

Role of the Heat Shock Response and Molecular Chaperones in Oncogenesis and Cell Death

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Exposure of cells to conditions of environmental stress—including heat shock, oxidative stress, heavy metals, or pathologic conditions, such as ischemia and reperfusion, inflammation, tissue damage, infection, and mutant proteins associated with genetic diseases—results in the inducible expression of heat shock proteins that function as molecular chaperones or proteases. Molecular chaperones are a class of proteins that interact with diverse protein substrates to assist in their folding, with a critical role during cell stress to prevent the appearance of folding intermediates that lead to misfolded or otherwise damaged molecules. Consequently, heat shock proteins assist in the recovery from stress either by repairing damaged proteins (protein refolding) or by degrading them, thus restoring protein homeostasis and promoting cell survival. The events of cell stress and cell death are linked, such that molecular chaperones induced in response to stress appear to function at key regulatory points in the control of apoptosis. On the basis of these observations—and on the role of molecular chaperones in the regulation of steroid aporeceptors, kinases, caspases, and other protein remodeling events involved in chromosome replication and changes in cell structure—it is not surprising that the heat shock response and molecular chaperones have been implicated in the control of cell growth. In this review, we address some of the molecular and cellular events initiated by cell stress—the interrelationships between stress signaling, cell death, and oncogenesis—and chaperones as potential targets for cancer diagnosis and treatment. [J Natl Cancer Inst 2000;92:1564–72]

CELLULAR RESPONSE TO STRESS

The heat shock response was discovered in 1962 by Ritossa (1), who observed a pattern of *Drosophila* salivary gland chromosome puffs that were induced in response to transient exposures to elevated temperatures. Since then, efforts from a large number of investigators have shown that the heat shock response is ubiquitous and highly conserved—in all organisms from bacteria to plants and animals—as an essential defense mechanism for protection of cells from a wide range of harmful conditions, including heat shock, alcohols, inhibitors of energy metabolism, heavy metals, oxidative stress, fever, or inflammation (2,3). Stress-inducing agents often affect the redox state and hydration of the cell, which, in turn, causes increased levels of misfolded proteins that may be deleterious by virtue of their altered biologic activities.

The cellular response to stress is represented at the molecular level by the induced synthesis of heat shock proteins (Hsps), of which molecular chaperones and proteases represent two well-

characterized families of proteins. Whereas molecular chaperones function in protein folding, translocation, and refolding of intermediates, proteases, such as the ubiquitin-dependent proteasome, ensure that damaged and short-lived proteins are degraded efficiently. Exposure of cells to acute and chronic stress shifts the protein-folding equilibrium, such that molecular chaperones are directed toward the capture of folding intermediates to prevent misfolding and aggregation and to facilitate refolding or degradation (4–6). Hsps have been classified into six major families according to their molecular size: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small heat shock proteins (Table 1). Within each gene family are members that are constitutively expressed, inducibly regulated, and/or targeted to different compartments. For example, Hsp90 functions in both the cytosolic and nuclear compartments, whereas Grp94 performs an analogous function in the endoplasmic reticulum. Likewise, members of the Hsp70 family exhibit complex patterns of growth-regulated and stress-induced gene expression and are targeted to different subcellular compartments. For example, Hsc70 (heat shock constitutive 70) and Hsp70 proteins are cytosolic and nuclear, whereas Grp78 (glucose-regulated protein 78) is localized to the endoplasmic reticulum and mHsp70 (mitochondrial Hsp70)/Grp74 (glucose-regulated protein 74) is a mitochondrial-localized protein.

The cellular response to stress has been an invaluable tool for investigating the mechanisms and dynamics of inducible gene expression in eukaryotes (2,3). The molecular analysis of Hsp genes identified the heat shock element (HSE)—a stress-responsive promoter element essential for heat shock inducibility—which comprises multiple adjacent inverted arrays of the binding site (5'-nGAAn-3'). HSEs are positioned at various distances upstream of the site of transcription initiation; in vertebrates, inducible transcription requires the *de novo* binding of heat shock transcription factors (HSFs) transiently to the HSEs (7,8). Whereas vertebrates and plants have at least four members of the HSF gene family, only a single HSF is expressed in *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Drosophila*. In human cells, three HSFs (HSF1, HSF2, and HSF4) have been characterized (7,8). HSF1 is ubiquitously expressed and has the principal role in the stress-induced expression of Hsp genes and appears functionally equivalent to *Drosophila* HSF.

