Cell Death and Differentiation (2002) 9, 483–485

www.nature.com/cdd

News and Commentary

Size matters: of the small HSP27 and its large oligomers

C Garrido*,[1]

[1] INSERM 517. Faculty of Medicine, University of Burgundy, 7 Boulevard Jeanne D'Arc, 21033 Dijon, France
* Corresponding author: Tel: 33 3 80 39 33 53; Fax: 33 3 80 39 34 34; E-mail: cgarrido@u-bourgogne.fr
DOI: 10.1038/sj/cdd/44001005

Stress or heat shock proteins, like the Bcl-2 family of proteins, include anti- and pro-apoptotic members whose expression could determine cell fate in response to death stimuli.[1] The small heat shock protein HSP27, similar to Bcl-2, has strong anti-apoptotic properties and functions at multiple steps of the apoptotic signaling pathway. In addition, both proteins modulate reactive oxygen species (ROS) and glutathione levels. However, the comparison stops there.

Structurally, HSP27 is characterized by a well conserved C-terminal domain with homology to α-crystallins from the vertebrate eye, a flexible C-terminal tail and a weakly conserved N-terminal domain containing a hydrophobic WDPF motif, necessary for the protein oligomerization.[2] This characteristic feature of HSP27 endows the protein with the ability to form large oligomers (up to 800 Kda). HSP27 oligomerization is a dynamic process that depends on the physiology of the cells, the phosphorylation status of the protein and exposure to stress. The dimer of HSP27 seems to be the building block for the multimeric complex of the protein. HSP27 can be phosphorylated at three serine residues and its dephosphorylation enhances oligomerization. This phosphorylation is a reversible process, catalyzed by the MAPKAP kinases 2 and 3, that can be induced by a variety of stress including differentiating agents, mitogens, inflammatory cytokines such as TNFα and IL-1β, hydrogen peroxide and other oxidants.[2] We have recently shown that HSP27 phosphorylation is important for the regulation of its oligomerization, but only in exponentially growing cells cultured *in vitro*. However, in tumor cells growing *in vivo* or grown at confluence *in vitro*, cell–cell contact induces the formation of large oligomers independently of the phosphorylation status of the protein.[3]

HSP27 is expressed in many cell types and tissues, at specific stages of differentiation and development,[2] and the failure to obtain knock-out mice suggests that this protein is essential for development. HSP27 is a powerful ATP-independent chaperone *in vitro*, that inhibits aggregation and promotes the refolding of denatured proteins.[4] When overexpressed in tumor cells, this protein protects against cell death triggered by various stimuli. Paradoxically, such stimuli often induce HSP27 overexpression, providing an example of how pro-apoptotic stimuli can elicit protective responses when delivered below a threshold level. The protective effect of HSP27 applies to both necrosis and apoptosis, depending on the stress stimulus and the cell type. For example, HSP27 protects murine L929 fibrosarcoma and NIH3T3-ras fibroblasts from necrosis induced by TNFα.[5] The apoptotic pathways inhibited by HSP27 include those induced by staurosporine, agonist of the fas death receptor (also called CD95 and APO-1), growth factor deprivation, hydrogen peroxide, sodium arsenite, hyperthermia, UV radiation and anticancer drugs.[1] Several mechanisms may account for HSP27's cytoprotective activity. The resistance may result from the ability of HSP27 to accelerate the recovery from the stress-induced blockage of RNA and protein synthesis, and nuclear protein aggregation.[6] In addition, the protein could enhance the antioxidant defense of cells by increasing cellular glutathione content.[5] The cytoprotective effect of HSP27 has also been attributed to its capacity to bind and stabilize actin polymerization.[7] We have recently shown that HSP27 interferes with the cytochrome *c*-dependent mitochondrial apoptotic pathway. However, unlike Bcl-2, it does not affect cycochrome *c* release from the mitochondria but binds to the released cytochrome *c* (but not AIF) in the cytosol.[8,9] The heme group of cytochrome *c* is necessary but not sufficient for this interaction. The region between amino-acids 51 and 141 of HSP27 is indispensable for the interaction with cytochrome *c* and dimerization of the protein is required. This specific interaction has functional consequences, since it prevents formation of the apoptosome and thereby caspase activation.[9] HSP27 has also been shown to affect the death receptor mediated apoptotic pathway. HSP27 interacts with Daxx to prevent Fas-mediated cell death.[10]

