ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 3 & 4, 2005

Forum Review

Hsp27 Consolidates Intracellular Redox Homeostasis by Upholding Glutathione in Its Reduced Form and by Decreasing Iron Intracellular Levels

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ABSTRACT

Small stress proteins [small heat shock proteins (sHsps)] are molecular chaperones that modulate the ability of cells to respond to oxidative stress. The current knowledge concerning the protective mechanism generated by the expression of mammalian heat shock protein-27 (Hsp27) that allows cells to increase their resistance to oxidative stress is presented. We describe the effects mediated by Hsp27 expression toward crucial enzymes such as glucose-6-phosphate dehydrogenase and glutathione reductase that uphold glutathione in its reduced form. New data are presented showing that the expression of sHsps correlates with a drastic decrease in the intracellular level of iron, a catalyzer of hydroxyl radical ($OH^{\bullet}$) generation. A decreased ability of sHsps expressing cells to concentrate iron will therefore end up in a decreased level of oxidized proteins. In addition, we propose a role of Hsp27 in the presentation of oxidized proteins to the proteasome degradation machinery. We also present an analysis of several Hsp27 mutants that suggests that the C-terminal part of this stress protein is essential for its protective activity against oxidative stress. *Antioxid. Redox Signal.* 7, 414–424.

INTRODUCTION

SMALL STRESS PROTEINS [also denoted small heat shock proteins (sHsps)] share a sequence (the α-crystallin domain) in their C-terminal moiety with mammalian α-crystallin polypeptides. sHsps were first characterized in *Drosophila* as proteins that displayed increased synthesis following heat shock or conditions or agents that alter protein folding (6). Families of sHsps have been characterized in different organisms (22). The human family contains 10 polypeptides, but only a subset of them are true heat shock proteins. In mammals, the most studied sHsps are heat shock protein-27 (Hsp27) (also called HspB1) and αB-crystallin (HspB3). Several reports have described an increased cellular resistance to various stress in response to sHsp overexpression. Protection has been observed in the case of heat shock (47), oxidative stress (2–4, 6, 10, 56), cancer chemotherapy agents (30, 31, 38, 69, 81), and inflammatory mediators such as tumor necrosis factor-α (TNFα) (59, 93). Evidence has been obtained that sHsps share an ATP-independent chaperone activity (25, 43, 44) and are able to bind misfolded polypeptides. This creates reservoirs of folding intermediates (24, 49) counteracting the formation of deleterious protein aggregates. Folding intermediates are then renatured by ATP-dependent protein chaperones (Hsp70, Hsp40, Hsp90, and cochaperones) and/or degraded by the ubiquitin-proteasome machinery. sHsp expression was also found to counteract different apoptotic processes such as those induced by agents that do not trigger an oxidative stress (9) or that take place during early differentiation (62). For example, because of the existence of an apoptotic signaling pathway linking cytoskeleton damages to mitochondria, the ability of Hsp27 to protect F-actin network integrity interferes with the release of cytochrome *c* from mitochondria (75). Hsp27 also binds cytochrome *c* once it is released from the mitochondria and inhibits its ability to promote apoptosome activation (15).

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Hsp27 can also interfere with procaspase 3 activation (70) and can modulate DAXX (18) and Akt (80) signaling mechanisms.

The present report deals with recent observations concerning the ability of mammalian sHsps to modulate intracellular redox status and oxidoresistance. New data are presented concerning different Hsp27 mutants and the ability of sHsps to down regulate iron intracellular levels.

DYNAMIC CHANGES IN THE INTRACELLULAR LOCALIZATION OF sHsps

Mammalian Hsp27 exists in a number of dynamic states depending on the physiological state of the cell. In the absence of nonionic detergents, biochemical fractionation revealed that Hsp27 partitioned approximatively equally between the soluble and insoluble fractions, whereas in the presence of detergent this polypeptide was in the soluble fraction. Indirect immunofluorescence experiments confirmed that, in cells grown at normal temperature, Hsp27 is present in the cytosol and also at the level of detergent-sensitive structures such as membranes (8). When cells are exposed to a drastic heat shock treatment, Hsp27 has the tendancy to concentrate transiently within the nucleus, where it associates with detergent-resistant structures (8). In contrast, oxidative stress abolishes the presence of Hsp27 in detergent-sensitive insoluble fractions (58), hence suggesting that in oxidative conditions Hsp27 is essentially cytosolic.

EXPRESSION OF EXOGENOUS sHsps CAN GENERATE A PRO-REDUCED STATUS IN MAMMALIAN CELLS

In normal growing conditions, cells such as murine fibroblasts (*i.e.,* murine L929 or NIH 3T3-ras cells) are devoid of constitutive expression of either Hsp27 or αB-crystallin. These cells are extremely sensitive to oxidative stress and used as standards for TNFα cytotoxicity. Stable transfections of these cells were achieved using expression vectors bearing the coding sequence of different sHsps under the control of a constitutive promoter. In these cell lines, we and others have observed that the expression of either human Hsp27, murine Hsp27, *Drosophila* Hsp27, or mammalian αB-crystallin upregulated total glutathione level and significantly decreased the basal level of intracellular reactive oxygen species (ROS) (11, 60, 74, 79). However, this redox state modulation depends on the cellular content of sHsps and is therefore difficult to detect in cells that express a high level of sHsps. For example, in L929 fibroblasts, the level of glutathione was not further increased and reached a plateau value when exogenous Hsp27 accumulation reached ~1 ng/μg of total cellular proteins (59). Another example concerns HeLa cells that constitutively express a high level of endogenous Hsp27 (~3 ng/μg of total proteins). In these cells, a transfection-mediated increase in Hsp27 levels did not significantly modify glutathione and ROS levels. In human colorectal cancer HT-29 cells, the overexpression of Hsp27 down-regulated ROS without altering the reduced glutathione (GSH) level (31). An Hsp27-mediated down-regulation of ROS was also observed in monkey COS-7 cells (W. Firdaus and A.-P. Arrigo, manuscript in preparation).

Some interesting observations have been made concerning the mechanism that generates a pro-reducing state in response to the expression of sHsps. First, it has been reported that the expression of Hsp27 increases the level of extracellular superoxide produced by CCL39 fibroblasts (87). This phenomenon may be related to the protection of NADPH-oxidase activity by Hsp27 and is compatible with the generation of a pro-reduced state inside the cell.

Second, a careful analysis of several key enzymes that regulate intracellular redox state was performed (79). This study showed that in murine L929 cells, the expression of human Hsp27 significantly increases the activity of glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase, and glutathione transferase (Fig. 1). G6PDH, the first enzyme of the pentose phosphate cycle, has the interesting property of reducing $NADP^+$ to $NADPH(H)^+$ and thus is the key enzyme that provides the reducing power of the cell (Fig. 1). In this respect, we and others have shown that genetically manipulated L929 and HeLa cells expressing high levels of G6PDH display an increased level of GSH and show oxidoresistance (79, 83). Hence, the possibility exists that the modulation of GSH and ROS levels observed in Hsp27-expressing cells results from the increased G6PDH activity detected in these cells. The oxidoresistance generated by high G6PDH activity may then be responsible for the protection observed at the level of cell morphology, cytoskeletal architecture, and mitochondrial membrane potential (74, 77, 79). Further analysis revealed that the cellular content of G6PDH was increased as a consequence of the presence of large aggregates of Hsp27 (61, 78). How the large oligomers of Hsp27 increase G6PDH levels is unknown. It can be excluded that this phenomenon results from a transcriptionally regulated event because similar levels of G6PDH mRNA were detected in normal and Hsp27-expressing L929 cells. Hsp27 may then affect G6PDH half-life or the translability of G6PDH mRNA. These hypotheses should be tested because several reports have suggested an important role of sHsps in the control of protein turnover (71, 72) and mRNA translability (20, 68).

One major problem encountered by *in vivo* analysis is that Hsp27 knockout mice are not viable. This lethality is linked to the fact that embryonic stem cells that are unable to express Hsp27 during early differentiation undergo apoptosis (62). Recently, a very interesting study from Ivor Benjamin's laboratory provided evidence that the modulation of the intracellular redox state by sHsp was not restricted to cell cultures, but was also observed in animals (95). Using heat shock factor 1 (Hsf1) knockout mice as the model, these authors observed a strong reduction in the constitutive expression of Hsp27 and αB-crystallin in cardiac cells (72% in the case of Hsp27 and 34% in the case of αB-crystallin). In these *hsf1*$^{-/-}$ cardiac cells, the down-regulation of these sHsps correlated with a 40% decrease in the ratio of GSH to oxidized glutathione (GSSG) and with a 43% increase in the level of superoxide ions. These phenomena were also associated with a 34% decreased activity of G6PDH. Taken together, these ob-

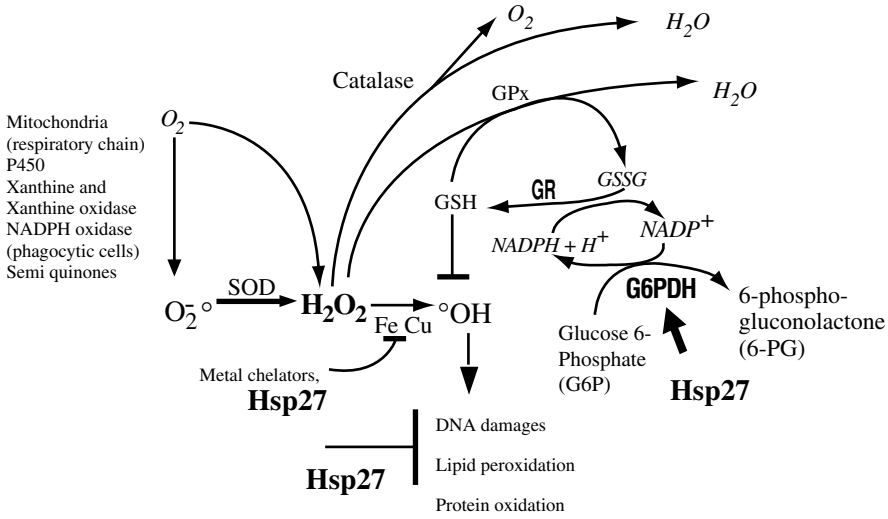

FIG. 1. Illustration of Hsp27 putative mode of action during oxidative stress. Hsp27 generates a pro-reduced state inside the cell by modulating the activity of G6PDH, glutathione reductase (GR), and glutathione transferase. Hsp27 also decreases the intracellular level of iron and therefore interferes with the formation of hydroxyl radical (OH•) via the Fenton reaction. This leads to a decreased level of ROS, increased level of GSH, and the upholding of this redox modulator in its reduced form. GPx, glutathione peroxidase; $O_2^{\bullet}$, superoxide ion; SOD, superoxide dismutase.