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Table 1. Brief summary of the nomenclature, location, and function of the major heat shock protein (Hsp) families*

| Family | Organism | Chaperones | Location | Functions (reference Nos.) |
|------------|----------------------|--------------------------------------|-----------------|---|
| Hsp100 | <i>E. coli</i> | ClpA,B,C | Cytosol | Role in stress tolerance; helps the resolubilization of heat-inactivated proteins from insoluble aggregates (82). |
| | <i>S. cerevisiae</i> | HSP104 | Cytosol | |
| Hsp90 | <i>E. coli</i> | HtpG | Cytosol | Role in signal transduction (e.g., interaction with steroid hormone receptors, tyrosine kinases, serine/threonine kinases); refolds and maintains proteins <i>in vitro</i> ; autoregulation of the heat shock response; role in cell cycle and proliferation (54,55,115–120). |
| | <i>S. cerevisiae</i> | HSP83 | Cytosol | |
| | Mammals | HSP90 | Cytosol | |
| | | GRP94 | ER | |
| Hsp70 | <i>E. coli</i> | DnaK | Cytosol | Roles in lambda phage replication; autoregulation of the heat shock response; interaction with nascent chain polypeptides; functions in interorganellar transport; roles in signal transduction; refolds and maintains denatured proteins <i>in vitro</i> ; role in cell cycle and proliferation; antiapoptotic activity; potential antigen-presenting molecule in tumor cells (43–46,86–95). |
| | | <i>S. cerevisiae</i> | Ssa 1–4 | |
| | Mammals | Ssb 1,2 | Cytosol | |
| | | Kar2 | ER | |
| | | Ssc1 | Mitochondria | |
| | | HSC70 | Cytosol/nucleus | |
| | | HSP70 | Cytosol/nucleus | |
| | | BIP | ER | |
| | | mHSP70 | Mitochondria | |
| | | groEL | Cytosol | |
| Hsp60 | <i>E. coli</i> | groEL | Cytosol | Refolds and prevents aggregation of denatured proteins <i>in vitro</i> ; may facilitate protein degradation by acting as a cofactor in proteolytic systems; role in the assembly of bacteriophages and Rubisco (an abundant protein in the chloroplast) (82). |
| | <i>S. cerevisiae</i> | HSP60 | Mitochondria | |
| | Plants | Cpn60 | Chloroplasts | |
| | Mammals | HSP60 | Mitochondria | |
| Hsp40 | <i>E. coli</i> | dnaJ | Cytosol | Essential cochaperone activity with Hsp70 proteins to enhance rate of adenosine triphosphatase activity and substrate release (43–47). |
| | <i>S. cerevisiae</i> | Ydj1 | Cytosol/nucleus | |
| | Mammals | Hdj1 and Hdj2 | | |
| Small Hsps | <i>E. coli</i> | lbp A and B | Cytosol | Suppresses aggregation and heat inactivation of proteins <i>in vitro</i> ; confers thermotolerance through stabilization of microfilaments; antiapoptotic activity (96,97). |
| | <i>S. cerevisiae</i> | HSP27 | Cytosol | |
| | Mammals | α A and α B crystallin | Cytosol | |
| | | HSP27 | Cytosol | |

**E. coli* = *Escherichia coli*, *S. cerevisiae* = *Saccharomyces cerevisiae*, and ER = endoplasmic reticulum.

HSF2 is activated during specific stages of development, in hemin-induced cell differentiation, and during inhibition of the ubiquitin-dependent proteasome (8). HSF4 is expressed in a tissue-specific manner and displays constitutive DNA-binding activity, and at least one isoform of HSF4 acts as an inhibitor of stress-induced gene expression (7,8). HSF3, which, to date, has been characterized only in avian cells, is activated along with HSF1 by stress to broaden the range of conditions and temperatures over which the heat shock response can be activated and exhibits a co-dependency for regulation of both HSFs (8).

