligomer construction and chaperone activity, whereas the flexible C-terminal extension stabilizes quaternary structure and enhances protein/substrate complex solubility. sHSP polypeptides assemble into dynamic oligomers which undergo subunit exchange and they bind a wide range of cellular substrates. As molecular chaperones, the sHSPs protect protein structure and activity, thereby preventing disease, but they may contribute to cell malfunction when perturbed. For example, sHSPs prevent cataract in the mammalian lens and guard against ischemic and reperfusion injury due to heart attack and stroke. On the other hand, mutated sHSPs are implicated in diseases such as desmin-related myopathy and they have an uncertain relationship to neurological disorders including Parkinson's and Alzheimer's disease. This review explores the involvement of sHSPs in disease and their potential for therapeutic intervention.

Within the molecular chaperone family, sHSPs constitute a structurally divergent group characterized by a conserved sequence of 80–100 amino acid residues termed the α -crystallin domain [1–8]. The α -crystallin domain, duplicated in the unusual example of parasitic flatworms (Platyhelminthes) [9], is located toward a highly flexible, variable, C-terminal extension, and is usually preceded by a poorly conserved N-terminal region. The molecular mass of sHSP subunits ranges from 12 to 43 kDa, and they assemble into large, dynamic complexes up to 1 MDa. sHSP secondary structure is dominated by β -strands with limited α -helical content, and β -sheets within the α -crystallin domain mediate dimer formation. Crystallization of

two sHSPs has contributed significantly to the description of oligomerization, quaternary structure, subunit exchange, and chaperone activity. Characterization of a highly conserved arginine is also an important outcome of crystallization and related studies because mutation of this residue has profound effects on sHSP function and contributes to certain diseases [10–16].

The sHSPs are molecular chaperones, storing aggregation prone proteins as folding competent intermediates and conferring enhanced stress resistance on cells by suppressing aggregation of denaturing proteins, actions associated with oligomerization and subunit exchange [17–20]. Functional studies of the sHSPs are

Abbreviations17-AAG, 17-allylamino-17-demethoxygeldanamycin; A β , amyloid- β ; AGE, advanced glycation end-product; ALS, amyotrophic lateral sclerosis; CAT, cancer/testis antigen; GFAP, glial fibrillary acidic protein; HMM, high molecular weight; IFN- γ , interferon- γ ; MS, multiple sclerosis; sHSP, small heat shock protein; SOD, superoxide dismutase.

more limited than for other chaperones, but this is changing as the application of genomics and proteomics reveals sHSP characteristics and their medical importance emerges. In this context, 10 sHSPs, termed HspB1–10, many of which are constitutively present at high levels in muscle and implicated in disease, are found in humans [2,21–23]. Intracellular quantities and cellular localizations of sHSPs change in response to development, physiological stressors such as anoxia/hypoxia, heat and oxidation, and in relation to pathological status. sHSPs interact with many essential cell structures and it follows from such promiscuity that functional disruption and inappropriate association of these molecular chaperones with substrates will foster disease. Therefore, this review considers the role of sHSPs in several human medical conditions and it ends with a discussion of their therapeutic potential.

sHSPs and cataract

sHSP mutation and post-translational change contribute to cataract development in the mammalian lens, a transparent organ with refractive characteristics specialized to focus visible light [5,24–28]. Lens tissue derives from cells containing large amounts of densely packed proteins known as α -, β - and γ -crystallins, which function for the lifespan of an organism and are essential for vision. Lens transparency, viscosity and refractive index depend on crystallins, their interactions with one another, with membranes [13,29], and with cell components such as actin [30] and the intermediate filament proteins CP49 and filensin [31]. α -crystallins maintain lens transparency by serving interdependently as structural elements and molecular chaperones. As α -crystallin chaperoning capability declines, lens proteins are more likely to aggregate, a characteristic linking cataract to other protein folding diseases [24]. That is, amyloid fibrils arise in solutions of bovine lens α -, β - and γ -crystallins under mild denaturing conditions, as might happen upon sHSP post-translational modification, leading to aggregation in the presence of reduced chaperoning ability [32]. What is more, post-translational changes reduce crystallin solubility, contributing to less effective protein packing. The evidence strongly favors the belief that perturbation of α A- and α B-crystallin reduces lens transparency and generates cataract, the leading cause of blindness worldwide. As these aberrant processes become better understood through continued study of the α -crystallins, methods to counter cataract development are certain to emerge.

Cataract and α -crystallin post-translational changes

Posttranslational modifications of α A- and α B-crystallin, including truncation [33–37], deamidation [36, 38–42], oxidation [40,43–46], glycation [46–53], phosphorylation [33] and racemization/isomerization [54, 55], promote cataract formation in aging organisms through modification of chaperone activity and solubility [24,35,40,41,47,56]. α -crystallin post-translational changes, with a corresponding effect on lens transparency, occur during diabetes where chaperone activity decreases in reverse correlation to glucose levels [52]. Glycation, the nonenzymatic addition of sugars to proteins, is enhanced in rat and human lenses during diabetes, causing protein cross-linking and advanced glycation end-products (AGE), a change engendered by methylglyoxal interaction with lysine and arginine residues [51]. Glycation *in vitro* limits the chaperone activity of human, calf and rabbit lens α -crystallins [46,51], as does methylglyoxal treatment of calf lens in organ culture, with corresponding reduction in protein stability [48,49]. However, in other studies, glycation of C-terminal lysines does not disrupt α -crystallin chaperoning [53] and activity increases when the protein is modified *in vitro* [48,50], suggesting in contrast to prevailing theories that post-translational modifications are an aging related protective mechanism for long-lived lens proteins.

Demonstrating definitive causal relationships between sHSP post-translational modifications and function is difficult, a problem confounding the analyses of other proteins such as tubulin [57,58], but progress has been made. Truncated α -crystallin from lenses of ICR/f rats, a strain with hereditary cataract, exhibits reduced chaperone activity against heat-induced aggregation of β L-crystallin from the same source [35]. Truncated α -crystallin functional loss can be rationalized in light of sHSP N- and C-terminal region properties, and reduced chaperoning links truncation to cataract. α -crystallin deamidation involves the nonenzymatic conversion of asparagine to either aspartate or isoaspartate, and glutamine becomes glutamic acid, prevalent changes during cataract formation and aging [36]. The use of site-directed mutagenesis to generate variants N146D and N78D/N146D demonstrates deamidation significantly impacts bacterially produced human α B-crystallin, whereas the single modification N78D has little effect [38]. In comparison to wild type, oligomer size increases and chaperone activity decreases in N146D and N78D/N146D mutants, suggesting deamidation disrupts lens α B-crystallin

packing and chaperoning, thereby compounding the role of this post-translational change as a causative agent of cataract. Mutations N101D, N123D, and N101D/N123D of human α A-crystallin also reduce chaperone action and enlarge oligomers, with N101D effects greater than N123D [39]. Negative charges introduced by deamidation disturb tertiary structure, contributing to functional changes and to cataract. Site-directed mutagenesis was employed to examine oxidation of α A-crystallin, a protein with two cysteine residues [44] and where intrapolypeptide disulfides [45] and mixed glutathione disulfides [59] curtail chaperone activity. Exposing wild-type α -crystallin and mutants C113I, C142I and C131I/C142I to hydrogen peroxide demonstrates disulfide-dependent dimerizations are less important in production of high molecular mass (HMM) protein aggregates accompanying cataract than are secondary structural changes generated upon tryptophan and tyrosine oxidation. Additionally, α -crystallin dimerization promoted by calcium-activated transglutaminase eliminates chaperone activity, suggesting a role in reduced lens transparency and cataract [56]. Oxidation and transglutaminase induced cross-linking may coordinately transform lens α -crystallin chaperone activity and packing, magnifying the consequences of these changes and promoting cataract formation more than anticipated.

Evidence linking cataract and α -crystallin post-translational changes is compelling, but there are examples of extensive α -crystallin modification before disease appears, and cataract associated protein changes may occur subsequent to lens α -crystallin denaturation rather than before [24,42]. In spite of these observations, the prevalence of post-translational changes in lens α -crystallins argues forcefully for a major role in cataract and their study remains important if the disease is to be fully understood. Potential exists for development of therapeutic applications such as the use of carnosine to disaggregate glycated α -crystallin [47] and employing agents that prevent post-translational changes [40].

Cataract and α -crystallin mutations

The mutation responsible for autosomal dominant congenital cataract, a common cause of infant blindness, localizes to the α A-crystallin gene (*CRYAA*) [60]. An R116C substitution renders α A-crystallin defective in chaperone function [11–13], but impaired chaperoning may not completely explain cataract development [10,61]. Another dominant mutation in human α A-crystallin associated with cataract, R49C, is the first shown to lie outside the α -crystallin domain [61]. This change causes lens central core nuclear opacities,

as does the R116C mutation. However, in contrast to R116C α A-crystallin, the R49C variant localizes to the cell nucleus and the cytoplasm, superficially suggesting a relationship to neurodegenerative disorders characterized by intranuclear glutamine-repeats [61]. The α B-crystallin gene, *CRYAB*, described later in the context of desmin-related myopathy, is associated with cataract when possessing an R120G mutation [15, 62,63]. α B-Crystallin R120 corresponds to α A-crystallin R116 and both are conserved α -crystallin domain arginines. R120G α B-crystallin permits intermediate filament self association *in vitro*, although binding of the modified protein to filaments increases in comparison to wild-type α B-crystallin [15,16,64], and this may encourage cataract.