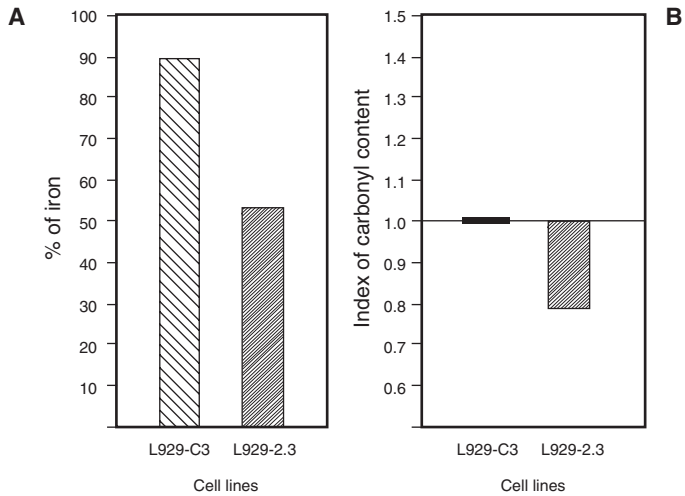

FIG. 2. Hsp27 decreases intracellular iron level and protein carbonyl content. (**A**) Determination of the intracellular concentration of iron determined by the colorimetric method of Fish (26). Stable transformants of L929 murine fibroblasts expressing (L929–2.3) or not expressing (L929-C2) 0.2 ng of human Hsp27/μg of total cellular proteins (79) have been analyzed [L929–2.3 cells are also indicated as L929–27–3-97 cells in Préville *et al.* (79)]. Cells were washed and scraped from the dish in phosphate-buffered saline, and the cell pellets were resuspended in 1 ml of water. An equivalent of 2 mg of total protein was used per assay. Buffer A (500 μl) (0.6 *M* HCl, 0.142 *M* $KMnO_4$) was added, and the samples were incubated for 2 h at 60°C. This procedure allowed iron to be released and solubilized. Buffer B (100 μl) was then added {6.5 m*M* Ferrozine [disodium 3-(2-pyridyl)-5,6-bis(4-phenylsulfonate)-1,2,4-triazine]; 3.1 m*M* neocuproine (2,9-dimethyl-1,10-phenanthroline), 2 *M* ascorbic acid, and 5 *M* ammonium acetate]}. After 60 min of incubation, absorbance of Ferrozine–Fe(II) complex was read at 562 nm. In addition to Fe(II), only Cu(I) and Co(II) can form a colored complex with Ferrozine. Neocuproine is added to the reaction mixture to chelate Cu(I). This chelator allows a quantification of iron with <5% error in the presence of up to a 2:1 weight ratio of Cu:Fe. Co(II) interferes <5% in the iron assay for quantities of Co(II) up to a 5:1 weight ratio over Fe. Similar analysis was performed using 1 ml of the growth medium as starting material. The ratio between the level of iron present in the cell extract and that observed in the growth medium was calculated. The corresponding percentage is presented. All chemicals were from Sigma–Aldrich (St-Quentin-Fallavier, France). (**B**) Protein carbonyl content, which is taken as presumptive evidence of an irreversible protein oxidative modification, was measured using 2,4-dinitrophenylhydrazine (51) as already described (79). Index of carbonyl content was calculated as the ratio between the value determined in Hsp27-expressing cells and that measured in control cells.

servations indicate that the constitutive expression of sHsps is directly and functionally linked to the maintenance of redox homeostasis and antioxidative defenses at normal temperature.

As iron is an important factor in the cellular damage generated by oxidative stress, we have analyzed the intracellular level of this metal in cells that do or do not express sHsps. Iron catalyzes the Haber–Weiss/Fenton reactions that lead to the formation of the highly reactive and toxic hydroxyl radical ($OH^{\bullet}$, see Fig. 1) (37, 89). The ability of cells to concentrate iron will therefore facilitate the formation of intracellular $OH^{\bullet}$. High levels of intracellular iron thus stimulate oxidative damage and have been associated with a number of oxidative injury-dependent, age-related conditions and diseases (63, 76, 92). We have observed that the expression of human Hsp27 in murine L929 cells decreased the intracellular level of iron by almost 50% (Fig. 2A). We made a similar observation in genetically modified HeLa cells expressing αB-crystallin, a polypeptide that is normally not constitutively expressed in these cells (S. Virot and A.-P. Arrigo, manuscript in preparation). Moreover, oxyblot analysis (88) revealed a decrease in the level of oxidized proteins in L929 cells expressing Hsp27 (79) (Fig. 2B) and in HeLa cells expressing α-crystallin (S. Virot and A.-P. Arrigo, manuscript in preparation). Hence, at least two sHsps appear to share the ability to decrease intracellular iron levels and to interfere with the level of proteins oxidized as a consequence of $OH^{\bullet}$ activity. This observation is of prime importance because *in vivo* iron deprivation or chelation acts as a potent antioxidant, preventing oxidative stress in tissues and organs. Iron chelators have also been described to favor successful ageing in general (76). The mechanism responsible for decreasing iron intracellular level is unknown, but could be related to an inhibitory effect of sHsps toward the action of iron-regulatory proteins (IRP-1,2) (17, 54). IRP-1,2, whose action is sensitive to cellular iron concentration, are activated and bind stem-loop untranslated regions (IRE regions) of transferrin and ferritin mRNAs, hence activating and inhibiting their translations, respectively. This concerted mechanism permits a fine tuning of iron homeostasis in the cell. It is likely that IRP-1,2 sense Fe(II) and that Fe(II) oxidation to Fe(III) by hydrogen peroxide (H_2O_2) treatments of the cells sets up a program for increasing iron uptake (17, 54). Whether sHsps modulate the translation of transferrin and ferritin mRNAs will merit investigation because it could represent the second example of an mRNA translation modulation by sHsp expression that generates a pro-reduced state in cells.

The question remains as to whether the modulation of the intracellular redox status by sHsps results from the biochemical action of these proteins or an adaptation of the cell to their presence. In other words, could it be that sHsps are prooxidant proteins that generate a drastic antioxidant defense of the cell, such as a decrease in the intracellular level of iron? Among the experiments that are in favor of this hypothesis, one can cite those describing a sensitization of the cells to oxidative stress. For example, Hsp27 overexpression sensitizes a squamous carcinoma cell line (91), as well as KMST-6 human immortalized fibroblasts (1) and a sub species of L929 fibrosarcoma cells (53), to oxidative stress. Hence, the cellular context in which Hsp27 is expressed appears important. However, care should be taken in analyzing these results because it is difficult to compare studies devoid of Hsp27 expression quantification. For example, in murine L929 fibroblasts, a low level of expression of Hsp27 generates oxidoresistance, whereas a high level of this protein is usually toxic and sensitizes cells to oxidative stress.

sHsps PROTECT AGAINST OXIDATIVE STRESS-INDUCED CYTOTOXICITY

Depending on its intensity, oxidative stress can induce two types of cell death process. In the case of cells exposed to moderate levels of ROS, apoptosis is usually observed probably because of the depletion of GSH, which triggers the mitochondrial apoptotic pathway leading to caspase activation (99). In contrast, high levels of ROS drastically oxidize proteins, lipids, and nucleic acids. This oxidation process triggers cell necrosis, which, *in vivo*, can lead to inflammation (42). Apoptosis is inhibited by a high level of ROS, which decreases ATP levels and inhibits the activation of caspases (50, 67, 84).

Several reports have described a protection against the deleterious effects induced by oxidative stress in cells (L929, NIH 3T3-ras, CHO, HeLa, colorectal cancer HT-29) expressing sHsps (human Hsp27, murine Hsp27, rodent Hsp27, *Drosophila* Hsp27, and mammalian αB-crystallin) (31, 40, 41, 59, 73, 93). This protection correlated with a significant decrease in the intracellular burst of ROS generated by oxidative stress (H_2O_2, menadione, TNFα) (58–61, 82). This modulation of ROS production was found to be glutathione-dependent because sHsps cannot protect against the oxidative stress generated by drugs that interfere with GSH activity or synthesis, such as buthionine sulfoximine or diethyl maleate (60). Hence, ROS-dependent phenomena, such as lipid peroxidation, protein oxidation, nuclear factor-κB activation, and disruption of F-actin architecture, were decreased by the expression of Hsp27 (60). Further analysis revealed that the pattern of irreversibly oxidized proteins (carbonylation of side chains) in response to H_2O_2 treatment observed in Hsp27-expressing cells was different from that observed in control cells pretreated with the antioxidant drug glutathione ethyl ester (79). This suggests that Hsp27 may act in different ways: (a) by exerting unspecific protections (upholding of glutathione in its reduced form, decrease of intracellular iron and ROS) and (b) by acting at the level of specific proteins to either avoid their oxidation and/or induce their rapid degradation once they are irreversibly oxidized. Among the different enzymes protected by Hsp27 expression one can cite glutathione *S*-transferase and glutathione peroxidase that are involved in the ROS detoxification machinery (79).