Genes encoding HSPs are also transcriptionally regulated by a variety of physiologic processes not typically associated with cell stress, including the cell cycle, cell proliferation, and differentiation (9–11). These observations have led to suggestions that Hsp70 and Hsp90 may also have critical functions during cell growth, specifically associated with the cell cycle and the proliferative response (12–15). Hsp expression is also modulated by many of the conditions leading to apoptosis (16–20) and is associated with pathologic states, including ischemia, fever, inflammation, infection, and cancer (21–25). Altered expression of Hsps has also been reported for nearly all classes of tumors; these observations, while intriguing, have not resolved whether the association with the pathogenesis of cancer is causal or correlative. Since stress-signaling events must initiate two interconnected yet opposing pathways, for survival and for apoptosis, we propose that cell stress and cell death are likely to have multiple points of regulatory cross-talk. The balance between these two pathways depends on the specific nature and intensity of stress such that the expression level and activity of individual components of the pathways will determine, ultimately, the fate of the cell. Because a prominent characteristic of tumor cells is their

resistance to cell death, the ability of Hsps to protect cells from apoptosis should stimulate further study.

CANCER AND THE DEREGULATED EXPRESSION OF HSP GENES

Cells or tissues from a wide range of tumors have been shown to express atypical levels of one or more Hsps (21,22). Such observations have led to suggestions that Hsps could be used as biomarkers. For example, Hsp expression in breast or gastric cancer is associated with poor prognosis and resistance to chemotherapy or radiation therapy (22–24,26). While these observations beg the question whether the altered expression of a particular Hsp is due to the suboptimal cellular environment in the hypoxic poorly vascularized tumor, the aberrant expression of Hsps via their pleiotropic activities as molecular chaperones affords the cancer cell with an opportunity to alter the properties of numerous key proteins, such as transcription factors and cell-signaling molecules. Consequently, the altered levels of Hsps could lead to the loss of control of cell growth and inhibitory effects on apoptosis.

Increased levels of Hsp27, relative to its level in nontransformed cells, have also been detected in a number of cancers, such as breast cancer, endometrial cancer, and leukemia (22,26). In addition, the analysis of the pattern of Hsp27 phosphorylation in tumor cells appears distinct and characteristic as compared with the phosphorylation pattern of Hsp27 in primary untransformed cells (26). Consequently, it has been proposed that the diversity of Hsp27 phosphorylation isoforms could also represent useful tumor markers, although a more comprehensive analysis of tissue samples will be required. The Hsp27 gene,

which contains an imperfect estrogen-responsive element (27), can be induced by estrogen in breast tissue (26). However, the relationship between estrogen regulation of Hsp27 and its aberrant expression in hormone response tumors, while provocative, is not a consistent predictor of response to hormonal treatment, since only a subset of estrogen-positive breast tumors expresses high levels of Hsp27 (26).

Elevated expression of members of the Hsp70 family has also been reported in high-grade malignant tumors (28–31). In breast tumors, elevated expression of Hsp70 is associated with short-term disease-free survival, metastasis, and poor prognosis among patients treated with combined chemotherapy, radiation therapy, and hyperthermia (28,32,33). Other members of the Hsp family—including Hsp90 α , Hsp90 β , and Hsp60—are also overexpressed in breast tumors, lung cancer, leukemias, and Hodgkin's disease (24,34–36). The molecular basis for overexpression of Hsps in tumor cells has not been well studied and may have multiple molecular etiologies, for example, associated with the complexity of the promoter region of the human Hsp70 gene (21). In the adenovirus-transformed human embryonic kidney 293 cell line, overexpression of the endogenous Hsp70 gene is associated with the presence of the potent adenovirus transactivator E1a (37–39); in contrast, in human A431 carcinoma cells, increased expression of the Hsp90 β gene is due, at least in part, to gene amplification (40).

LESSONS FROM VIRAL STRATEGIES AND THE REGULATION OF CELLULAR STRESS GENES

During the course of viral infection, the expression of heat shock genes is induced by activation of the cellular stress response. Adenovirus infection induces transcription of the cellular Hsp70 and Hsp90 α genes through binding of the viral E1A protein to CBP/p300, a component of the basal transcriptional machinery (9,38,41,42). Although the molecular mechanisms by which certain viruses activate cellular stress genes have been elucidated, the specific requirements for elevated levels of chaperones in the viral life cycle remain uncharacterized. Certainly, it would not be unexpected that the rapid burst in protein components required for assembly of the virion would require parallel expression of molecular chaperones to ensure proper assembly of a macromolecular structure. Among the first examples of viral activation of host cellular stress responses was the demonstration that bacteriophage lambda utilizes components of the Hsp70 chaperone machinery (dnaK and dnaJ) to assemble the multiprotein complex at the origin of replication and to ensure the sequence of events for chromosome replication (43–45). This example suggests that the cellular stress response serves the infectious agent, not only to utilize the chaperone machinery for virus formation, as a means to manipulate the host cell, but also perhaps to allow the virus to escape various forms of surveillance.