As a prelude to examination of protein recognition by modified α -crystallins, results obtained by mammalian two-hybrid analyses demonstrate that interaction of α A- and α B-crystallin with one another is about three times stronger than the engagement of either chaperone with the prominent lens proteins, β B2-crystallin or γ C-crystallin [65,66]. Moreover, α B-crystallin self-interaction occurs essentially independent of the polypeptide's N-terminus, but self-association of α A-crystallin requires this domain [66]. Attachment of R116C α A-crystallin to Hsp27 and α B-crystallin increases in comparison to wild type, while binding to γ C-crystallin and β B2-crystallin decreases. Reaction of R120G α B-crystallin with β B2-crystallin is moderately enhanced, but there is no change in recognition of γ C-crystallin and Hsp27, and association with α A- and α B-crystallin declines. The altered interplay with other crystallins illustrates that R116C α A-crystallin and R120G α B-crystallin, both observed in congenital cataract, maintain lens protein solubility less effectively and promote cataract development.

Lens size drops off in mice homozygous for α A-crystallin gene loss [α A (–/–)], a characteristic correlated with 50% reduction in lens epithelial cell growth and enhanced sensitivity to apoptotic death [67,68]. The lenses of α A (–/–) mice become opaque with age and contain many inclusion bodies reactive with antibody to α B-crystallin, but not to β - and γ -crystallin, suggesting an important role for α A-crystallin in maintaining lens transparency [69]. Over-expression of α A-crystallin protects stably transfected cells against UVA radiation, whereas α A (–/–) lens epithelial cells have greater sensitivity to photo-oxidative stress, exhibiting more apoptosis and actin filament modifications. Synthesis of exogenous human α A-crystallin in lens epithelial cells of the same species counters UVB-induced apoptosis by favoring action of the AKT kinase pathway, potentially explaining results obtained with knock-out mice

[70]. α B-Crystallin ($-/-$) mice develop skeletal muscle dystrophy but not cataract [71] and they are hyperproliferative, with tetraploid or higher ploidy cells and enhanced susceptibility to apoptosis [72,73]. α B-Crystallin may protect cells from genomic instability. In contrast to the situation with α A-crystallin depletion, there is no apparent effect on the actin cytoskeleton in α B-crystallin ($-/-$) mice, but abnormal mitotic spindles occur, demarcating a relationship between α B-crystallin and tubulin. Interestingly, synthesis of exogenous α B-crystallin in human lens epithelial cells hinders UVA-induced activation of the RAF/MEK/ERK signal transduction pathway and reduces apoptosis substantially, implicating the chaperone in protection against programmed cell death [70].

sHSPs and desmin-related myopathy

An R120G mutation in α B-crystallin, an abundant protein in nonocular tissues such as skeletal and cardiac muscle [2,21–23], gives rise to inherited, adult onset, desmin-related myopathy, a neuromuscular disorder where desmin, an intermediate filament protein, aggregates with α B-crystallin [63]. The mutation disrupts α B-crystallin structure, chaperone activity and intermediate filament interaction, demonstrating the functional importance of residue R120 [14–16,62,74]. This was the first sHSP mutation shown to cause inherited human muscle disease, but two additional dominant negative α B-crystallin mutations have since been linked to myofibrillar myopathy, but not cardiomyopathy [75]. The α B-crystallin C-terminus is truncated by 13 residues in one case and 25 in another, a region important for sHSP solubilization, chaperone activity and oligomer formation.

R120G α B-crystallin synthesis in hearts of transgenic mice induces desmin-related cardiomyopathy [74,76], potentiating desmin and α B-crystallin aggregation, myofibril derangement, compromised muscle action, and heart failure. Study of transgenic mice containing mutations in both desmin and α B-crystallin signifies that the sHSP prevents aggregation of misfolded desmin [77]. A nuclear role for α B-crystallin during cardiomyopathy is also possible because the R120G mutant inhibits speckle formation by the wild-type chaperone in several transfected cell lines [78]. Speckles are thought to participate in RNA transcription and splicing. Cardiomyocyte transfection with adenovirus encoding R120G α B-crystallin promotes microtubule-dependent production of intracellular aggregates [79]. These structures, appearing in cardiomyocytes of dilated and hypertrophic cardiomyopathies, are characteristic of amyloid-related

neurodegenerative conditions, indicating relationships between these two major types of disease and implying common roles for aggregate-associated sHSPs. Furthermore, aggregates stain weakly for desmin, suggesting the concept of desmin-related cardiomyopathies as desmin-based should be reconsidered [79]. In line with this proposal, R120G α B-crystallin localizes to insoluble inclusions when expressed in transiently transfected HeLa cells [80]. These inclusions lack the type III intermediate filament proteins, desmin and vimentin, differing from previously described aggregates because ubiquitin is absent and formation is microtubule-independent. These HeLa cell inclusions are solubilized by Hsp27 coexpression, indicating R120G α B-crystallin is chaperoned. R120G α B-crystallin is disorganized and aggregate-like inclusions develop in cultured nonmuscle cells deficient in desmin, again demonstrating inclusion body construction independent of intermediate filaments [62]. Interestingly, inclusion body formation is slowed by α B-crystallin, Hsp27 and HspB8, offering a molecular explanation for the delayed adult-onset of desmin-related myopathy through chaperone action.

sHSPs and ischemia/reperfusion injury

Ischemia/reperfusion injury to cells during heart attack and stroke is far reaching and includes protein/enzyme denaturation, perturbation of oxidoreductive status, mitochondrial deterioration, cytoskeleton disruption and membrane lipid peroxidation [81]. sHSP overexpression in transgenic animals and cultured cardiomyocytes, the latter by transfection with adenovirus vectors, shields heart cells against apoptosis and necrosis upon ischemia/reperfusion injury [74,81–84]. Over expressed wild-type and nonphosphorylatable Hsp27 were equally effective in safeguarding contractile activity and cell integrity, as determined by retention of creatine kinase activity in transgenic mice hearts during ischemia/reperfusion [81]. sHSP phosphorylation status may have little influence on the ability of Hsp27 to protect myocardial cells of these transgenic mice during ischemia/reperfusion, although nonphosphorylatable Hsp27 variants produce larger oligomers on average than wild type, a trend accentuated by the stress of ischemia/reperfusion, and there is a potential effect on how well cells cope with oxidative stress.

Gene deletion experiments indicate sHSPs defend cells against ischemia/reperfusion injury. That is, the hearts of double knock-out mice lacking the abundant sHSPs, α B-crystallin and HspB2, develop as expected under nonstress conditions and show normal contractility [85]. However, when exposed to ischemia and

reperfusion, hearts from these animals display reduced contractility and less glutathione, accompanied by greater necrosis and apoptosis due to free radical production. The need for either or both α B-crystallin and HspB2 for optimal recovery from heart attack is apparent. Phosphorylated Hsp20, known to associate with and stabilize actin [86], and α B-crystallin [87], arrest β -agonist-induced apoptosis experienced by heart failure patients, probably by inhibiting caspase-3 activation. Five mammalian sHSPs, namely α B-crystallin (HspB5), MKBP (HspB2), Hsp25 (HspB1), Hsp20 (HspB6) and cv Hsp (HspB7) translocate from heart cell cytosol to myofibrils during ischemia, with varying localization to Z-lines, I-bands, and intercalated discs. Binding to microfibrils is tight and sHSPs may save stressed heart cells from harm by stabilizing sarcomeres [36,88,89]. Microtubule preservation by α B-crystallin, but not Hsp27, occurs during ischemia [90], but the role played by microtubule disruption in cell injury is uncertain, possibly representing a reversible situation with minor implications for patient survival [91].

sHSPs and neurological disease

Maintaining the appropriate intracellular complement of functional proteins depends upon proteolytic enzymes and molecular chaperones [92]. If either one or both malfunction, potential exists for tissue-specific build-up of protein aggregates termed amyloid. Such accumulations typify neurodegenerative or 'conformational' diseases, of which Parkinson's, Alzheimer's and other tauopathies, Huntington's, amyotrophic lateral sclerosis (ALS), and the prion disorders, are examples [93–102]. Deposits are fibrillar, enriched in β -pleated sheet, and some contain neurofilament proteins as in desmin-related myopathy inclusions and Parkinson's associated Lewy bodies. Protein deposits observed in neurological diseases may be harmful, beneficial or of no consequence.

Alzheimer's is characterized by amyloid- β peptide (A β) in extracellular senile plaques and tau in neurofibrillary tangles, aggregates that are major morphological indicators of the disease [103]. Alzheimer's disease is the most common tauopathy, a group of familial neurodegenerative conditions distinguished by intracellular filamentous bodies composed of tau, a low molecular weight microtubule-associated protein [104]. Neurons are the predominant location of tau pathology in Alzheimer's, but glial pathology manifests in corticobasal degeneration and progressive supranuclear palsy. Increased α B-crystallin, and to a lesser extent Hsp27, appear in the latter, conceivably in response to aberrant tau. α B-Crystallin and Hsp27,

up-regulated in Alzheimer's brains and localizing to astrocytes and degenerating neurons [104–109], interact with A β and occur in amyloid plaque, thereby affecting amyloid production [107,110,111].