Hsp27 ABILITY TO PROTECT AGAINST OXIDATIVE STRESS IS REGULATED BY ITS STRUCTURAL ORGANIZATION

To approach the mechanism of Hsp27 action, the functional consequences of two prominent features of this poly-

peptide, oligomerization and phosphorylation, have been considered.

sHsps are oligomeric proteins that display dynamic changes in their oligomerization profile when cells are exposed to environmental changes (5, 7, 8, 29, 55). For example, in normal growth conditions, human Hsp27 is cytosolic and forms heterodispersed oligomers with native size ranging from 60 to 800 kDa. However, when cells are starved and arrested in G_o, only the small oligomers of Hsp27 are detected (55). In cells exposed to heat shock, Hsp27 large oligomers transiently accumulate and, depending on the intensity of the stress, can be recovered inside the nucleus (8). This phenomenon is followed by the rapid accumulation of Hsp27 in the form of small oligomers as a consequence of Hsp27 phosphorylation. Of interest, in cells made thermotolerant to the heat stress, no changes in the structural organization and phosphorylation of Hsp27 are observed (8), hence suggesting that these changes are directly correlated with the intracellular damages induced by heat shock. Similarly, the protective activity of Hsp27 in response to oxidative stress (TNFα, H_2O_2) correlates with the formation of large structures containing this protein. This phenomenon is followed by the phosphorylation-dependent dissociation of Hsp27 large structures (58, 61, 79). In cells overexpressing the antioxidant enzyme selenoglutathione peroxidase, no changes in Hsp27 structural organization and phospho-isoform composition are observed in response to oxidative stress (57). This suggests that the intracellular burst of ROS observed in response to oxidative stress is responsible for the changes in Hsp27 structural organization and phosphorylation.

In cells exposed to heat shock, Hsp27 large oligomers have been described to act as reservoirs that facilitate the renaturation of misfolded proteins by other chaperones, such as the ATP-dependent chaperones Hsp70, Hsp40, and Hsp90 (24, 49). However, in the case of irreversibly oxidized proteins, selective proteolysis is triggered (33). Hence, the transient accumulation of Hsp27 in the form of large oligomers probably activates the presentation of irreversibly oxidized proteins to the ubiquitin-independent 20S proteasome (4, 10), which is known to have a high affinity for oxidized proteins (33, 34, 85). This phenomenon counteracts the accumulation of proteolysis-resistant large aggregates of oxidized proteins (lipofuscin) that block proteasome activity (13) and are extremely deleterious to the cell (86).

Hsp27 phosphorylation during heat or oxidative stress is regulated by the stress kinase pathway. Murine Hsp27 is phosphorylated at serine residues 15 and 86 (27), whereas human Hsp27 contains three phosphorylated serines residues, 15, 78, and 82 (48). Hsp27 phosphorylation is mediated by numerous stimuli that include heat shock, TNFα, and other forms of oxidative stress (6) and is catalyzed by mitogen-activated protein kinase-activated protein kinase 2 and 3 (MAPKAPK2-3) (19, 39, 52, 90) and/or by the inactivation of specific phosphatases (28, 36).

Several studies have demonstrated that phosphorylation generates the formation of small oligomers of Hsp27 (45, 61, 82). For example, inhibition of p38 kinase by the SB203580 inhibitor did not alter the formation of Hsp27 large oligomers in response to oxidative stress, but inhibited their dissociation later (78). Moreover, studies performed with nonphosphorylatable or phosphorylated mimicry mutants led to the conclusion that the large unphosphorylated oligomers represent the active form of Hsp27 that modulates ROS and glutathione levels and displays *in vitro* chaperone activity (61, 88).

Hence, the intracellular burst of ROS generated early during oxidative stress induces the formation of large oligomers of Hsp27 that bear chaperone and anti-ROS activities. The concomitant stimulation of MAPKAPK2-3 kinase, which phosphorylates Hsp27, then leads to the subsequent formation of small oligomers that are observed when the oligomerization pressure mediated by the ROS burst is decreasing. These small oligomeric forms may either inactivate Hsp27 chaperone activity or induce its recycling through dynamic deaggregation–oligomerization of the protein.

The dynamic changes in Hsp27 oligomerization and phosphorylation may also play a role in the protection of the cytoskeleton because the F-actin microfilament network is one of the earliest targets of oxidative stress (12, 21). In this respect, unphosphorylated Hsp27 small oligomers have been described to inhibit *in vitro* F-actin polymerization (14, 65). *In vivo* Hsp27 also appears to modulate F-actin filament dynamics (35, 66) and protects F-actin against oxidative stress-induced dissociation (40), probably by upholding glutathione in its reduced form (77) and decreasing iron intracellular levels.

ANALYSIS OF Hsp27 MUTANTS

We have obtained several mutants of human Hsp27 (point mutations or deletions) (see Fig. 3). These mutants were transiently expressed in HeLa cells and their protective ability against a 24-h oxidative stress performed with 600 μ*M* H_2O_2 has been analyzed. Figure 4 compares the protective activity

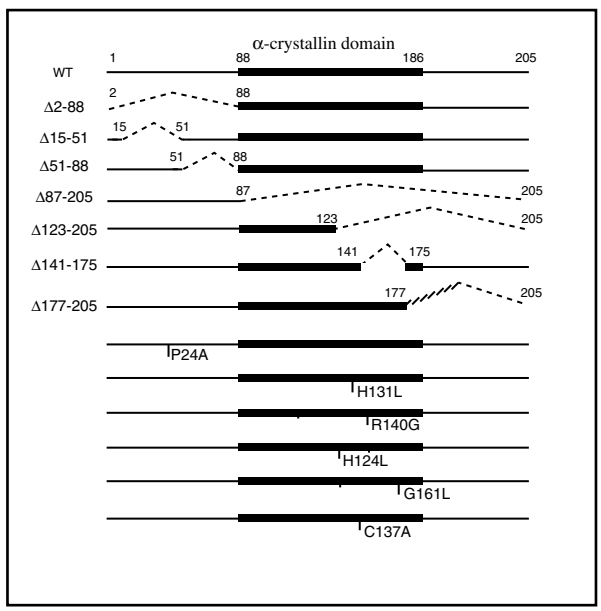

FIG. 3. Schematic illustration of human Hsp27 mutants. Point mutations, as well as deletions, are indicated. Black bars correspond to the α-crystallin domain shared by sHsps.