HSPS, ONCOGENES, AND p53

Simian virus 40 (SV40) large T antigen, a prototypic viral oncogene, has recently been shown to contain a J domain that corresponds to a region that is conserved among all cellular DnaJ/Hsp40 molecular chaperones and is essential for chaperone activity and interaction with the Hsp70 chaperone machine (46–48). Evidence to suggest a link between chaperones and deregulated cell growth is that forced overexpression of chaperones by stable transfection in cultured cells or in transgenic

animals results in cellular transformation and tumor formation. Overexpression of either Hsp27 or Hsp70 increases the tumorigenic potential of rodent cells in syngeneic hosts and the metastatic potential of human breast cancer cells in nude mice (49,50). Additional intriguing observations are that overexpression of Hsp70 alone in primary cells can lead to transformation (51); likewise, when Hsp70 overexpression is turned off, the transformed phenotype is reversed. Consistent with these observations, overexpression of human Hsp70 in transgenic mice results in the development of T-cell lymphomas (52).

How do these observations relate to the molecular induction of Hsp levels in response to stress as a molecular event that must occur frequently during the life of a cell and that stress exposure itself is not known to result directly in the transformed phenotype or increased risk to transformation? A likely explanation is that forced overexpression of Hsps may complement the transformed phenotype by altering the activities of key regulatory proteins, but this overexpression, by itself, is insufficient to cause cellular transformation. The process of cellular transformation may utilize components of the cellular stress response machinery to alter the conformation and/or activities of mutant tumor suppressor proteins. Likewise, aberrant levels of molecular chaperones may potentiate the transforming activity of oncogenes, such as mutant p53, and interfere with the stress-signaling mechanism, thus perturbing a cellular defense mechanism that would lead normally to the elimination of transformed cells by apoptosis.

A plausible role for Hsps in tumorigenesis is as a modifier of protein activities, in particular, components of the cell cycle machinery, kinases, and other proteins implicated in cancer progression. Hsp90 interacts with tyrosine kinase oncogene products pp60-v-src, fes, and fgr to form highly stable complexes (53–56). Interaction with Hsp90 alters the half-life of pp60-v-src and modulates its kinase activity and substrate specificity (54), leading to the hypothesis that altered levels of Hsp90 detected in tumor cells may be associated with the oncogenic activity of this kinase. Hsp70 has been detected in complexes with proteins, including SV40 large T antigen, adenovirus E1A protein, cellular c-myc, and the tumor suppressor protein p53 (also known as TP53) (57–59). Such interactions have been suggested to alter protein activities by altering protein conformation or association with other proteins and also to modulate the half-life by modulating ubiquitination and targeted degradation. Since c-myc has also been implicated in the expression of the Hsp70 gene (60,61), this suggests a mechanism in which the cell responds to elevated levels of c-myc protein by inducing the synthesis of Hsp70, which, in turn, interacts with c-myc to inhibit its transforming ability.

The association of mutant forms of p53 that transform cells with both cytosolic and nuclear localized chaperones Hsp70/Hsc70 affords intriguing insights on a possible mechanism in which genetic mutations in p53 that affect its conformation utilize the chaperone machinery to facilitate and stabilize these altered conformational states (59,62). Wild-type p53 is essential for the negative regulation of the cell cycle and regulation of apoptosis via its function as a transcriptional regulator (63–66). Among the p53 target genes identified to date, those relevant to the antiproliferative function of p53 include bax (67), Hsp70 (68), and c-fos (69). Mutation or deletion of one allele of the p53 gene and deletion of the second allele lead to cell proliferation and transformation and are among the most common genetic

alterations in human cancer (62,65,66,70,71). Mutant p53 lacks many of the properties of its wild-type counterpart and exhibits a longer half-life that allows accumulation to much higher levels (65,66). The accumulation of mutant p53 is considered to be an effective marker of poor prognosis in breast (72) and gastric (73) cancers.