Mass spectrometry reveals that three Hsp16 family members, in addition to other molecular chaperones, coimmunoprecipitate with human A β in transgenic *Caenorhabditis elegans* [112]. sHSP expression is induced by the presence of A β , which is associated with progressive worm paralysis, and the proteins colocalize intracellularly, suggesting a role for molecular chaperones in A β toxicity and metabolism. Human recombinant α B-crystallin also interacts with A β *in vitro*, and as shown by thioflavin T fluorescence and far-CD measurements, α B-crystallin promotes β -sheet formation by A β [110]. Samples were not examined by electron microscopy during this work, so α B-crystallin effects on A β fibril formation and aggregation, although indicated by A β secondary structural changes, are unknown. Thioflavine T fluorescence assays and electron microscopy demonstrated that human Hsp27 inhibits A β amyloidogenesis *in vitro* much more effectively than α -crystallin, which is almost without effect [113]. Nonetheless, study of Hsp27 suggests aging-related reduction in chaperone activity contributes to Alzheimer's pathogenesis. α B-Crystallin inhibits A β fibril formation *in vitro*, although β -sheet content and neuronal toxicity of A β preparations increase. Possibly, α B-crystallin/A β complexes maintain A β as a toxic nonfibrillar protein and A β toxicity is independent of fibril formation. In this scenario, sHSPs exacerbate rather than diminish, Alzheimer's symptoms [111].

sHSPs have been investigated in neurological diseases other than Alzheimer's, but to lesser extents. The childhood leukodystrophy, Alexander's disease, manifests amplified expression of Hsp27 and α B-crystallin in the brain, and astrocytes display Rosenthal fibers where α B-crystallin and Hsp27 interact with glial fibrillary acidic protein (GFAP) [108,109,114,115]. Augmented α B-crystallin discriminates neurons in Creutzfeldt–Jakob disease and spinal cord astrocytes in amyotrophic lateral sclerosis (ALS) [108]. α B-Crystallin binds mutated Cu/Zn-superoxide dismutase (SOD-1) characteristic of familial ALS [116]. Moreover, a mouse model of familial ALS displays down-regulation of sHSPs in motor neurons and up-regulation in astrocytes. Mouse Hsp25 colocalizes with mutant SOD-1 [117], similar to results obtained with a cultured neuronal cell line [118]. Interaction with mutant, but not wild-type SOD-1 may limit antiapoptotic potential and decrease cell protection by Hsp25. In another example, Hsp27 and α B-crystallin appear in Parkinson's disease

with severe dementia [119]. sHSPs and neurological diseases are evidently linked, but consequences are uncertain. Chaperoning can prevent or promote aggregate creation, and either outcome may be favorable or unfavorable, depending on the disease. As a case in point, formation of huntingtin-containing inclusion bodies in Huntington's disease encourages cell survival, whereas monomers and small inclusion bodies of huntingtin, a protein possessing abnormal polyQ repeats, are toxic, an effect potentially mediated by transcription factor destabilization [96,99,120]. Prevention of abnormal protein aggregation obviously does not always benefit cells, an observation with important implications when choosing therapeutic approaches to neurological diseases.

Nerve demyelination presents in multiple sclerosis (MS), a chronic autoimmune neurological condition involving brain and spinal cord inflammation. T cells from MS patients express a dominant response to α B-crystallin, a major autoantigen affiliated with central nervous system myelin, the disease target [121,122]. In contrast to healthy individuals, α B-crystallin resides in oligodendrocytes and astrocytes [122] and α B-crystallin mRNA is the most prevalent transcript found uniquely in MS plaques [123]. Moreover, MS characteristics are influenced by the α B-crystallin genotype with promoter polymorphisms affecting the disease [124]. α B-Crystallin is not thought to cause demyelination directly, but may enhance the inflammatory response and its effects. Antibodies to α B-crystallin and other elevated proteins could serve as confirmation markers for MS diagnosis, and this will assist in disease treatment [125].

sHSP mutations are linked to distal motor neuropathies, genetically heterogeneous diseases of the peripheral nervous system bringing about nerve degeneration and distal limb muscle atrophy [126–128]. HspB8 (Hsp22) mutation K141N exists in two families with distal hereditary motor neuropathy and a second mutation, K141E, is found in two other pedigrees [127]. K141 dwells in the α -crystallin domain and is equivalent to α A-crystallin R116 and α B-crystallin R120, amino acid residues described previously as associated with human disease. The K141N mutant of HspB8 binds more strongly to HspB1 than does its wild-type counterpart, and when expressed in cultured COS cells the K141N variant dramatically increases cytoplasmic and perinuclear aggregate number. Neuronal N2a cell viability is compromised by K141E HspB8 and less so by the K141N mutant. It is not known if neuronal aggregates form in distal motor neuropathies, nor is HspB8 function understood, however, mutations to K141 are linked to motor neuro-

pathies. Mutations S135F, R127W, T151I and P182L in HspB1 (Hsp27) were subsequently discovered in families with distal hereditary motor neuropathy [128]. Individuals with the genetically and clinically heterogeneous syndrome, Charot–Marie–Tooth disease, the most common inherited motor and sensory neuropathy, contain HspB8 K141N, as in distal hereditary motor neuropathy [126], as well as S135F and R136W in HspB1 [128]. All HspB1 mutations, with exception of P182L in the C-terminal extension, are quartered in the α -crystallin domain near residue R140. Neuronal N2a cells transfected with S135F HspB1 are less viable than cells expressing wild-type HspB1, symptomatic of distal motor neuropathies and Charot–Marie–Tooth disease being caused by mutation induced, premature axonal degeneration. Multi-nucleated cells almost double upon expression of the S135F HspB1 mutant and intermediate filament arrangement is affected adversely in an adrenal carcinoma cell line, implicating cytoskeleton disruption in these diseases.

sHSPs and cancer

Based on the consequences of molecular chaperone induction in diseased (stressed) cells, the relationship between cancer and sHSPs is worthy of examination. One area receiving attention is sHSP value in clinical prognosis of individual cancers and of cancers at different developmental stages. By example, a strong correlation exists between lymph node involvement and high α B-crystallin levels in primary breast carcinoma specimens, but measuring only the sHSP inadequately predicts patient outcome [129]. Elevated Hsp27 expression indicates good prognosis in other studies [130,131], contrasting results where increased sHSP indicates aggressive tumor behavior and poor prognosis [132–139], findings that undoubtedly reflect differences between cancers and experimental methods. Interestingly, HspB9, a testis cell-specific mammalian sHSP under normal circumstances, occurs in tumors of several tissues and may be a cancer/testis antigen (CAT) [140]. CATs include many proteins typically synthesized in primitive germ cells; malignant transformation reactivates CAT genes and the proteins reappear in tumors. CAT effects on disease progression and their worth in prognosis are unknown. Overall, sHSPs tend to lack reliability as prognostic indicators for cancers, but the approach has use especially as sHSPs and other proteins indicating poor prognosis are potential therapeutic targets.

sHSPs modulate metastatic potential and tumor progression. Enhanced Hsp27 expression in human

melanoma cell lines decreases invasiveness, reduces matrix metalloproteinases *in vitro* and eliminates production of $\alpha\text{v}\beta 3$ integrin, a protein missing in normal melanocytes but often manufactured during the invasive phase [141]. Hsp27 over expression in melanoma cells prevents E-cadherin loss, and synthesis of the adhesion molecule MUC18/MCAM, which correlates with metastatic potential, is disrupted [142]. The cumulative data indicate Hsp27 slows A375 melanoma cell growth *in vitro*, lowers tumor appearance rate in mice [143] and inhibits tumor progression. In another example, Hsp27 increases MDA-MB-231 breast cancer cell metastasis [135]. Concurrently, MMP-9, a zinc dependent endoprotease capable of degrading several extracellular matrix proteins and enhancing tumor cell invasion, is amplified, while Yes, a Src tyrosine kinase related to cell adhesion and invasion, declines. Reconstitution of Yes in Hsp27 over-expressing cells by transfection reduces MMP-9, signifying mediation of Hsp27 effects by the Yes signaling cascade. Intriguingly, enhancing chondrocyte Hsp25 lowers growth rate, modifies morphology, lessens adhesion and disrupts differentiation, but leaves actin distribution unaffected. These observations have implications for metastatic potential as reduced adhesion leads to cell release from tumors and spreading throughout the organism [144].

sHSP induced drug resistance is of concern for patients undergoing cancer chemotherapy [145,146]. Rat sarcoma cells exhibit less cell death than either rat lymphoma or mouse breast carcinoma cells upon treatment with the anticancer drugs doxorubicin and lovastatin [132]. Among the three cancers, sarcoma cells possess the most Hsp25, the rodent equivalent of human Hsp27, and the protein builds up upon drug treatment, suggestive of a role in cell survival. In another case, a murine melanoma line of low metastatic potential over-expressing Hsp25 displays enhanced susceptibility to interleukin stimulated dDX-5⁺ natural killer cells, thought to perform malignant disease immune surveillance and control. In contrast, a related murine melanoma cell line with high metastatic potential and enhanced Hsp25 expression is no more susceptible to interleukin stimulated natural killer cells than controls not over expressing the sHSP [147]. The difference is apparently unrelated to Hsp25 surface display because protein prevalence at the cytoplasmic membrane is independent of metastatic potential and over-expression. Such findings demonstrate difficulties in extrapolating the implications of sHSP effects from one cancer to another while hinting at treatments. sHSP associated diseases are summarized in Table 1.