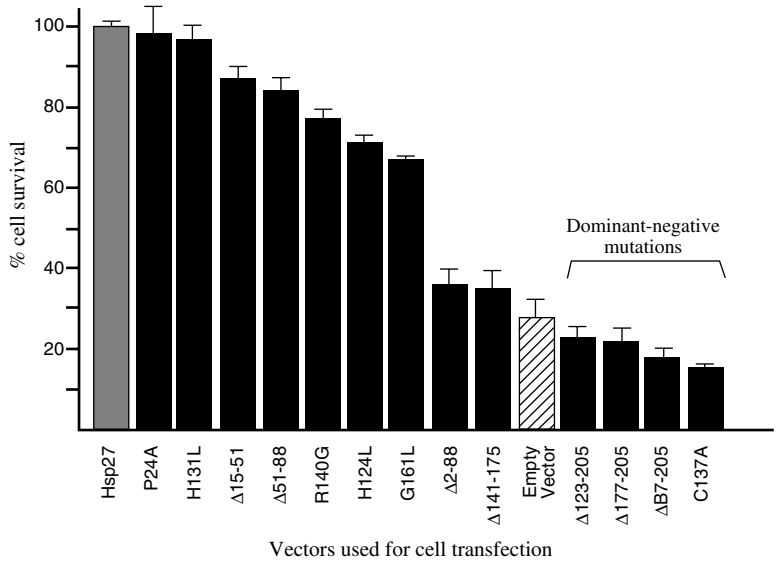

FIG. 4. Protection mediated by wild type and mutants of Hsp27 against H_2O_2 cytotoxicity. *Construction of Hsp27 vectors.* The cDNA encoding human Hsp27 was inserted into the *Eco*RI site of pSVK3 vector (Pharmacia, Saint-Quentin-en-Yvelines, France) or pCI-neo vector (Promega, Charbonnieres, France). Empty vector corresponds to either plain pSVK3 or pCI-neo vector. The mutants described in this study were constructed as follows: (A) Mutants constructed in Hsp27-bearing pSVK3 vector using the following PCR primers. H131L (substitution of histidine 131 by a leucine residue): sense 5'-CAGGACGAGCTCGGCTA CATCT-3' and antisense 5'-AGATGTAGCCGAGCTCGTCCTG-3'. H124L (substitution of histine 124 by a leucine residue): sense 5'-ACCGGCAAGCTTGAGGAGCGGC-3' and antisense 5'-GCCGCTCCTCAAGCTTGCCGG-3'. P24A (substitution of proline 24 by an alanine residue): sense 5'-CGACTGGTACGCGCATAGCCG-3' and antisense 5'-CGGCTATGCGCGTA CCAGTCG-3'. G161L (substitution of glycine 161 by a leucine residue): sense 5'-GTCCCCTGAGCTCACACTGACC-3' and antisense 5'-GGTCAGTGTGAGCTCAGGGGAC-3'. DNA amplification was followed by restriction and ligation steps. The Δ2–88 deletion was constructed by introducing two *Sph*I sites in the Hsp27 coding sequence using the following primers: sense 5'-GGGGTCTCGGGCATGCGGCACACTG-3' and antisense 5'-GCGGCGCTCGGGCATGCTGGCTC-3'. Following restriction and ligation steps, the deletion was generated by digestion with *Sph*I. The Δ15–51 deletion was generated by digestion with *Pvu*II followed by a ligation step. (B) Mutants generated in Hsp27-bearing pCI-neo vector using the Stratagene Quickchange™ site-directed mutagenesis kit (Stratagene Europe, Amsterdam Zuidoost, The Netherlands). C137A (substitution of the unique cysteine residue of Hsp27 by an alanine residue), PCR primers: sense 5'-GGCTACATCTCCCGGGCCTTCACGCGGAAATACACG-3' and antisense 5'-CGTCTATTTCCGCGTGAAGGCCCGGGAGATGTAGCC-3'. R140G (substitution of arginine 140 by glycine): sense 5'-CCCGGTGCTTCACGGGGAAATACACGCTGCCC-3' and antisense 5'-GGGCAGCGTGTATTTCCCCGTGAAGCAC CGGG-3'. The Δ51–88 deletion was constructed using the following primers: sense 5'-GGTTAGGCGGCAGCAGCCGGCACAC TGCGGACCG-3' and antisense 5'-CGGTCCGCAGTGTGCCGGCTGCTGCCGCCTAACC-3'. The Δ141–175 deletion was generated with the following primers: sense 5'-CCGGTGCTTCACGCGGAACGAGATCACCATCC-3' and antisense 5'-GGATGGT GATCTCGTTCCGCGTGAAGCACCGG-3'. For the Δ87–205, Δ123–205, and Δ177–205 deletions, we used the following primers containing an *Eco*RI site: sense 5'-CGGAATTCATGACCGAGCGCCGCGTCCCC-3' and antisense 5'-GGAATTCTTA CGAGACCCCGCTGC-3', 5'-GGAATTCTTAGCCGGTGATCTCCACC-3' and 5'-GGAATTCTTAGGACTGCGTGGCTAGC-3', respectively. The deleted Hsp27 genes were then cloned in the *Eco*RI site of pCI-neo plasmid. *Transfection experiments:* HeLa cells transiently expressing wild type (gray plot) or mutants (black plots) of human Hsp27 were exposed to 600 μM H_2O_2 for 24 h. Control cells transfected with the corresponding empty vector were also analyzed (hatched bar). Cell survival was estimated by crystal violet staining. Percentage of cell survival was calculated by comparing the values obtained for the different mutants with that observed for wild-type Hsp27 (100%). Mutants that induced a sensitization to H_2O_2 were considered as dominant negative, probably because they interfere with the protective activity of endogenous Hsp27. Standard deviations are indicated (n = 6).

of the different mutants to that of wild-type Hsp27. This figure clearly shows that a hydrophobic replacement at position 131 (H131L mutant) that should alter the major hydrophilic peak seen in the α-crystallin domain had no effect. The hydrophobic replacement at position 124 (H124L mutant) or 161 (G161L mutant) was more effective. Analysis of the N-terminal part comprised of amino acids (AA) 15–88 revealed that this region is not of prime importance for protecting the cell against H_2O_2. In contrast, the C-terminal part of Hsp27 appears essential. Some mutants (in the C-terminal part of the protein, as well as the C137A point mutation) render the cell hypersensitive to H_2O_2. As sHsps of different origins can form oligomeric complexes (96), mosaic structures are probably formed that inactivate endogenous Hsp27 of HeLa cells. These mutants are therefore considered as dominant negative. Concerning the different point mutations that interfered with Hsp27 protective activity, we have already reported that the triple mutation of the phosphorylated serine sites (serines 15, 78, and 82) does not protect against oxidative stress (82). Of interest, mammalian Hsp27 polypeptides contain only one cysteine residue (position 141 in murine and 137 in human) that is highly suceptible to oxidation. It is seen in Fig. 4 that

the substitution of this cysteine by an alanine residue (C137A mutant) renders HeLa cells extremely sensitive to H_2O_2. The mutation impaired Hsp27 ability to form dimers and induced a dominant-negative effect (C. Diaz-Latoud, E. Buache, E. Javouhey, and A.-P. Arrigo, *Antioxid Redox Signal* 7: 436–445, 2005). The role of the unique cysteine residue of Hsp27 is unclear because the oligomerization of this protein does not appear to rely on disulfite formation (97), but to require phosphorylation-sensitive interactions at the N-terminus (46). However, disulfite formation between two Hsp27 polypeptides and Hsp27 *S*-thiolation have been observed, especially in oxidative stress conditions (23, 97, 98).

A comparison of the results described in Fig. 4 with those obtained when cells are exposed to etoposide (an apoptotic agent inhibiting topoisomerase II) reveals different domains of Hsp27 that protect against these agents (Table 1). For example, the region comprised of AA 51–88 is essential for the binding to cytochrome *c* and for the protection against apoptosis (15). In contrast, the Δ51–88 deletion only weakly alters Hsp27 protective activity against H_2O_2 induced cell death. The C-terminal part of the protein (AA 141–205) also shows differential protective activity against etoposide or H_2O_2. Hence, different domains appear to be involved in the protection against H_2O_2 or etoposide. As Hsp27 appears to protect against oxidative stress through its chaperone activity (82), several other activities of Hsp27 may be required to control the mitochondrial apoptotic pathway.

Table 1. Protection Mediated by Wild-Type and Mutant Hsp27 Against H_2O_2 and Etoposide Cytotoxicity

	% cell death	
Mutations in human Hsp27	*H_2O_2*	*Etoposide*
Wild type	21.3 ± 4.0	37 ± 4.9
P24A	22.2 ± 4.9	ND
H131L	22.3 ± 3.5	33 ± 4.6
C137A	88.3 ± 4.1	92 ± 4.3
R140G	39.0 ± 6.9	ND
H124L	43.5 ± 5.0	ND
G161L	47.2 ± 3.5	ND
Δ1–88	68.6 ± 2.0	97.4 ± 4.3
Δ15–50	31.7 ± 1.6	40 ± 2.4
Δ51–88	35.1 ± 2.6	110 ± 4.1
Δ141–175	78.2 ± 4.1	49 ± 3.3
Δ123–205	82.3 ± 1.0	87 ± 6.5
Δ177–205	82.7 ± 7.0	34 ± 3.1
Δ87–205	85.8 ± 4.8	92 ± 4.3
Empty vector	100.0 ± 0.0	100.0 ± 0.0

HeLa cells transiently expressing wild type or mutants of human Hsp27 were exposed for 24 h to 600 μ*M* H_2O_2 or 500 μ*M* etoposide. Control cells transfected with the corresponding empty vector were also analyzed. Cell death was estimated by crystal violet staining. Percentage of cell death was obtained from the ratio between the values obtained for the wild type and the different mutants and the value determined when transfection was performed with the empty vector (100%). Standard deviations are indicated (n = 6). ND, not determined; Δ, deletion.

CONCLUSIONS AND PERSPECTIVES

Hsp27 is a negative regulator of programmed cell death with a broad spectrum of action. In addition to its antiapoptotic effect, Hsp27 also appears to play key roles in pathological situations where its expression can either aggravate the pathological state (*i.e.,* cancer cells with impaired apoptosis) (16, 32) or be beneficial to protect against oxidative stress, inflammation, or acute cell death (as, for example, in the case of neurodegenerescence and asthma pathologies where high levels of Hsp27 have been detected) (64, 94). We can therefore be confident that future studies will bring us a better understanding of the molecular mechanisms induced by this chaperone protein that modulate key enzymes of the ROS–glutathione pathway and intracellular iron levels.

ACKNOWLEDGMENTS

This work was supported by the Association pour la recherche sur le cancer (grant no. 4602) and the Région Rhône-Alpes (Thématique Cancer).

ABBREVIATIONS

AA, amino acids; G6PDH, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; Hsf1, heat shock factor 1; Hsp27, heat shock protein-27; IRP, iron regulatory protein; MAPKAPK2-3, mitogen-activated protein kinase-activated protein kinase 2 and 3; $OH^{\bullet}$, hydroxyl radical; ROS, reactive oxygen species; sHsp, small stress protein or small shock protein; TNFα, tumor necrosis factor-α.