Confluent cells are more resistant than proliferative cells to cell death induced by different anti-cancer agents. Confluence induces the accumulation of HSP27, the multimerization of the protein, its cellular relocalization and a drastic decrease in ROS cellular content.[11] Since confluent cells imitate better than proliferative cells the growth conditions within solid tumors, HSP27 could contribute to the high resistance of solid tumors to chemotherapeutic drugs. Clinically, in a number of cancers such as breast cancer, endometrial cancer and leukemia, an increased level of HSP27, relative to its level in non-transformed cells, has been detected.[2] The fact that HSP27 is often highly expressed in cells or tissues from a wide range of tumors supports the hypothesis that this protein could limit the efficacy of cancer therapy. Similarly, the cytoprotective effect of HSP27 on tumor cells could influence their tumorigenicity. Experimental models support this hypothesis since HSP27 increases the tumorigenic potential of rodent cells in syngeneic host.[3,12] However, clinicopathological and immunohistochemical studies attempting to correlate the level of HSP27 in tumor cells with tumor progression and clinical outcome provided contra-

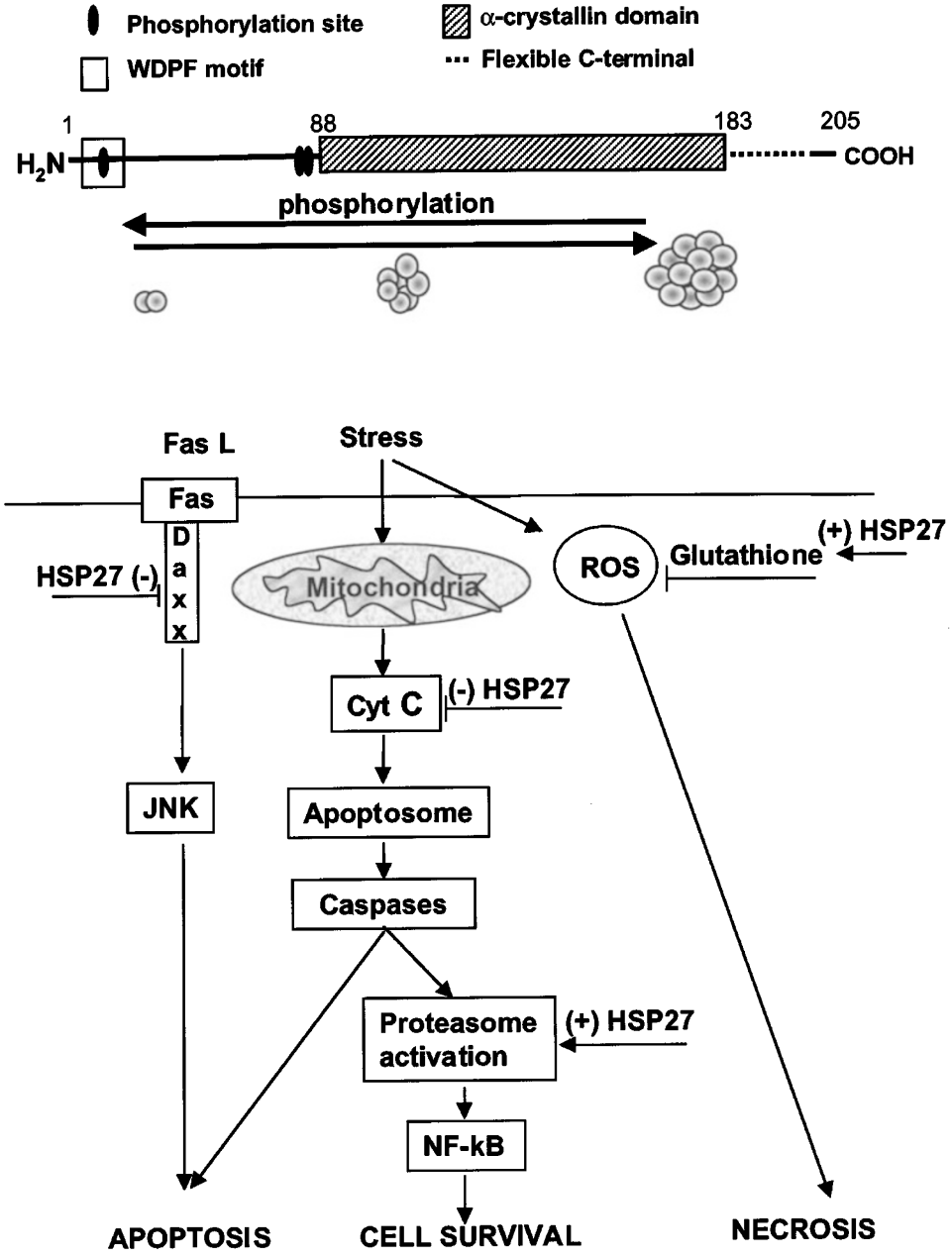

Figure 1 Schematic representation of HSP27 structural organization and proposed protective function (see text). A minus sign denotes negative regulation and a plus sign indicates positive regulation