Therapeutic implications of sHSPs and other molecular chaperones

Temperature induced synthesis of sHSPs protects against ischemia/reperfusion damage to the heart, brain, and kidney [148]. Hsp27 microinjection enhances neuron survival upon stress exposure and reduces apoptosis, demonstrating the protein's importance in cell maintenance [149]. sHSPs prevent aggregation of oxidized and damaged proteins as organism's age, extending life-span and delaying disease onset [150]. These observations suggest sHSP utility as early diagnostic markers and therapeutic targets. Novel approaches include the use of reagents that modify chaperones structurally and functionally, the modulation of signaling pathways regulating sHSP properties such as phosphorylation, and changing the level of sHSP synthesis [26].

Suppression of sHSPs indicating poor cancer prognosis could be important for treatment. For example, the down regulation of Hsp27 by interferon- γ (IFN- γ) in oral squamous cell carcinoma lines enhances drug effectiveness [134]. Hsp27 is thought to protect against drug induced apoptosis and once either removed or reduced by IFN- γ exposure, cells gain sensitivity to anticancer drugs such as cisplatin. The importance of combination therapy consisting of sHSP reduction and drug exposure is demonstrated, however, INF- γ induced lowering of Hsp27 may be specific to oral squamous cell carcinomas, consequently limiting this potential therapeutic approach. The metabolite, pantethine, increases α -crystallin chaperone activity and aids prevention of rat lens opacification [26,151]. Other therapeutic possibilities include alteration of cellular Ca^{2+} balance through membrane transport protein effectors and changing sHSP function by nucleotide and anti-inflammatory drug application [26]. SAPK2/p38 kinase stimulation leads to sHSP phosphorylation and oligomer size alteration [152], suggesting that drug-dependent regulation of kinases and phosphatases improves sHSP protection [26]. Hsp20 phosphorylation at serine 16 guards against agonist induced cardiac apoptosis, implicating the sHSP as a therapeutic target in treatment of heart failure [86]. The development of pharmaceuticals which modify and/or stimulate sHSPs is feasible and this depends on more extensive characterization of chaperone sites interacting with metabolites, nucleotides and drugs.

The therapeutic application of sHSPs is further suggested by study of other molecular chaperones, with disruption of HSPs that protect deregulated intracellular signaling proteins and transcription factors

Table 1. sHSP modifications associated with disease. Many diseases are associated with changes to sHSPs occurring either as a result of mutation or by post-translational changes, and these are outlined below and described in the text of the review. In addition, changes in the amounts of sHSPs, unaccompanied by a structural change in the protein per se, are observed in cancers and neurological diseases such as Alzheimer's, Alexander's, Creutzfeldt-Jakob, amyotrophic lateral sclerosis, Parkinson's and multiple sclerosis. These diseases are described in the review but not listed in the table. Δ C13, Δ C25, mutations resulting in loss of 13 and 25 amino acid residues, respectively, from the C-terminus of α B-crystallin. Hsp22, HspB8; Hsp25/27, HspB1.

Disease	sHSP modification		
	Post-translation change	Mutation	References
Cataract	Truncation		[33–37]
	Deamidation		[36,38–42]
	Oxidation		[40,43–46]
	Glycation		[46–53]
	Phosphorylation		[33]
	Racemization/isomerization		[54,55]
Desmin-related myopathy		α A-crystallin R116C	[60]
		α A-crystallin R49C	[61]
		α B-crystallin R120G	[16]
		α B-crystallin R120G	[14–16,62,63,74]
		α B-crystallin Δ C13	[75]
Desmin-related cardiomyopathy		α B-crystallin Δ C25	[75]
Distal hereditary motor neuropathies		α B-crystallin R120G	[74,76]
		Hsp25 S135F	[128]
		Hsp25 R127W	[128]
		Hsp25 T151I	[128]
		Hsp25 P182L	[128]
		Hsp22 K141N	[127]
		Hsp22 K141E	[127]
		Hsp25 S135F	[128]
Charot-Marie-Tooth disease		Hsp25 R136W	[128]
		Hsp22 K141N	[126]

involved in malignant phenotypes, as examples [153,154]. Perturbation of high-affinity Hsp90 in tumors, but not healthy cells, causes ubiquitination and proteasomal degradation of chaperone binding proteins, enhancing drug antitumor activity. The first Hsp90-reactive drug to reach phase I trials, 17-allyl-amino-17-demethoxygeldanamycin (17-AAG, NSC 330507), modifies this molecular chaperone while exhibiting limited human toxicity. The hydroxylamine derivative, arimoclomol, delays ALS progression in mice with Cu/Zn superoxide dismutase-1 mutations and induces synthesis of Hsp70 and Hsp90, but not Hsp27 [155]. The hydroxylamine derivatives potentiate HSP expression during stress by prolonging the time heat shock transcription factor-1 (HSF-1) binds gene promoters, presumably increasing HSPs and protecting cells from protein misfolding. The macrocyclic antifungal antibiotic, radicicol, induces HSP expression in neonatal rat cardiomyocytes and shelters cells from the effects of simulated ischemia [156]. Radicicol frees HSF-1 from Hsp90. In contrast to many Hsp90 clients, liberated HSF-1 evades degradation, undergoes activation and enhances HSP gene expression, thereby

inducing heat shock response. The HSPs increased upon radicicol exposure of rat neonatal cardiomyocytes are unknown, but protection from simulated ischemia is independent of Hsp90 over-expression [156]. Stimulation of HSP synthesis by drug-induced disruption of Hsp90 may promote sHSP synthesis leading to beneficial therapeutic effects.

sHSP delivery by gene therapy is being tested in animal models and a catheter-based clinical approach for infusion of adenoviral vectors has promise for treatment of congestive heart failure [157]. In a procedural variation, recombinant adeno-associated virus vectors containing an extracellular superoxide dismutase (SOD) are administered by intramyocardial injection, yielding long lasting protection against ischemia/reperfusion injury in rats [158]. Pre-emptive gene therapy strategies, where SOD or other therapeutic proteins are produced in patients at high risk for ischemic/reperfusion injury associated with coronary artery disease and related chronic ailments, hold medical potential.

Extracellular HSPs indicate necrosis, inducing significant immune response upon cell surface receptor

recognition and initiating internal signaling cascades. Many peptides generated by degradation of self and nonself bind HSPs noncovalently, indicating cells of origin and cause of destruction, while effectively stimulating the immune system [159–164]. Tumor cell HSPs and client proteins/peptides have been used to synthesize oncoprotein vaccines, and when injected into patients immune responses against cells containing HSP-associated proteins are promoted, an approach that may facilitate cancer treatment. The delivery of constitutively active HSF-1 enhances tumor cell HSP expression and augments tumor immunoantigenicity, perhaps by limiting phagocytosis of apoptotic cells [161]. If HSF-1 is employed therapeutically only one gene must be introduced to effect expression of several HSP genes, all with the capacity to enhance HSP synthesis and immunogenicity. sHSPs have also been considered for delivery of antigens and the design of vaccines directed against protein targets in HIV infection [163]. The therapeutic implications associated with HSPs, are provocative, and efforts to exploit molecular chaperones, including the sHSPs, in disease amelioration are underway.

Conclusions

sHSPs were described previously as the ‘forgotten chaperones’, but this is no longer true. Two sHSPs have been crystallized, opening the door to more informed interpretation of results obtained by site-directed mutagenesis and other molecular probing. The functions of sHSP domains and individual amino acid residues are becoming clearer, as is the molecular basis of oligomerization. The implications of oligomer assembly and disassembly as chaperoning prerequisites are under study, sHSP substrates have been identified, and the role of ATP-dependent chaperones in substrate release and refolding revealed. sHSPs operate in the front lines of cell defense, protecting proteins during stress and providing opportunities for salvage. As molecular chaperones, sHSPs have the potential to guard cells from disease, but when perturbed or as residents of aberrant cells, they may promote disease. For example, sHSPs defend against ischemia, oxidative damage and apoptosis, but post-translational modifications and gene mutations cause cataract and desmin-related myopathies. Disease involvement suggests therapeutic exploitation of sHSPs, but this remains poorly explored, as is generally true for other HSPs. However, as sHSPs are better understood, opportunities for disease prevention and treatment become more apparent, and this, along with their fundamental

importance in stress physiology, means that sHSPs will not be forgotten for some time to come.