REFERENCES

1. Arata S, Hamaguchi S, and Nose K. Effects of the overexpression of the small heat shock protein, Hsp27, on the sensitivity of human fibroblast cells exposed to oxidative stress. *J Cell Physiol* 163: 458–465, 1995.
2. Arrigo A-P. Small stress proteins: chaperones that act as regulators of intracellular redox state and programmed cell death. *J Biol Chem* 379: 19–26, 1998.
3. Arrigo A-P. sHsp as novel regulators of programmed cell death and tumorigenicity. *Pathol Biol (Paris)* 48: 280–288, 2000.
4. Arrigo A-P. Hsp27: novel regulator of intracellular redox state. *IUBMB Life* 52: 303–307, 2001.
5. Arrigo A-P and Ducasse C. Expression of the anti-apoptotic protein Hsp27 during both the keratinocyte differentiation and dedifferentiation of HaCat cells: expression linked to changes in intracellular protein organization? *Exp Gerontol* 37: 1247–1255, 2002.
6. Arrigo A-P and Landry J. Expression and function of the low-molecular-weight heat shock proteins. In: *The Biology of Heat Shock Proteins and Molecular Chaperones*, edited

by Morimoto RI, Tissieres A, and Georgopoulos C). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1994, pp. 335–373.
7. Arrigo A-P and Welch W. Characterization and purification of the small 28,000-dalton mammalian heat shock protein. *J Biol Chem* 262: 15359–15369, 1987.
8. Arrigo A-P, Suhan JP, and Welch WJ. Dynamic changes in the structure and intracellular locale of the mammalian low-molecular-weight heat shock protein. *Mol Cell Biol* 8: 5059–5071, 1988.
9. Arrigo A-P, Paul C, Ducasse C, Manero F, Kretz-Remy C, Virot S, Javouhey E, Mounier N, and Diaz-Latoud C. Small stress proteins: novel negative modulators of apoptosis induced independently of reactive oxygen species. *Prog Mol Subcell Biol* 28: 185–204, 2002.
10. Arrigo A-P, Paul C, Ducasse C, Sauvageot O, and Kretz-Remy C. Small stress proteins: modulation of intracellular redox state and protection against oxidative stress. *Prog Mol Subcell Biol* 28: 171–184, 2002.
11. Baek SH, Min JN, Park EM, Han MY, Lee YS, Lee YJ, and Park YM. Role of small heat shock protein HSP25 in radioresistance and glutathione-redox cycle. *J Cell Physiol* 183: 100–107, 2000.
12. Becker J, Mezger V, Courgeon A, and Best-Belpomme M. On the mechanism of action of H_2O_2 in the cellular stress. *Free Radic Res Commun* 12: 455–460, 1991.
13. Bence NF, Sampat RM, and Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292: 1552–1555, 2001.
14. Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, and Lutsch G. Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. *J Biol Chem* 269: 20780–20784, 1994.
15. Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E, and Garrido C. Hsp27 negatively regulates cell death by interacting with cytochrome *c*. *Nat Cell Biol* 2: 645–652, 2000.
16. Bruey JM, Paul C, Fromentin A, Hilpert S, Arrigo AP, Solary E, and Garrido C. Differential regulation of HSP27 oligomerization in tumor cells grown in vitro and in vivo. *Oncogene* 19: 4855–4863, 2000.
17. Cairo G and Pietrangelo A. Iron regulatory proteins in pathobiology. *Biochem J* 352: 241–250, 2000.
18. Charette SJ, Lavoie JN, Lambert H, and Landry J. Inhibition of Daxx-mediated apoptosis by heat shock protein 27. *Mol Cell Biol* 20: 7602–7612, 2000.
19. Cuenda A, Rouse J, Doza Y, Meier R, Cohen P, Gallagher T, Young P, and Lee J. SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett* 364: 229–233, 1995.
20. Cuesta R, Laroia G, and Schneider RJ. Chaperone Hsp27 inhibits translation during heat shock by binding eIF4G and facilitating dissociation of cap-initiation complexes. *Genes Dev* 14: 1460–1470, 2000.
21. Dalle-Donne I, Rossi R, Milzani A, Di Simplicio P, and Colombo R. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. *Free Radic Biol Med* 31: 1624–1632, 2001.
22. De Jong WW, Caspers GJ, and Leunissen JA. Genealogy of the alpha-crystallin—small heat-shock protein superfamily. *Int J Biol Macromol* 22: 151–162, 1998.
23. Eaton P, Fuller W, and Shattock MJ. *S*-Thiolation of HSP27 regulates its multimeric aggregate size independently of phosphorylation. *J Biol Chem* 277: 21189–21196, 2002.
24. Ehrnsperger M, Graber S, Gaestel M, and Buchner J. Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. *EMBO J* 16: 221–229, 1997.
25. Ehrnsperger M, Gaestel M, and Buchner J. Analysis of chaperone properties of small Hsp's. *Methods Mol Biol* 99: 421–429, 2000.
26. Fish WW. Rapid colorimetric micromethod for the quantitation of complexed iron in biological samples. *Methods Enzymol* 158: 357–364, 1988.
27. Gaestel M, Schroder W, Benndorf R, Lippman C, Buchner K, Hucho F, Erdmann VA, and Bielka H. Identification of the phosphorylation sites of the murine small heat shock protein hsp 25. *J Biol Chem* 266: 14721–14724, 1991.
28. Gaestel M, Benndorf R, Hayess K, Priemer E, and Engel K. Dephosphorylation of the small heat shock protein hsp25 by calcium/calmodulin-dependent (type 2B) protein phosphatase. *J Biol Chem* 267: 21607–21611, 1992.
29. Garrido C. Size matters: of the small HSP27 and its large oligomers. *Cell Death Differ* 9: 483–485, 2002.
30. Garrido C, Mehlen P, Fromentin A, Hammann A, Assem M, Arrigo A-P, and Chauffert B. Inconstant association between 27-kDa heat-shock protein (Hsp27) content and doxorubicin resistance in human colon cancer cells. The doxorubicin-protecting effect of Hsp27. *Eur J Biochem* 237: 653–659, 1996.
31. Garrido C, Ottavi P, Fromentin A, Hammann A, Arrigo AP, Chauffert B, and Mehlen P. HSP27 as a mediator of confluence-dependent resistance to cell death induced by anticancer drugs. *Cancer Res* 57: 2661–2667, 1997.
32. Garrido C, Fromentin A, Bonnotte B, Favre N, Moutet M, Arrigo AP, Mehlen P, and Solary E. Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. *Cancer Res* 58: 5495–5499, 1998.
33. Grune T and Davies KJ. Breakdown of oxidized proteins as a part of secondary antioxidant defenses in mammalian cells. *Biofactors* 6: 165–172, 1997.
34. Grune T, Reinheckel T, and Davies K. Degradation of oxidized proteins in mammalian cells. *FASEB J* 11: 526–534, 1997.
35. Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, and Landry J. Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. *J Cell Sci* 110: 357–368, 1997.
36. Guy G, Cairns J, Ng S, and Tan Y. Inactivation of a redox-sensitive protein phosphatase during the early events of tumor necrosis factor/interleukin-1 signal transduction. *J Biol Chem* 268: 2141–2148, 1993.
37. Halliwell B and Gutteridge J. Role of iron in oxygen radical reactions. *Methods Enzymol* 105: 47–56, 1984.

38. Huot J, Roy G, Lambert H, Chretien P, and Landry J. Increased survival after treatments with anticancer agents of Chinese hamster cells expressing the human M_r 27,000 heat shock protein. *Cancer Res* 51: 5245–5252, 1991.
39. Huot J, Lambert H, Lavoie JN, Guimond A, Houle F, and Landry J. Characterization of 45-kDa/54-kDa HSP27 kinase, a stress sensitive kinase which may activate the phosphorylation-dependent protective function of mammalian 27-kDa heat shock protein HSP27. *Eur J Biochem* 227: 416–427, 1995.
40. Huot J, Houle F, Spitz DR, and Landry J. HSP27 phosphorylation-mediated resistance against actin fragmentation and cell death induced by oxidative stress. *Cancer Res* 56: 273–279, 1996.
41. Huot J, Houle F, Marceau F, and Landry J. Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. *Circ Res* 80: 383–392, 1997.
42. Jacobson MD. Reactive oxygen species and programmed cell death. *Trends Biochem Sci* 21: 83–86, 1996.
43. Jakob U and Buchner J. Assisting spontaneity: the role of Hsp90 and small Hsps as molecular chaperones. *Trends Biochem Sci* 19: 205–211, 1994.
44. Jakob U, Gaestel M, Engels K, and Buchner J. Small heat shock proteins are molecular chaperones. *J Biol Chem* 268: 1517–1520, 1993.
45. Kato K, Hasegawa K, Goto S, and Inaguma Y. Dissociation as a result of phosphorylation of an aggregated form of the small stress protein, hsp27. *J Biol Chem* 269: 11274–11278, 1994.
46. Lambert H, Charette SJ, Bernier AF, Guimond A, and Landry J. HSP27 multimerization mediated by phosphorylation-sensitive intermolecular interactions at the amino terminus. *J Biol Chem* 274: 9378–9385, 1999.
47. Landry J, Chretien P, Lambert H, Hickey E, and Weber LA. Heat shock resistance conferred by expression of the human HSP 27 gene in rodent cells. *J Cell Biol* 109: 7–15, 1989.
48. Landry J, Lambert H, Zhou M, Lavoie JN, Hickey E, Weber LA, and Anderson CW. Human HSP 27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. *J Biol Chem* 267: 794–803, 1992.
49. Lee GJ, Roseman AM, Saibil HR, and Vierling E. A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. *EMBO J* 16: 659–671, 1997.
50. Lelli JL Jr, Becks LL, Dabrowska MI, and Hinshaw DB. ATP converts necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic Biol Med* 25: 694–702, 1998.
51. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, and Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 186: 464–478, 1990.
52. Ludwig S, Engel K, Hoffmeyer A, Sithanandam G, Neufeld B, Palm D, Gaestel M, and Rapp UR. 3pK, a novel mitogen-activated protein (MAP) kinase-activated protein kinase, is targeted by three MAP kinase pathways. *Mol Cell Biol* 16: 6687–6697, 1996.
53. Mairesse N, Horman S, Mosselmans R, and Galand P. Antisense inhibition of the 27 kDa heat shock protein production affects growth rate and cytoskeletal organization in MCF-7 cells. *Cell Biol Int* 20: 205–212, 1996.
54. Martins EA, Robalinho RL, and Meneghini R. Oxidative stress induces activation of a cytosolic protein responsible for control of iron uptake. *Arch Biochem Biophys* 316: 128–134, 1995.
55. Mehlen P and Arrigo A-P. The serum-induced phosphorylation of mammalian hsp27 correlates with changes in its intracellular localization and levels of oligomerization. *Eur J Biochem* 221: 327–334, 1994.
56. Mehlen P, Briolay J, Smith L, Diaz-Latoud C, Pauli D, and Arrigo A-P. Analysis of the resistance to heat and hydrogen peroxide stresses in COS cells transiently expressing wild type or deletion mutants of the *Drosophila* 27-kDa heat-shock protein. *Eur J Biochem* 215: 277–284, 1993.
57. Mehlen P, Kretz-Remy C, Briolay J, Fostan P, Mirault ME, and Arrigo AP. Intracellular reactive oxygen species as apparent modulators of heat-shock protein 27 (hsp27) structural organization and phosphorylation in basal and tumour necrosis factor alpha-treated T47D human carcinoma cells. *Biochem J* 312: 367–375, 1995.
58. Mehlen P, Mehlen A, Guillet D, Préville X, and Arrigo A-P. Tumor necrosis factor-α induces changes in the phosphorylation, cellular localization, and oligomerization of human hsp27, a stress protein that confers cellular resistance to this cytokine. *J Cell Biochem* 58: 248–259, 1995.
59. Mehlen P, Préville X, Chareyron P, Briolay J, Klemenz R, and Arrigo A-P. Constitutive expression of human hsp27, *Drosophila* hsp27, or human alpha B-crystallin confers resistance to TNF- and oxidative stress-induced cytotoxicity in stably transfected murine L929 fibroblasts. *J Immunol* 154: 363–374, 1995.
60. Mehlen P, Préville X, Kretz-Remy C, and Arrigo A-P. Human hsp27, *Drosophila* hsp27 and human αB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these protein against TNFα-induced cell death. *EMBO J* 15: 2695–2706, 1996.
61. Mehlen P, Hickey E, Weber L, and Arrigo A-P. Large unphosphorylated aggregates as the active form of hsp27 which controls intracellular reactive oxygen species and glutathione levels and generates a protection against TNFα in NIH-3T3-ras cells. *Biochem Biophys Res Commun* 241: 187–192, 1997.
62. Mehlen P, Mehlen A, Godet J, and Arrigo A-P. hsp27 as a switch between differentiation and apoptosis in murine embryonic stem cells. *J Biol Chem* 272: 31657–31665, 1997.
63. Mello-Filho AC and Meneghini R. Iron is the intracellular metal involved in the production of DNA damage by oxygen radicals. *Mutat Res* 251: 109–113, 1991.
64. Merendino AM, Paul C, Vignola AM, Costa MA, Melis M, Chiappara G, Izzo V, Bousquet J, and Arrigo A-P. Heat shock protein-27 protects human bronchial epithelial cells against oxidative stress-mediated apoptosis: possible implication in asthma. *Cell Stress Chaperones* 7: 269–280, 2002.
65. Miron T, Vancompernolle K, Vandekerckhove J, Wilchek M, and Geiger B. A 25-kD inhibitor of actin polymeriza-