dictory results.[2] It is possible that in human tumors the protective effect of HSP27 may be bypassed by a variety of other modulators, under certain circumstances.

How does HSP27 accomplish all this? The oligomerization/phosphorylation status of the protein seems to determine its association with different cellular partners and therefore its biochemical functions. Purified phosphorylated monomers, as opposed to the non-phosphorylated form, bind to actin and behave as actin cap-binding proteins. This binding was shown to inhibit actin polymerization *in vitro*, an effect that might account for the ability of the protein to modulate filament stability and reorganization *in vivo*, e.g. in endothelial cells.[7] On the other hand, only large oligomers of HSP27 have demonstrated an ATP-independent chaperone activity *in vitro* by absorbing heat denatured proteins on its surface, preventing their aggregation and keeping them in a folding competent state.[13] Subsequent phosphorylation-dependent dissociation of multimeric HSP27 could clear protein folding intermediates from HSP27 to allow other chaperone molecules such as HSP70 to renature these proteins. We have demonstrated *in vitro* and *in vivo*, that large oligomers of HSP27 are the active form responsible for the general caspase-dependent anti-apoptotic effect of HSP27.[3] Similarly, only large

oligomers have the capacity to decrease ROS content.[13] In contrast, only phosphorylated HSP27, and therefore most likely small oligomers, associate with Daxx.[10] Thus, it appears that multimerization/phosphorylation probably alters the conformation of the protein and hence its capacity to interact with a given protein.

Are the different biochemical effects of HSP27 related to its chaperone activity? It seems unlikely. First, while small phosphorylated oligomers of HSP27 have the capacity to associate to certain proteins with some physiological repercussions at least in cultured cells, only large oligomers show chaperone activity. Secondly, even though large oligomers are the active form of the protein for its anti-apoptotic as well as chaperone properties, different regions of the protein seem involved. While the 33 amino acids of N-terminal region, adjacent to the highly conserved α-crystallin domain of the protein, are dispensable for its chaperone activity *in vitro*,[14] this region is essential for HSP27 binding to cytochrome *c*.[9] In conclusion, HSP27 biochemical functions may be modulated by its oligomeric status which is a highly dynamic process. Depending on the cellular needs, HSP27 could modulate its interaction to various cellular partners by shifting towards small or large oligomers. Phosphorylation, cell–cell contact and probably other factors regulate this equilibrium. The different protein–protein interactions, not always directly related to its chaperone function, may determine the biochemical properties of the protein and the fate of the cell. Further studies are needed to unravel the fine mechanisms of such interactions and will provide invaluable information on the pathophysiological role of HSP27.

Acknowledgements

I thank E Solary, S Gurbixani and A Pance for their valuable suggestions.

1. Garrido *et al.* (2001) Biochem. Biophys. Res. Commun. 286: 433–442
2. Garrido *et al.* (2000) Recent Res. Devel. Cancer 2: 105–114
3. Bruey *et al.* (2000) Oncogene 19: 4855–4863
4. Ehrnsperger *et al.* (1997) EMBO J. 16: 221–229
5. Mehlen *et al.* (1996) EMBO J. 15: 2695–2706
6. Carper *et al.* (1997) J. Cell Biochem. 66: 153–164
7. Lavoie *et al.* (1995) Mol. Cell Biol. 15: 505–516
8. Garrido *et al.* (1999) FASEB J. 13: 2061–2070
9. Bruey *et al.* (2000) Nat. Cell Biol. 2: 645–652
10. Charrette *et al.* (2000) Mol. Cell Biol. 20: 7602–7612
11. Garrido *et al.* (1997) Cancer Res. 57: 2661–2669
12. Garrido *et al.* (1998) Cancer Res. 58: 5495–5499
13. Rogalla *et al.* (1999) J. Biol. Chem. 274: 18947–18956
14. Gio *et al.* (2000) Biochem. Biophys. Res. Commun. 270: 183–189