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References

- 1 MacRae TH (2000) Structure and function of small heat shock/ α -crystallin proteins: established concepts and emerging ideas. *Cell Mol Life Sci* **57**, 899–913.
- 2 Frank E, Madsen O, van Rheede T, Ricard G, Huynen MA & de Jong WW (2004) Evolutionary diversity of vertebrate small heat shock proteins. *J Mol Evol* **59**, 792–805.
- 3 Augusteyn RC (2004) α -Crystallin: a review of its structure and function. *Clin Exp Optom* **87**, 356–366.
- 4 Laksanalamai P & Robb FT (2004) Small heat shock proteins from extremophiles: a review. *Extremophiles* **8**, 1–11.
- 5 Horwitz J (2003) Alpha-crystallin. *Exp Eye Res* **76**, 145–153.
- 6 Narberhaus F (2002) α -Crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. *Microbiol Mol Biol Rev* **66**, 64–93.
- 7 Sun W, Van Montagu M & Verbruggen N (2002) Small heat shock proteins and stress tolerance in plants. *Biochim Biophys Acta* **1577**, 1–9.
- 8 Scharf K-D, Siddique M & Vierling E (2001) The expanding family of *Arabidopsis thaliana* small heat stress proteins and a new family of proteins containing α -crystallin domains (Acd proteins). *Cell Stress Chaperones* **6**, 225–237.
- 9 Kappé G, Aquilina JA, Wunderink L, Kamps B, Robinson CV, Garate T, Boelens WC & de Jong WW (2004) Tsp36, a tapeworm small heat-shock protein with a duplicated α -crystallin domain, forms dimers and tetramers with good chaperone-like activity. *Proteins Struct Funct Bioinform* **57**, 109–117.
- 10 Bera S & Abraham EC (2002) The α A-crystallin R116C mutant has a higher affinity for forming heteroaggregates with α B-crystallin. *Biochemistry* **41**, 297–305.
- 11 Andley UP, Patel HC & Xi J-H (2002) The R116C mutation in α A-crystallin diminishes its protective ability against stress-induced lens epithelial cell apoptosis. *J Biol Chem* **277**, 10178–10186.

- 12 Shroff NP, Cherian-Shaw M, Bera S & Abraham EC (2000) Mutation of R116C results in highly oligomerized α A-crystallin with modified structure and defective chaperone-like function. *Biochemistry* **39**, 1420–1426.
- 13 Cobb BA & Petrash JM (2000) Structural and functional changes in the α A-crystallin R116C mutant in hereditary cataracts. *Biochemistry* **39**, 15791–15798.
- 14 Kumar LVS, Ramakrishna T & Rao ChM (1999) Structural and functional consequences of the mutation of a conserved arginine residue in α A and α B crystallins. *J Biol Chem* **274**, 24137–24141.
- 15 Bova MP, Yaron O, Huang Q, Ding L, Haley DA, Stewart PL & Horwitz J (1999) Mutation R120G in α B-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function. *Proc Natl Acad Sci USA* **96**, 6137–6142.
- 16 Perng MD, Muchowski PJ, van den IJssel P, Wu GJS, Hutcheson AM, Clark JI & Quinlan RA (1999) The cardiomyopathy and lens cataract mutation in α B-crystallin alters its protein structure, chaperone activity, and interaction with intermediate filaments *in vitro*. *J Biol Chem* **274**, 33235–33243.
- 17 Fu X & Chang Z (2004) Temperature-dependent subunit exchange and chaperone-like activities of Hsp16.3, a small heat shock protein from *Mycobacterium tuberculosis*. *Biochem Biophys Res Commun* **316**, 291–299.
- 18 Sobott F, Benesch JLP, Vierling E & Robinson CV (2002) Subunit exchange of multimeric protein complexes: real-time monitoring of subunit exchange between small heat shock proteins by using electrospray mass spectrometry. *J Biol Chem* **277**, 38921–38929.
- 19 Gu L, Abulimiti A, Li W & Chang Z (2002) Monodisperse HSP16.3 nonamer exhibits dynamic dissociation and reassociation, with the nonamer dissociation prerequisite for chaperone-like activity. *J Mol Biol* **319**, 517–526.
- 20 Bova MP, Huang Q, Ding L & Horwitz J (2002) Subunit exchange, conformational stability, and chaperone-like function of the small heat shock protein 16.5 from *Methanococcus jannaschii*. *J Biol Chem* **277**, 38468–38475.
- 21 Golenhofen N, Perng MD, Quinlan RA & Drenkhahn D (2004) Comparison of the small heat shock proteins α B-crystallin, MKBP, HSP25, HSP20, and cvHSP in heart and skeletal muscle. *Histochem Cell Biol* **122**, 415–425.
- 22 Kappé G, Franck E, Verschuure P, Boelens WC, Leunissen JAM & de Jong WW (2003) The human genome encodes 10 α -crystallin-related small heat shock proteins: HspB1–10. *Cell Stress Chaperones* **8**, 53–61.
- 23 Taylor RP & Benjamin IV (2005) Small heat shock proteins: a new classification scheme in mammals. *J Mol Cell Cardiol* **38**, 433–444.
- 24 Bloemendal H, de Jong W, Jaenicke R, Lubsen NH, Slingsby C & Tardieu A (2004) Ageing and vision: structure, stability and function of lens crystallins. *Prog Biophys Mol Biol* **86**, 407–485.
- 25 Horwitz J (2000) The function of alpha-crystallin in vision. *Sem Cell Dev Biol* **11**, 53–60.
- 26 Clark JI & Muchowski PJ (2000) Small heat-shock proteins and their potential role in human disease. *Curr Opin Struct Biol* **10**, 52–59.
- 27 Clark JI, Matsushima H, David LL & Clark JM (1999) Lens cytoskeleton and transparency: a model. *Eye* **13**, 417–424.
- 28 McAvoy JW, Chamberlain CG, de Iongh RU, Hales AM & Lovicu FJ (1999) Lens development. *Eye* **13**, 425–437.
- 29 Cobb BA & Petrash JM (2000) Characterization of α -crystallin-plasma membrane binding. *J Biol Chem* **275**, 6664–6672.
- 30 Weinreb O, Dovrat A, Dunia I, Benedetti EL & Bloemendal H (2001) UV-A-related alterations of young and adult lens water-insoluble α -crystallin, plasma membranous and cytoskeletal proteins. *Eur J Biochem* **268**, 536–543.
- 31 Quinlan RA, Sandilands A, Procter JE, Prescott AR, Hutcheson AM, Dahm R, Gribbon C, Wallace P & Carter JM (1999) The eye lens cytoskeleton. *Eye* **13**, 409–416.
- 32 Meehan S, Berry Y, Luisi B, Dobson CM, Carver JA & MacPhee CE (2004) Amyloid fibril formation by lens crystallin proteins and its implications for cataract formation. *J Biol Chem* **279**, 3413–3419.
- 33 Kamei A, Takamura S, Nagai M & Takeuchi N (2004) Phosphoproteome analysis of hereditary cataractous rat lens α -crystallin. *Biol Pharm Bull* **27**, 1923–1931.
- 34 Kamei A, Iwase H & Masuda K (1997) Cleavage of amino acid residue(s) from the N-terminal region of α A- and α B-crystallins in human crystalline lens during aging. *Biochem Biophys Res Commun* **231**, 373–378.
- 35 Takeuchi N, Ouchida A & Kamei A (2004) C-terminal truncation of α -crystallin in hereditary cataractous rat lens. *Biol Pharm Bull* **27**, 308–314.
- 36 Srivastava OP & Srivastava K (2003) Existence of deamidated α B-crystallin fragments in normal and cataractous human lenses. *Mol Vis* **9**, 110–118.
- 37 Takemoto LJ (1997) Changes in the C-terminal region of alpha-A crystallin during human cataractogenesis. *Int J Biochem Cell Biol* **29**, 311–315.
- 38 Gupta R & Srivastava OP (2004) Effect of deamidation of asparagine 146 on functional and structural properties of human lens α B-crystallin. *Invest Ophthalmol Vis Sci* **45**, 206–214.