tion is a low molecular mass heat shock protein. *J Cell Biol* 114: 255–261, 1991.
66. Mounier N and Arrigo AP. Actin cytoskeleton and small heat shock proteins: how do they interact? *Cell Stress Chaperones* 7: 167–176, 2002.
67. Nicotera P, Leist M, and Ferrando-May E. Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol Lett* 102–103: 139–142, 1998.
68. Nover L, Scharf K-D, and Neumann D. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol Cell Biol* 9: 1298–1308, 1989.
69. Oesterreich S, Weng C-N, Qiu M, Hilsenbeck SG, Osborne CK, and Fuqua SW. The small heat shock protein hsp27 is correlated with growth and drug resistance in human breast cancer cell lines. *Cancer Res* 53: 4443–4448, 1993.
70. Pandey P, Farber R, Nakazawa A, Kumar S, Bharti A, Nalin C, Weichselbaum R, Kufe D, and Kharbanda S. Hsp27 functions as a negative regulator of cytochrome *c*-dependent activation of procaspase-3. *Oncogene* 19: 1975–1981, 2000.
71. Parcellier A, Gurbuxani S, Schmitt E, Solary E, and Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochem Biophys Res Commun* 304: 505–512, 2003.
72. Parcellier A, Schmitt E, Gurbuxani S, Seigneurin-Berny D, Pance A, Chantome A, Plenchette S, Khochbin S, Solary E, and Garrido C. HSP27 is a ubiquitin-binding protein involved in I-kappaBalpha proteasomal degradation. *Mol Cell Biol* 23: 5790–5802, 2003.
73. Park YM, Han MY, Blackburn RV, and Lee YJ. Overexpression of HSP25 reduces the level of TNF alpha-induced oxidative DNA damage biomarker, 8-hydroxy-2'-deoxyguanosine, in L929 cells. *J Cell Physiol* 174: 27–34, 1998.
74. Paul C and Arrigo A-P. Comparison of the protective activities generated by two survival proteins: Bcl-2 and Hsp27 in L929 murine fibroblasts exposed to menadione or staurosporine. *Exp Gerontol* 35: 757–766, 2000.
75. Paul C, Manero F, Gonin S, Kretz-Remy C, Virot S, and Arrigo A-P. Hsp27 as a negative regulator of cytochrome C release. *Mol Cell Biol* 22: 816–834, 2002.
76. Polla AS, Polla LL, and Polla BS. Iron as the malignant spirit in successful ageing. *Ageing Res Rev* 2: 25–37, 2003.
77. Préville X, Gaeste M, and Arrigo A-P. Phosphorylation is not essential for protection of L929 cells by Hsp25 against H_2O_2-mediated disruption of actin cytoskeleton, a protection which appears related to the redox change mediated by Hsp25. *Cell Stress Chaperones* 3: 177–187, 1998.
78. Préville X, Schultz H, Knauf U, Gaestel M, and Arrigo A P. Analysis of the role of Hsp25 phosphorylation reveals the importance of the oligomerization state of this small heat shock protein in its protective function against TNFalpha- and hydrogen peroxide-induced cell death. *J Cell Biochem* 69: 436–452, 1998.
79. Preville X, Salvemini F, Giraud S, Chaufour S, Paul C, Stepien G, Ursini MV, and Arrigo AP. Mammalian small stress proteins protect against oxidative stress through their ability to increase glucose-6-phosphate dehydrogenase activity and by maintaining optimal cellular detoxifying machinery. *Exp Cell Res* 247: 61–78, 1999.
80. Rane MJ, Pan Y, Singh S, Powell DW, Wu R, Cummins T, Chen Q, McLeish KR, and Klein JB. Heat shock protein 27 controls apoptosis by regulating akt activation. *J Biol Chem* 278: 27828–27835, 2003.
81. Richards EH, Hickey E, Weber LA, and Master JR. Effect of overexpression of the small heat shock protein HSP27 on the heat and drug sensitivities of human testis tumor cells. *Cancer Res* 56: 2446–2451, 1996.
82. Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, Paul C, Wieske M, Arrigo AP, Buchner J, and Gaestel M. Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. *J Biol Chem* 274: 18947–18956, 1999.
83. Salvemini F, Franze A, Iervolino A, Filosa S, Salzano S, and Ursini MV. Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. *J Biol Chem* 274: 2750–2757, 1999.
84. Samali A, Nordgren H, Zhivotovsky B, Peterson E, and Orrenius S. A comparative study of apoptosis and necrosis in HepG2 cells: oxidant-induced caspase inactivation leads to necrosis. *Biochem Biophys Res Commun* 255: 6–11, 1999.
85. Sitte N, Merker K, and Grune T. Proteasome-dependent degradation of oxidized proteins in MRC-5 fibroblasts. FEBS Lett 440: 399–402, 1998.
86. Sitte N, Huber M, Grune T, Ladhoff A, Doecke WD, Von Zglinicki T, and Davies KJ. Proteasome inhibition by lipofuscin/ceroid during postmitotic aging of fibroblasts. *FASEB J* 14: 1490–1498, 2000.
87. Souren JE, Van Aken H, and Van Wijk R. Enhancement of superoxide production and protection against heat shock by HSP27 in fibroblasts. *Biochem Biophys Res Commun* 227: 816–821, 1996.
88. Stadtman ER. Protein oxidation and aging. *Science* 257: 1220–1224, 1992.
89. Starke PE and Farber JL. Ferric iron and superoxide ions are required for the killing of cultured hepatocytes by hydrogen peroxide. Evidence for the participation of hydroxyl radicals formed by an iron-catalyzed Haber–Weiss reaction. *J Biol Chem* 260: 10099–10104, 1985.
90. Stokoe D, Engel K, Campbell D, Cohen P, and Gaestel M. Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. *FEBS Lett* 313: 307–313, 1992.
91. Trautinger F, Kokesch C, Herbacek I, Knobler RM, and Kindas-Mugge I. Overexpression of the small heat shock protein, hsp27, confers resistance to hyperthermia, but not to oxidative stress and UV-induced cell death, in a stably transfected squamous cell carcinoma cell line. *J Photochem Photobiol* 39: 90–95, 1997.
92. Von Zglinicki T and Brunk UT. Intracellular interactions under oxidative stress and aging: a hypothesis. *Z Gerontol* 26: 215–220, 1993.
93. Wang G, Klostergaard J, Khodadadian M, Wu J, Wu TW, Fung KP, Carper SW, and Tomasovic SP. Murine cells transfected with human Hsp27 cDNA resist TNF-induced cytotoxicity. *J Immunother Emphasis Tumor Immunol* 19: 9–20, 1996.

94. Wyttenbach A, Sauvageot O, Carmichael J, Diaz-Latoud C, Arrigo AP, and Rubinsztein DC. Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum Mol Genet* 11: 1137–1151, 2002.
95. Yan LJ, Christians ES, Liu L, Xiao X, Sohal RS, and Benjamin IJ. Mouse heat shock transcription factor 1 deficiency alters cardiac redox homeostasis and increases mitochondrial oxidative damage. *EMBO J* 21: 5164–5172, 2002.
96. Zantema A, Verlaam-De Vries M, Maasdam D, Bol S, and van der Eb A. Heat shock protein 27 and αB-crystallin can form a complex, which dissociates by heat shock. *J Biol Chem* 267: 12936–12941, 1992.
97. Zavialov A, Benndorf R, Ehrnsperger M, Zav'yalov V, Dudich I, Buchner J, and Gaestel M. The effect of the intersubunit disulfide bond on the structural and functional properties of the small heat shock protein Hsp25. *Int J Biol Macromol* 22: 163–173, 1998.
98. Zavialov AV, Gaestel M, Korpela T, and Zav'yalov VP. Thiol/disulfide exchange between small heat shock protein 25 and glutathione. *Biochim Biophys Acta* 1388: 123–132, 1998.
99. Zucker B, Hanusch J, and Bauer G. Glutathione depletion in fibroblasts is the basis for apoptosis-induction by endogenous reactive oxygen species. *Cell Death Differ* 4: 388–395, 1997.

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Received for publication September 25, 2004; accepted October 9, 2004.

This article has been cited by:

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2. Ling Huang, Jie Xiang, Jiazhou Liu, Tingzhao Rong, Jing Wang, Yanli Lu, Qilin Tang, Wen Wen, Moju Cao. 2012. Expression characterization of genes for CMS-C in maize. *Protoplasma* **249**:4, 1119-1127. [CrossRef]
3. Aparna Vidyasagar, Shannon R Reese, Omeed Hafez, Ling-Jin Huang, William F Swain, Lynn M Jacobson, Jose R Torrealba, Pierre-Emmanuel Chammas, Nancy A Wilson, Arjang Djamali. 2012. Tubular expression of heat-shock protein 27 inhibits fibrogenesis in obstructive nephropathy. *Kidney International* . [CrossRef]
4. G. F. Dilly, C. R. Young, W. S. Lane, J. Pangilinan, P. R. Girguis. 2012. Exploring the limit of metazoan thermal tolerance via comparative proteomics: thermally induced changes in protein abundance by two hydrothermal vent polychaetes. *Proceedings of the Royal Society B: Biological Sciences* **279**:1741, 3347-3356. [CrossRef]
5. Delphine Peric, Jean Labarre, François Chevalier, Germain Rousselet. 2012. Impairing the microRNA biogenesis pathway induces proteome modifications characterized by size bias and enrichment in antioxidant proteins. *PROTEOMICS* **12**:14, 2295-2302. [CrossRef]
6. Qiang Wan, Ilson Whang, Jehee Lee. 2012. Molecular and functional characterization of HdHSP20: A biomarker of environmental stresses in disk abalone Haliotis discus discus. *Fish & Shellfish Immunology* **33**:1, 48-59. [CrossRef]
7. Serena Carra, Valeria Crippa, Paola Rusmini, Alessandra Boncoraglio, Melania Minoia, Elisa Giorgetti, Harm H. Kampinga, Angelo Poletti. 2012. Alteration of protein folding and degradation in motor neuron diseases: Implications and protective functions of small heat shock proteins. *Progress in Neurobiology* **97**:2, 83-100. [CrossRef]
8. Aparna Vidyasagar, Nancy A Wilson, Arjang Djamali. 2012. Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. *Fibrogenesis & Tissue Repair* **5**:1, 7. [CrossRef]
9. Pragathi Pallepati, Diana A. Averill-Bates. 2011. Activation of ER stress and apoptosis by hydrogen peroxide in HeLa cells: Protective role of mild heat preconditioning at 40°C. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1813**:12, 1987-1999. [CrossRef]
10. Jose Luis Lopez Guerra, Qingyi Wei, Xianglin Yuan, Daniel Gomez, Zhensheng Liu, Yan Zhuang, Ming Yin, Minghuan Li, Li-E Wang, James D. Cox, Zhongxing Liao. 2011. Functional promoter rs2868371 variant of HSPB1 associates with radiation-induced esophageal toxicity in patients with non-small-cell lung cancer treated with radio(chemo)therapy. *Radiotherapy and Oncology* **101**:2, 271-277. [CrossRef]
11. Shivali Duggal, Jan E. Brinchmann. 2011. Importance of serum source for the in vitro replicative senescence of human bone marrow derived mesenchymal stem cells. *Journal of Cellular Physiology* **226**:11, 2908-2915. [CrossRef]
12. Diana Lebherz-Eichinger, Hendrik J. Ankersmit, Stefan Hacker, Hubert Hetz, Oliver Kimberger, Elisabeth M. Schmidt, Thomas Reiter, Walter H. Hörl, Martin Haas, Claus G. Krenn, Georg A. Roth. 2011. HSP27 and HSP70 serum and urine levels in patients suffering from chronic kidney disease. *Clinica Chimica Acta* . [CrossRef]
13. Emna El Golli-Bennour, Hassen Bacha. 2011. Hsp70 expression as biomarkers of oxidative stress: Mycotoxins' exploration. *Toxicology* **287**:1-3, 1-7. [CrossRef]
14. Alessandra Stacchiotti, Giovanni Li Volti, Antonio Lavazza, Ilaria Schena, Maria Francesca Aleo, Luigi Fabrizio Rodella, Rita Rezzani. 2011. Different role of Schisandrin B on mercury-induced renal damage in vivo and in vitro. *Toxicology* **286**:1-3, 48-57. [CrossRef]
15. Adrienne T. Black, Patrick J. Hayden, Robert P. Casillas, Diane E. Heck, Donald R. Gerecke, Patrick J. Sinko, Debra L. Laskin, Jeffrey D. Laskin. 2011. Regulation of Hsp27 and Hsp70 expression in human and mouse skin construct models by caveolae following exposure to the model sulfur mustard vesicant, 2-chloroethyl ethyl sulfide. *Toxicology and Applied Pharmacology* **253**:2, 112-120. [CrossRef]
16. Ruicheng Hu, Qing Ouyang, Aiguo Dai, Shuangxiang Tan, Zhiqiang Xiao, Cene Tang. 2011. Heat Shock Protein 27 and Cyclophilin A Associate with the Pathogenesis of COPD. *Respirology* no-no. [CrossRef]
17. B Gibert, E Hadchity, A Czekalla, M-T Aloy, P Colas, C Rodriguez-Lafrasse, A-P Arrigo, C Diaz-Latoud. 2011. Inhibition of heat shock protein 27 (HspB1) tumorigenic functions by peptide aptamers. *Oncogene* . [CrossRef]
18. Po Yee Chiu , Philip Y. Lam , Hoi Yan Leung , Pou Kuan Leong , Chung Wah Ma , Qing Tao Tang , Kam Ming Ko . 2011. Co-Treatment with Shengmai San-Derived Herbal Product Ameliorates Chronic Ethanol-Induced Liver Damage in Rats. *Rejuvenation Research* **14**:1, 17-23. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]