- 39 Gupta R & Srivastava OP (2004) Deamidation affects structural and functional properties of human α A-crystallin and its oligomerization with α B-crystallin. *J Biol Chem* **279**, 44258–44269.
- 40 Takemoto L & Boyle D (1998) The possible role of α -crystallins in human senile cataractogenesis. *Int J Biol Macromol* **22**, 331–337.
- 41 Takemoto L & Boyle D (1998) Deamidation of specific glutamine residues from alpha-A crystallin during aging of the human lens. *Biochemistry* **37**, 13681–13685.
- 42 Takemoto L & Boyle D (1998) Determination of the *in vivo* deamidation rate of asparagine-101 from alpha-A crystallin using microdissected sections of the aging human lens. *Exp Eye Res* **67**, 119–120.
- 43 Fujii N, Uchida H & Saito T (2004) The damaging effect of UV-C irradiation on lens α -crystallin. *Mol Vis* **10**, 814–820.
- 44 Chen S-J, Sun T-X, Akhtar NJ & Liang JJ-N (2001) Oxidation of human lens recombinant α A-crystallin and cysteine-deficient mutants. *J Mol Biol* **305**, 969–976.
- 45 Cherian-Shaw M, Smith JB, Jiang X-Y & Abraham EC (1999) Intrapolypeptide disulfides in human α A-crystallin and their effect on chaperone-like function. *Mol Cell Biochem* **199**, 163–167.
- 46 Cherian M & Abraham EC (1995) Decreased molecular chaperone property of α -crystallins due to post-translational modifications. *Biochem Biophys Res Commun* **208**, 675–679.
- 47 Seidler NW, Yeorgans GS & Morgan TG (2004) Carnosine disaggregates glycosylated α -crystallin: an *in vitro* study. *Arch Biochem Biophys* **427**, 110–115.
- 48 Kumar MS, Reddy PY, Kumar PA, Surolia I & Reddy GB (2004) Effect of dicarbonyl-induced browning on α -crystallin chaperone-like activity: physiological significance and caveats of *in vitro* aggregation assays. *Biochem J* **379**, 273–282.
- 49 Kumar MS, Mrudula T, Mitra N & Reddy GB (2004) Enhanced degradation and decreased stability of eye lens α -crystallin upon methylglyoxal modification. *Exp Eye Res* **79**, 577–583.
- 50 Nagaraj RM, Oya-Ito T, Padayatti PS, Kumar R, Mehta S, West K, Levison B, Sun J, Crabb JW & Padival AK (2003) Enhancement of chaperone function of α -crystallin by methylglyoxal modification. *Biochemistry* **42**, 10746–10755.
- 51 Derham BK & Harding JJ (2002) Effects of modifications of α -crystallin on its chaperone and other properties. *Biochem J* **364**, 711–717.
- 52 Thampi P, Zarina S & Abraham EC (2002) α -Crystallin chaperone function in diabetic rat and human lenses. *Mol Cell Biochem* **229**, 113–118.
- 53 Blakytyn R, Carver JA, Harding JJ, Kilby GW & Sheil MM (1997) A spectroscopic study of glycosylated bovine α -crystallin: investigation of flexibility of the C-terminal extension, chaperone activity and evidence for diglycation. *Biochim Biophys Acta* **1343**, 299–315.
- 54 Fujii N, Awakura M, Takemoto L, Inomata M, Takata T, Fujii N & Saito T (2003) Characterization of α A-crystallin from high molecular weight aggregates in the normal human lens. *Mol Vis* **9**, 315–322.
- 55 Fujii N, Matsumoto S, Hiroki K & Takemoto L (2001) Inversion and isomerization of Asp-58 residue in human α A-crystallin from normal aged and cataractous lenses. *Biochim Biophys Acta* **1549**, 179–187.
- 56 Shridas P, Sharma Y & Balasubramanian D (2001) Transglutaminase-mediated cross-linking of α -crystallin: structural and functional consequences. *FEBS Lett* **499**, 245–250.
- 57 Westermann S & Weber K (2003) Post-translational modifications regulate microtubule function. *Nat Rev Mol Cell Biol* **4**, 938–947.
- 58 MacRae TH (1997) Tubulin post-translational modifications. Enzymes and their mechanisms of action. *Eur J Biochem* **244**, 265–278.
- 59 Cherian M, Smith JB, Jiang X-Y & Abraham EC (1997) Influence of protein-glutathione mixed disulfide on the chaperone-like function of α -crystallin. *J Biol Chem* **272**, 29099–29103.
- 60 Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW & Weleber RG (1998) Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene. *CRYAA Hum Mol Genet* **7**, 471–474.
- 61 Mackay DS, Andley UP & Shiels A (2003) Cell death triggered by a novel mutation in the alphaA-crystallin gene underlies autosomal dominant cataract linked to chromosome 21q. *Eur J Hum Genet* **11**, 784–793.
- 62 Zobel ATC, Loranger A, Marceau N, Thériault JR, Lambert H & Landry J (2003) Distinct chaperone mechanisms can delay the formation of aggregates by the myopathy-causing R120G α B-crystallin mutant. *Hum Mol Genet* **12**, 1609–1620.
- 63 Vicart P, Caron A, Guicheney P, Li Z, Prévost M-C, Faure A, Chateau D, Chapon F, Tomé F, Dupret J-M, Paulin D & Fardeau M (1998) A missense mutation in the α B-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet* **20**, 92–95.
- 64 Perng MD, Cairns L, van den IJssel P, Prescott A, Hutcheson AM & Quinlan RA (1999) Intermediate filament interactions can be altered by HSP27 and α B-crystallin. *J Cell Sci* **112**, 2099–2112.
- 65 Fu L & Liang JJ-N (2003) Alteration of protein-protein interactions of congenital cataract crystallin mutants. *Invest Ophthalmol Vis Sci* **44**, 1155–1159.
- 66 Fu L & Liang JJ-N (2002) Detection of protein-protein interactions among lens crystallins in a mammalian two-hybrid system assay. *J Biol Chem* **277**, 4255–4260.

- 67 Xi JH, Bai F & Andley UP (2002) Reduced survival of lens epithelial cells in the α A-crystallin-knockout mouse. *J Cell Sci* **116**, 1073–1085.
- 68 Andley UP, Song Z, Wawrousek EF & Bassnett S (1998) The molecular chaperone α A-crystallin enhances lens epithelial cell growth and resistance to UVA stress. *J Biol Chem* **273**, 31252–31261.
- 69 Brady JP, Garland D, Douglas-Tabor Y, Robison WG Jr, Groome A & Wawrousek EF (1997) Targeted disruption of the mouse α A-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein α B-crystallin. *Proc Natl Acad Sci USA* **94**, 884–889.
- 70 Liu J-P, Schlosser R, Ma W-Y, Dong Z, Feng H, Liu L, Huang X-Q, Liu Y & Li DW-C (2004) Human α A- and α B-crystallins prevent UVA-induced apoptosis through regulation of PKC α , RAF/MEK/ERK and AKT signaling pathways. *Exp Eye Res* **79**, 393–403.
- 71 Wawrousek EF & Brady JP (1998) α B-crystallin gene knockout mice develop a severe fatal phenotype late in life. *Invest Ophthalmol Vis Sci* **39**, S523.
- 72 Bai F, Xi JH, Wawrousek EF, Fleming TP & Andley UP (2003) Hyperproliferation and p53 status of lens epithelial cells derived from α B-crystallin knockout mice. *J Biol Chem* **278**, 36876–36886.
- 73 Andley UP, Song Z, Wawrousek EF, Brady JP, Bassnett S & Fleming TP (2001) Lensepithelial cells derived from α B-crystallin knockout mice demonstrate hyperproliferation and genomic instability. *FASEB J* **15**, 221–229.
- 74 Kumarapeli ARK & Wang X (2004) Genetic modification of the heart: chaperones and the cytoskeleton. *J Mol Cell Cardiol* **37**, 1097–1109.
- 75 Selcen D & Engel AG (2003) Myofibrillar myopathy caused by novel dominant negative α B-crystallin mutations. *Ann Neurol* **54**, 804–810.
- 76 Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, Hewett T & Robbins J (2001) Expression of R120G- α B-crystallin causes aberrant desmin and α B-crystallin aggregation and cardiomyopathy in mice. *Circ Res* **89**, 84–91.
- 77 Wang X, Klevitsky R, Huang W, Glasford J, Li F & Robbins J (2003) α B-Crystallin modulates protein aggregation of abnormal desmin. *Circ Res* **93**, 998–1005.
- 78 van den IJssel P, Wheelock R, Prescott A, Russell P & Quinlan RA (2003) Nuclear speckle localisation of the small heat shock protein α B-crystallin and its inhibition by the R120G cardiomyopathy-linked mutation. *Exp Cell Res* **287**, 249–261.
- 79 Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kaye R, Maloyan A & Robbins J (2004) Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. *Proc Natl Acad Sci USA* **101**, 10132–10136.
- 80 Ito H, Kamei K, Iwamoto I, Inaguma Y, Tsuzuki M, Kishikawa M, Shimada A, Hosokawa M & Kato K (2003) Hsp27 suppresses the formation of inclusion bodies induced by expression of R120G α B-crystallin, a cause of desmin-related myopathy. *Cell Mol Life Sci* **60**, 1217–1223.
- 81 Hollander JM, Martin JL, Belke DD, Scott BT, Swanson E, Krishnamoorthy V & Dillmann WH (2004) Overexpression of wild-type heat shock protein 27 and a nonphosphorylatable heat shock protein 27 mutant protects against ischemia/reperfusion injury in a transgenic mouse model. *Circulation* **110**, 3544–3552.
- 82 Vander Heide RS (2002) Increased expression of HSP27 protects canine myocytes from simulated ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* **282**, H935–H941.
- 83 Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH & Das DK (2001) Transgene overexpression of α B crystallin confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion. *FASEB J* **15**, 393–402.
- 84 Martin JL, Mestrlil R, Hilal-Dandan R, Brunton LL & Dillmann WH (1997) Small heat shock proteins and protection against ischemic injury in cardiac myocytes. *Circulation* **96**, 4343–4348.
- 85 Morrison LE, Whittaker RJ, Klepper RE, Wawrousek EF & Glembotski CC (2004) Roles for α B-crystallin and HSPB2 in protecting the myocardium from ischemia-reperfusion-induced damage in a KO mouse model. *Am J Physiol Heart Circ Physiol* **286**, H847–H855.
- 86 Fan G-C, Chu G, Mitton B, Song Q, Yuan Q & Kranias EG (2004) Small heat-shock protein Hsp20 phosphorylation inhibits β -agonist-induced cardiac apoptosis. *Circ Res* **94**, 1474–1482.
- 87 Morrison LE, Hoover HE, Thuerauf DJ & Glembotski CC (2003) Mimicking phosphorylation of α B-crystallin on serine-59 is necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis. *Circ Res* **92**, 203–211.
- 88 Golenhofen N, Ness W, Koob R, Htun P, Schaper W & Drenckhahn D (1998) Ischemia-induced phosphorylation and translocation of stress protein α B-crystallin to Z lines of myocardium. *Am J Physiol Heart Circ Physiol* **274**, H1457–H1464.
- 89 Yoshida K-i, Aki T, Harada K, Shama KMA, Kamoda Y, Suzuki A & Ohno S (1999) Translocation of HSP27 and MKBP in ischemic heart. *Cell Struct Funct* **24**, 181–185.
- 90 Bluhm WF, Martin JL, Mestrlil R & Dillmann WH (1998) Specific heat shock proteins protect microtubules during simulated ischemia in cardiac myocytes. *Am J Physiol Heart Circ Physiol* **275**, H2243–H2249.