19. Petra Seibold, Rebecca Hein, Peter Schmezer, Per Hall, Jianjun Liu, Norbert Dahmen, Dieter Flesch-Janys, Odilia Popanda, Jenny Chang-Claude. 2011. Polymorphisms in oxidative stress-related genes and postmenopausal breast cancer risk. *International Journal of Cancer* n/a-n/a. [CrossRef]
20. Amanda M. O'Reilly, R. William Currie, David B. Clarke. 2010. HspB1 (Hsp 27) Expression and Neuroprotection in the Retina. *Molecular Neurobiology* **42**:2, 124-132. [CrossRef]
21. Dayangku Fatiha Pengiran Burut, Anwar Borai, Callum Livingstone, Gordon Ferns. 2010. Serum heat shock protein 27 antigen and antibody levels appear to be related to the macrovascular complications associated with insulin resistance: a pilot study. *Cell Stress and Chaperones* **15**:4, 379-386. [CrossRef]
22. Yao-Ping Lin, Meng - Erh Hsu, Yi-Ying Chiou, Hung-Yi Hsu, Hiao-Chien Tsai, Yu-Ju Peng, Chi-Yu Lu, Chien-Yuan Pan, Wen-Chung Yu, Chen-Huan Chen, Chin-Wen Chi, Chao-Hsiung Lin. 2010. Comparative proteomic analysis of rat aorta in a subtotal nephrectomy model. *PROTEOMICS* **10**:13, 2429-2443. [CrossRef]
23. Evgeny V. Mymrikov, Olesya V. Bukach, Alim S. Seit-Nebi, Nikolai B. Gusev. 2010. The pivotal role of the #7 strand in the intersubunit contacts of different human small heat shock proteins. *Cell Stress and Chaperones* **15**:4, 365-377. [CrossRef]
24. Geneviève Morrow, Hyun-Ju Kim, Marie Le Pécheur, Sunil C. Kaul, Renu Wadhwa, Robert M. Tanguay. 2010. Protection from aging by small chaperones. *Annals of the New York Academy of Sciences* **1197**:1, 67-75. [CrossRef]
25. Pou Kuan Leong , Na Chen , Po Yee Chiu , Hoi Yan Leung , Chung Wah Ma , Qing Tao Tang , Kam Ming Ko . 2010. Long-Term Treatment with Shengmai San-Derived Herbal Supplement (Wei Kang Su) Enhances Antioxidant Response in Various Tissues of Rats with Protection Against Carbon Tetrachloride Hepatotoxicity. *Journal of Medicinal Food* **13**:2, 427-438. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
26. Yifeng Jia, Shiaw-Lin Wu, Jeff S. Isenberg, Shujia Dai, John M. Sipes, Lyndsay Field, Bixi Zeng, Russell W. Bandle, Lisa A. Ridnour, David A. Wink, Ramani Ramchandran, Barry L. Karger, David D. Roberts. 2010. Thiolutin inhibits endothelial cell adhesion by perturbing Hsp27 interactions with components of the actin and intermediate filament cytoskeleton. *Cell Stress and Chaperones* **15**:2, 165-181. [CrossRef]
27. Marzia Perluigi, Fabio Di Domenico, Carla Blarzino, Cesira Foppoli, Chiara Cini, Alessandra Giorgi, Caterina Grillo, Federico De Marco, David A Butterfield, Maria E Schinin#, Raffaella Coccia. 2010. Effects of UVB-induced oxidative stress on protein expression and specific protein oxidation in normal human epithelial keratinocytes: a proteomic approach. *Proteome Science* **8**:1, 13. [CrossRef]
28. Alessandra Stacchiotti, Giovanni Li Volti, Antonio Lavazza, Rita Rezzani, Luigi Fabrizio Rodella. 2009. Schisandrin B stimulates a cytoprotective response in rat liver exposed to mercuric chloride. *Food and Chemical Toxicology* **47**:11, 2834-2840. [CrossRef]
29. Alessandra Stacchiotti, Fausta Morandini, Francesca Bettoni, Ilaria Schena, Antonio Lavazza, Pier Giovanni Grigolato, Pietro Apostoli, Rita Rezzani, Maria Francesca Aleo. 2009. Stress proteins and oxidative damage in a renal derived cell line exposed to inorganic mercury and lead. *Toxicology* **264**:3, 215-224. [CrossRef]
30. Tanzeel Ahmed, Ashok K. Tripathi, Sanvidhan G. Suke, Vivek Kumar, Rafat S. Ahmed, Shukla Das, Basu Dev Banerjee. 2009. Role of HSP27 and reduced glutathione in modulating malathion-induced apoptosis of human peripheral blood mononuclear cells: Ameliorating effect of N-acetylcysteine and curcumin. *Toxicology in Vitro* **23**:7, 1319-1325. [CrossRef]
31. V. I. Kulinsky, L. S. Kolesnichenko. 2009. The glutathione system. II. Other enzymes, thiol-disulfide metabolism, inflammation, and immunity, functions. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry* **3**:3, 211-220. [CrossRef]
32. Feng Wang, Maohui Feng, Ping Xu, Han Xiao, Piye Niu, Xiaobo Yang, Yun Bai, Ying Peng, Pinfang Yao, Hao Tan, Robert M. Tanguay, Tangchun Wu. 2009. The level of Hsp27 in lymphocytes is negatively associated with a higher risk of lung cancer. *Cell Stress and Chaperones* **14**:3, 245-251. [CrossRef]
33. Bernadett Kalmar, Linda Greensmith. 2009. Induction of heat shock proteins for protection against oxidative stress. *Advanced Drug Delivery Reviews* **61**:4, 310-318. [CrossRef]
34. M. S. Allagui, R. Nciri, M. F. Rouhaud, J. C. Murat, A. El Feki, F. Croute, C. Vincent. 2009. Long-term Exposure to Low Lithium Concentrations Stimulates Proliferation, Modifies Stress Protein Expression Pattern and Enhances Resistance to Oxidative Stress in SH-SY5Y Cells. *Neurochemical Research* **34**:3, 453-462. [CrossRef]
35. LEI SHAO, RICARDO E. PEREZ, WILLIAM T. GERTHOFFER, WILLIAM E. TRUOG, DONG XU. 2009. Heat Shock Protein 27 Protects Lung Epithelial Cells From Hyperoxia-Induced Apoptotic Cell Death. *Pediatric Research* **65**:3, 328-333. [CrossRef]

36. Luis Da Silva-Azevedo, Sebastian Jähne, Christian Hoffmann, Daniel Stalder, Manfred Heller, Axel R. Pries, Andreas Zakrzewicz, Oliver Baum. 2009. Up-regulation of the peroxiredoxin-6 related metabolism of reactive oxygen species in skeletal muscle of mice lacking neuronal nitric oxide synthase. *The Journal of Physiology* **587**:3, 655-668. [CrossRef]
37. Agnese Viganò, Marilena Ripamonti, Sara De Palma, Daniele Capitanio, Michele Vasso, Robin Wait, Carsten Lundby, Paolo Cerretelli, Cecilia Gelfi. 2008. Proteins modulation in human skeletal muscle in the early phase of adaptation to hypobaric hypoxia. *PROTEOMICS* **8**:22, 4668-4679. [CrossRef]
38. Bertrand Friguet, Anne-Laure Bulteau, Isabelle Petropoulos. 2008. Mitochondrial protein quality control: Implications in ageing. *Biotechnology Journal* **3**:6, 757-764. [CrossRef]
39. Majid Ghayour-Mobarhan, Amirhossein Sahebkar, Seyyed Mohammad Reza Parizadeh, Mohsen Moohebati, Shima Tavallaie, Seyyed Mohammad RezaKazemi-Bajestani, Habib-Allah Esmaeili, Gordon Ferns. 2008. Antibody titres to heat shock protein 27 are elevated in patients with acute coronary syndrome. *International Journal of Experimental Pathology* **89**:3, 209-215. [CrossRef]
40. Marie-Thérèse Aloy, Elie Hadchity, Clara Bionda, Chantal Diaz-Latoud, Line Claude, Robert Rousson, André-Patrick Arrigo, Claire Rodriguez-Lafrasse. 2008. Protective Role of Hsp27 Protein Against Gamma Radiation–Induced Apoptosis and Radiosensitization Effects of Hsp27 Gene Silencing in Different Human Tumor Cells. *International Journal of Radiation Oncology*Biology*Physics* **70**:2, 543-553. [CrossRef]
41. André-Patrick Arrigo, Stéphanie Simon, Benjamin Gibert, Carole Kretz-Remy, Mathieu Nivon, Anna Czekalla, Dominique Guillet, Maryline Moulin, Chantal Diaz-Latoud, Patrick Vicart. 2007. Hsp27 (HspB1) and #B-crystallin (HspB5) as therapeutic targets. *FEBS Letters* **581**:19, 3665-3674. [CrossRef]
42. J S Isenberg, Y Jia, L Field, L A Ridnour, A Sparatore, P Soldato, A L Sowers, G C Yeh, T W Moody, D A Wink, R Ramchandran, D D Roberts. 2007. Modulation of angiogenesis by dithiolethione-modified NSAIDs and valproic acid. *British Journal of Pharmacology* **151**:1, 142-151. [CrossRef]
43. Anne Mulligan Tuttle, Julie Gauley, Norman Chan, John J. Heikkila. 2007. Analysis of the expression and function of the small heat shock protein gene, hsp27, in Xenopus laevis embryos. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **147**:1, 112-121. [CrossRef]
44. Alejandra Decanini, Curtis L. Nordgaard, Xiao Feng, Deborah A. Ferrington, Timothy W. Olsen. 2007. Changes in Select Redox Proteins of the Retinal Pigment Epithelium in Age-related Macular Degeneration. *American Journal of Ophthalmology* **143**:4, 607-615.e2. [CrossRef]
45. Wance J. J. Firdaus, Andreas Wyttenbach, Paola Giuliano, Carole Kretz-Remy, R. William Currie, André-Patrick Arrigo. 2006. Huntingtin inclusion bodies are iron-dependent centers of oxidative events. *FEBS Journal* **273**:23, 5428-5441. [CrossRef]
46. Giuseppe Filomeni , Maria R. Ciriolo . 2006. Redox Control of Apoptosis: An Update. *Antioxidants & Redox Signaling* **8**:11-12, 2187-2192. [Abstract] [Full Text PDF] [Full Text PDF with Links]
47. Joanna Joyner-Matos, Craig A. Downs, David Julian. 2006. Increased expression of stress proteins in the surf clam Donax variabilis following hydrogen sulfide exposure. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **145**:2, 245-257. [CrossRef]
48. Po Yee Chiu, Hoi Yan Leung, Michel K. T. Poon, Duncan H. F. Mak, Kam Ming Ko. 2006. (–)Schisandrin B is more potent than its enantiomer in enhancing cellular glutathione and heat shock protein production as well as protecting against oxidant injury in H9c2 cardiomyocytes. *Molecular and Cellular Biochemistry* **289**:1-2, 185-191. [CrossRef]
49. Gordon Ferns, Sedigheh Shams, Shahida Shafi. 2006. Heat shock protein 27: its potential role in vascular disease. *International Journal of Experimental Pathology* **87**:4, 253-274. [CrossRef]
50. Wance J. J. Firdaus, Andreas Wyttenbach, Chantal Diaz-Latoud, R. W. Currie, Andre-Patrick Arrigo. 2006. Analysis of oxidative events induced by expanded polyglutamine huntingtin exon 1 that are differentially restored by expression of heat shock proteins or treatment with an antioxidant. *FEBS Journal* **273**:13, 3076-3093. [CrossRef]
51. Michael R. Eman, Elsa Regan-Klapisz, Martijn W. H. Pinkse, Inge M. Koop, Johan Haverkamp, Albert J. R. Heck, Arie J. Verkleij, Jan A. Post. 2006. Protein expression dynamics during replicative senescence of endothelial cells studied by 2-D difference in-gel electrophoresis. *ELECTROPHORESIS* **27**:8, 1669-1682. [CrossRef]
52. Andre-Patrick Arrigo, Sophie Virot, Sylvain Chaufour, Wance Firdaus, Carole Kretz-Remy, Chantal Diaz-Latoud. 2005. Hsp27 Consolidates Intracellular Redox Homeostasis by Upholding Glutathione in Its Reduced Form and by Decreasing Iron Intracellular Levels. *ChemInform* **36**:39. . [CrossRef]
53. Kiyoshi Nose . 2005. Redox Control of Protein Trafficking. *Antioxidants & Redox Signaling* **7**:3-4, 303-307. [Citation] [Full Text PDF] [Full Text PDF with Links]