- 91 Vandroux D, Schaeffer C, Tissier C, Lalande A, Bès S, Rochette L & Athias P (2004) Microtubule alteration is an early cellular reaction to the metabolic challenge in ischemic cardiomyocytes. *Mol Cell Biochem* **258**, 99–108.
- 92 Goldberg AL (2003) Protein degradation and protection against misfolded or damaged proteins. *Nature* **426**, 895–899.
- 93 Dobson CM (2003) Protein folding and misfolding. *Nature* **426**, 884–890.
- 94 Muchowski PJ & Wacker JL (2005) Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci* **6**, 11–22.
- 95 Wacker JL, Zareie MH, Fong H, Sarikaya M & Muchowski PJ (2004) Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. *Nat Struct Mol Biol* **11**, 1215–1222.
- 96 Arrasate M, Mitra S, Schweitzer ES, Segal MR & Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805–810.
- 97 Selkoe DJ (2004) Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. *Nat Cell Biol* **6**, 1054–1061.
- 98 Forman MS, Trojanowski JQ & Lee VM-Y (2004) Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. *Nat Med* **10**, 1055–1063.
- 99 Landles C & Bates GP (2004) Huntingtin and the molecular pathogenesis of Huntington's disease. *EMBO Report* **5**, 958–963.
- 100 Ross CA & Poirier MA (2004) Protein aggregation and neurodegenerative disease. *Nat Med* **10**, S10–S17.
- 101 Tanaka M, Kim YM, Lee G, Junn E, Iwatsubo T & Mouradian MM (2004) Aggresomes formed by α -synuclein and synphilin-1 are cytoprotective. *J Biol Chem* **279**, 4625–4631.
- 102 Winklhofer KF, Henn IH, Kay-Jackson PC, Heller U & Tatzelt J (2003) Inactivation of parkin by oxidative stress and C-terminal truncations: a protective role of molecular chaperones. *J Biol Chem* **278**, 47199–47208.
- 103 Citron M (2004) Strategies for disease modification in Alzheimer's disease. *Nat Rev Neurosci* **5**, 677–685.
- 104 Dabir DV, Trojanowski JQ, Richter-Landsberg C, Lee VM-Y & Forman MS (2004) Expression of the small heat-shock protein α B-crystallin in tauopathies with glial pathology. *Am J Pathol* **164**, 155–166.
- 105 Yoo BC, Kim SH, Cairns N, Fountoulakis M & Lubec G (2001) Deranged expression of molecular chaperones in brains of patients with Alzheimer's disease. *Biochem Biophys Res Commun* **280**, 249–258.
- 106 Renkawek K, Voorter CEM, Bosman GJCGM, van Workum FPA & de Jong WW (1994) Expression of α B-crystallin in Alzheimer's disease. *Acta Neuropathol (Berl)* **87**, 155–160.
- 107 Shinohara H, Inaguma Y, Goto S, Inagaki T & Kato K (1993) α B-crystallin and HSP28 are enhanced in the cerebral cortex of patients with Alzheimer's disease. *J Neurol Sci* **119**, 203–208.
- 108 Iwaki T, Wisniewski T, Iwaki A, Corbin E, Tomokane N, Tateishi J & Goldman JE (1992) Accumulation of α B-crystallin in central nervous system glia and neurons in pathologic conditions. *Am J Pathol* **140**, 345–356.
- 109 Lowe J, McDermott H, Pike I, Spendllove I, Landon M & Mayer RJ (1992) α B-crystallin expression in nonlenticular tissues and selective presence in ubiquitinated inclusion bodies in human disease. *J Pathol* **166**, 61–68.
- 110 Liang JJ-N (2000) Interaction between β -amyloid and lens α B-crystallin. *FEBS Lett* **484**, 98–101.
- 111 Stege GJJ, Renkawek K, Overkamp PSG, Verschuure P, van Rijk AF, Reijnen-Aalbers A, Boelens WC, Bosman GJCGM & de Jong WW (1999) The molecular chaperone α B-crystallin enhances amyloid β neurotoxicity. *Biochem Biophys Res Commun* **262**, 152–156.
- 112 Fonte V, Kapulkin V, Taft A, Fluet A, Friedman D & Link CD (2002) Interaction of intracellular β amyloid peptide with chaperone proteins. *Proc Natl Acad Sci USA* **99**, 9439–9444.
- 113 Kudva YC, Hiddinga HJ, Butler PC, Mueske CS & Eberhardt NL (1997) Small heat shock proteins inhibit *in vitro* $A\beta_{1-42}$ amyloidogenesis. *FEBS Lett* **416**, 117–121.
- 114 Iwaki T, Iwaki A, Tateishi J, Sakaki Y & Goldman JE (1993) α B-crystallin and 27-kd heat shock protein are regulated by stress conditions in the central nervous system and accumulate in Rosenthal fibers. *Am J Pathol* **143**, 487–495.
- 115 Goldman JE & Corbin E (1991) Rosenthal fibers contain ubiquitinated α B-crystallin. *Am J Pathol* **139**, 933–938.
- 116 Shinder GA, Lacourse M-C, Minotti S & Durham HD (2001) Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. *J Biol Chem* **276**, 12791–12796.
- 117 Strey CW, Spellman D, Stieber A, Gonatas JO, Wang X, Lambris JD & Gonatas NK (2004) Dysregulation of stathmin, a microtubule-destabilizing protein, and up-regulation of Hsp25, Hsp27, and the antioxidant peroxiredoxin 6 in a mouse model of familial amyotrophic lateral sclerosis. *Am J Pathol* **165**, 1701–1718.
- 118 Okado-Matsumoto A & Fridovich I (2002) Amyotrophic lateral sclerosis: a proposed mechanism. *Proc Natl Acad Sci USA* **99**, 9010–9014.
- 119 Renkawek K, Stege GJJ & Bosman GJCGM (1999) Dementia, gliosis and expression of the small heat

- shock proteins Hsp27 and [alpha]B-crystallin in Parkinson's disease. *Neuroreport* **10**, 2273–2276.
- 120 Schaffar G, Breuer P, Boteva R, Behrends C, Tzvetkov N, Strippel N, Sakahira H, Siegers K, Hayer-Hartl M & Hartl FU (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol Cell* **15**, 95–105.
 - 121 Starckx S, Van den Steen PE, Verbeek R, van Noort JM & Opdenakker G (2003) A novel rationale for inhibition of gelatinase B in multiple sclerosis: MMP-9 destroys α B-crystallin and generates a promiscuous T cell epitope. *J Neuroimmunol* **141**, 47–57.
 - 122 van Noort JM, van Sechel AC, Bajramovic JJ, Ouagmirl ME, Polman CH, Lassmann H & Ravid R (1995) The small heat-shock protein α B-crystallin as candidate autoantigen in multiple sclerosis. *Nature* **375**, 798–801.
 - 123 Chabas D, Baranzini SE, Mitchell D, Bernard CCA, Rittling SR, Denhardt DT, Sobel RA, Lock C, Karpuij M, Pedotti R, Heller R, Oksenberg JR & Steinman L (2001) The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. *Science* **294**, 1731–1735.
 - 124 van Veen T, van Winsen L, Crusius JBA, Kalkers NF, Barkhof F, Peña AS, Polman CH & Uitdehaag BMJ (2003) α B-Crystallin genotype has impact on the multiple sclerosis phenotype. *Neurology* **61**, 1245–1249.
 - 125 Vojdani A, Vojdani E & Cooper E (2003) Antibodies to myelin basic protein, myelin oligodendrocytes peptide, α -B-crystallin, lymphocyte activation and cytokine production in patients with multiple sclerosis. *J Int Med* **254**, 363–374.
 - 126 Tang B-S, Zhao G-H, Luo W, Xia K, Cai F, Pan Q, Zhang R-X, Zhang F-F, Liu X-M, Chen B, Zhang C, Shen L, Jiang H, Long Z-G, Dai H- & P (2005) Small heat-shock protein 22 mutated in autosomal dominant Charcot-Marie-Tooth disease type 2L. *Hum Genet* **116**, 222–224.
 - 127 Irobi J, Van Impe K, Seeman P, Jordanova A, Dierick I, Verpoorten N, Michalik A, De Vriendt E, Jacobs A, Van Gerwen V, Vennekens K, Mazanec R, Tournev I, Hilton-Jones D, Talbot K, Kremensky I, Van Den Bosch L, Robberecht W, Vandekerckhove J, Van Broeckhoven C, Gettemans J, De Jonghe P & Timmerman V (2004) Hot-spot residue in small heat-shock protein 22 causes distal motor neuropathy. *Nat Genet* **36**, 597–601.
 - 128 Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, Van Impe K, Fedotov V, Dadali E, Auer-Grumbach M, Windpassinger C, Wagner K, Mitrovic Z, Hilton-Jones D, Talbot K, Martin J-J, Vasserman N, Tverskaya S, Polyakov A, Liem RKH, Gettemans J, Robberecht W, De Jonghe P & Timmerman V (2004) Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat Genet* **36**, 602–606.
 - 129 Chelouche-Lev D, Kluger HM, Berger AJ, Rimm DL & Price JE (2004) α B-crystallin as a marker of lymph node involvement in breast carcinoma. *Cancer* **100**, 2543–2548.
 - 130 Ungar DR, Hailat N, Strahler JR, Kuick RD, Brodeur GM, Seeger RC, Reynolds CP & Hanash SM (1994) Hsp27 expression in neuroblastoma: correlation with disease stage. *J Natl Can Inst* **86**, 780–784.
 - 131 Tetu B, Lacasse B, Bouchard HL, Lagace R, Huot J & Landry J (1992) Prognostic influence of HSP-27 expression in malignant fibrous histiocytoma: a clinicopathological and immunohistochemical study. *Can Res* **52**, 2325–2328.
 - 132 Ciocca DR, Rozados VR, Carrión FDC, Gervasoni SI, Matar P & Scharovsky OG (2003) Hsp25 and Hsp70 in rodent tumors treated with doxorubicin and lovastatin. *Cell Stress Chaperones* **8**, 26–36.
 - 133 Yonekura N, Yokota S, Yonekura K, Dehari H, Arata S, Kohama G & Fujii N (2003) Interferon- γ downregulates Hsp27 expression and suppresses the negative regulation of cell death in oral squamous cell carcinoma lines. *Cell Death Different* **10**, 313–322.
 - 134 Elpek GO, Karaveli Ş, Şimşek T, Keleş N & Aksoy NH (2003) Expression of heat-shock proteins hsp27, hsp70 and hsp90 in malignant epithelial tumour of the ovaries. *APMIS* **111**, 523–530.
 - 135 Hansen RK, Parra I, Hilsenbeck SG, Himelstein B & Fuqua SAW (2001) Hsp27-induced MMP-9 expression is influenced by the Src tyrosine protein kinase Yes. *Biochem Biophys Res Commun* **282**, 186–193.
 - 136 Bonkhoff H, Fixemer T, Hunsicker I & Remberger K (2000) Estrogen receptor gene expression and its relation to the estrogen-inducible HSP27 heat shock protein in hormone refractory prostate cancer. *Prostate* **45**, 36–41.
 - 137 Geisler JP, Geisler HE, Tammela J, Miller GA, Wiemann MC & Zhou Z (1999) A study of heat shock protein 27 in endometrial carcinoma. *Gynecol Oncol* **72**, 347–350.
 - 138 Aoyama A, Steiger RH, Fröhli E, Schäfer R, von Deimling A, Wiestler OD & Klemenz R (1993) Expression of α B-crystallin in human brain tumors. *Int J Cancer* **55**, 760–764.
 - 139 Thor A, Benz C, Moore D, 2nd Goldman E, Edgerton S, Landry J, Schwartz L, Mayall B, Hickey E & Weber LA (1991) Stress response protein (srp-27) determination in primary human breast carcinomas: clinical, histologic, and prognostic correlations. *J Natl Can Inst* **83**, 170–178.
 - 140 de Wit NJW, Verschuure P, Kappé G, King SM, de Jong WW, van Muijen GNP & Boelens WC (2004) Testis-specific human small heat shock protein HSPB9 is a cancer/testis antigen, and potentially interacts with the dynein subunit TCTEL1. *Eur J Cell Biol* **83**, 337–345.

- 141 Aldrian S, Trautinger F, Fröhlich I, Berger W, Micksche M & Kindas-Mügge I (2002) Overexpression of Hsp27 affects the metastatic phenotype of human melanoma cells *in vitro*. *Cell Stress Chaperones* **7**, 177–185.
- 142 Aldrian S, Kindas-Mügge I, Trautinger F, Fröhlich I, Gsur A, Herbacek I, Berger W & Micksche M (2003) Overexpression of Hsp27 in a human melanoma cell line: regulation of E-cadherin, MUC18/MCAM, and plasminogen activator (PA) system. *Cell Stress Chaperones* **8**, 249–257.
- 143 Kindas-Mügge I, Herbacek I, Jantschitsch C, Micksche M & Trautinger F (1996) Modification of growth and tumorigenicity in epidermal cell lines by DNA-mediated gene transfer of Mr 27,000 heat shock protein (hsp27). *Cell Growth Different* **7**, 1167–1174.
- 144 Favet N, Duverger O, Loones M-T, Poliard A, Kellermann O & Morange M (2001) Overexpression of murine small heat shock protein HSP25 interferes with chondrocyte differentiation and decreases cell adhesion. *Cell Death Different* **8**, 603–613.
- 145 Oesterreich S, Weng CN, Qiu M, Hilsenbeck SG, Osborne CK & Fuqua SA (1993) The small heat shock protein hsp27 is correlated with growth and drug resistance in human breast cancer cell lines. *Can Res* **53**, 4443–4448.
- 146 Huot J, Roy G, Lambert H, Chretien P & Landry J (1991) Increased survival after treatments with anticancer agents of Chinese hamster cells expressing the human Mr 27,000 heat shock protein. *Can Res* **51**, 5245–5252.
- 147 Jantschitsch C, Trautinger F, Klosner G, Gsur A, Herbacek I, Micksche M & Kindas-Mügge I (2002) Overexpression of Hsp25 in K1735 murine melanoma cells enhances susceptibility to natural killer cytotoxicity. *Cell Stress Chaperones* **7**, 107–117.
- 148 Treweek TM, Morris AM & Carver JA (2003) Intracellular protein unfolding and aggregation: the role of small heat-shock chaperone proteins. *Aust J Chem* **56**, 357–367.
- 149 Hopkins DA, Plumier J-CL & Currie RW (1998) Induction of the 27-kDa heat shock protein (Hsp27) in the rat medulla oblongata after vagus nerve injury. *Exp Neurol* **153**, 173–183.
- 150 Hsu A-L, Murphy CT & Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300**, 1142–1145.
- 151 Clark JI & Huang Q-L (1996) Modulation of the chaperone-like activity of bovine α -crystallin. *Proc Natl Acad Sci USA* **93**, 15185–15189.
- 152 Huot J, Houle F, Rousseau S, Deschesnes RG, Shah GM & Landry J (1998) SAPK2/p38-dependent F-actin reorganization regulates early membrane blebbing during stress-induced apoptosis. *J Cell Biol* **143**, 1361–1373.
- 153 Kamal A, Boehm MF & Burrows FJ (2004) Therapeutic and diagnostic implications of Hsp90 activation. *Trends Mol Med* **10**, 283–290.
- 154 Beliakoff J & Whitesell L (2004) Hsp90: an emerging target for breast cancer therapy. *Anti-Cancer Drugs* **15**, 651–662.
- 155 Kieran D, Kalmar B, Dick JRT, Riddoch-Contreras J, Burnstock G & Greensmith L (2004) Treatment with arimocloamol, a coinducor of heat shock proteins, delays disease progression in ALS mice. *Nat Med* **10**, 402–405.
- 156 Griffin TM, Valdez TV & Mestrl R (2004) Radicolol activates heat shock protein expression and cardioprotection in neonatal rat cardiomyocytes. *Am J Physiol Heart Circ Physiol* **287**, H1081–H1088.
- 157 Ding Z, Fach C, Sasse A, Gödecke A & Schrader J (2004) A minimally invasive approach for efficient gene delivery to rodent hearts. *Gene Ther* **11**, 260–265.
- 158 Agrawal RS, Muangman S, Layne MD, Melo L, Perrella MA, Lee RT, Zhang L, Lopez-Ilasaca M & Dzau VJ (2004) Pre-emptive gene therapy using recombinant adeno-associated virus delivery of extracellular superoxide dismutase protects heart against ischemic reperfusion injury, improves ventricular function and prolongs survival. *Gene Ther* **11**, 962–969.
- 159 Ehrnsperger M, Hergersberg C, Wienhues U, Nichtl A & Buchner J (1998) Stabilization of proteins and peptides in diagnostic immunological assays by the molecular chaperone Hsp25. *Anal Biochem* **259**, 218–225.
- 160 Srivastava P (2004) Heat shock proteins and immune response: methods to madness. *Methods* **32**, 1–2.
- 161 Gough MJ, Melcher AA, Crittenden MR, Sanchez-Perez L, Voellmy R & Vile RG (2004) Induction of cell stress through gene transfer of an engineered heat shock transcription factor enhances tumor immunogenicity. *Gene Ther* **11**, 1099–1104.
- 162 Kawanishi K, Shiozaki H, Doki Y, Sakita I, Inoue M, Yano M, Tsujinaka T, Shamma A & Monden M (1999) Prognostic significance of heat shock protein 27 and 70 in patients with squamous cell carcinoma of the esophagus. *Cancer* **85**, 1649–1657.
- 163 Brenner BG & Wainberg MA (1999) Heat shock protein-based therapeutic strategies against human immunodeficiency virus type 1 infection. *Infect Dis Obstet Gynecol* **7**, 80–90.
- 164 Suto R & Srivastava PK (1995) A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. *Science* **269**, 1585–1588.