Association of mutant p53 with Hsc70/Hsp70 was observed by direct biochemical characterization of p53 complexes, although it has not been established whether this event alone is associated with transformation. The increased stability of mutant p53 may be due to the formation of stable chaperone complexes (57,62,74). Overexpression of Hsp70/Hsc70 can suppress the transforming property of mutant p53 (75), suggesting that sequestration of mutant p53 by the chaperone could reduce the opportunity for wild-type and mutant p53s to associate, thereby allowing the wild-type protein to perform its antiproliferative activity (76). The interaction between mutant p53 and the Hsp70 chaperones could also result in conformational diversity of p53, thus influencing the interactions of mutant p53 with other client substrates. It has been shown that the DNA-binding activity of wild-type p53 can be activated *in vitro* by the *Escherichia coli* Hsp70 (62), which suggests that activation of p53 in normal cells could also be triggered by members of the Hsp70 family. Another provocative proposition is that Hsp70 could participate in the antigenic presentation of mutant p53, assisting its translocation from the nucleus to the cell surface, perhaps analogous to the role of the Hsc70/Hsp70 chaperones in clathrin-mediated exocytosis, in which the chaperone either would be released or play an immunogenic role. Indeed, some breast and lung cancer patients possess anti-p53 antibodies, and, with Hsp70-p53 complexes, can be detected in extracts from tumor tissues (77). Further understanding of these mechanisms may be of great interest for the development of anticancer strategies targeted to Hsp70 overexpression as a way to suppress p53-induced transformation.

HSPS AND CELL DEATH

Stress Responses and Apoptosis: Cross-talk and Checkpoints

Exposure of cells to stress activates a survival response via the induction of Hsps, yet, if the exposure to a specific stress is intensified, cell death will nevertheless occur, either by necrosis or apoptosis. Apoptosis, or programmed cell death, has been principally characterized in the context of embryonic morphogenesis and development (78–80). During the commitment or induction phase, a number of signals cause the cell to enter the apoptotic pathway by altering the balance of proapoptotic and antiapoptotic proteins that determine either susceptibility or resistance to apoptosis. Once the decision is made, cells enter the execution phase and activate a cascade of caspases (cysteine-containing aspartic acid-specific proteases) that cleave specific downstream targets and result in irreversible cellular degradation, organellar dysfunction, condensation of nuclear chromatin, cytoplasmic shrinkage, membrane blebbing, nuclear fragmentation, and formation of apoptotic bodies. Finally, the clearance stage involves phagocytosis and degradation of apoptotic bodies by macrophages or neighboring cells.

An increasing number of reports now reveal that the pathways leading to apoptosis and the stress response are linked. Heat shock and other stressful conditions that induce the heat shock response can also lead to apoptosis or necrosis, in part determined by the intensity and duration of the stress (81). How-

ever, the heat shock response can also protect against stress-induced cell death via a cell-protective process known as thermotolerance or cytoprotection, in which exposure of cells to mild stress conditions, sufficient to induce the expression and accumulation of Hsps, protects against a subsequent challenge from another stress that is, by itself, lethal (82). Since survival and death correspond to opposite cellular events, there should be multiple checkpoints and points of regulatory cross-talk to ensure that cells sustaining repairable molecular damage survive and that cells damaged beyond repair undergo cell death. There is increasing evidence that Hsps may function at multiple points in the apoptotic signaling pathway (Fig. 1), which suggests that constant titration occurs between these pathways, and that this balance can determine the fate of the stressed cell.

Hsps and Protection of Tumor Cells From Apoptosis

The proposal that Hsps interfere with apoptotic signaling is consistent with observations that high levels of Hsps are often detected in tumors (16–19). Apoptosis is the negative counterpart of proliferation; therefore, defects in apoptosis are associated with maintenance of the transformed state and cancer (78–80). In tumor cells, the intricate balance between proliferation and cell death shifts toward continued cell growth as a result of the expression of antiapoptotic proteins. Such proteins include members of the Bcl-2 family, members of the inhibitory of apoptosis protein family, and members of the HSP family—in particular, Hsp70 and Hsp27—that render tumor cells resistant to apoptosis (18,19,83,84).

Inducible Hsp70 has been suggested to have a multiple roles in cytoprotection against apoptosis; indeed, abrogation of Hsp70 expression by use of antisense oligonucleotides leads to inhibition of tumor cell proliferation and apoptosis (85). Consistent with this proposal, high levels of Hsp70 prevent stress-induced apoptosis. Elevated levels of Hsp70, attained in transient transfections or under the control of tetracycline-inducible promoters, reduce or block caspase activation and suppress mitochondrial damage and nuclear fragmentation (86,87). In this scenario, Hsp70 has been shown to inhibit apoptosis by preventing the recruitment of procaspases 9 and 3, to the apoptosome complex, thereby preventing the assembly of a functional apoptosome (88). Hsp70 has also been proposed to act on the apoptotic pathway at an earlier step by preventing JNK activation (86,89–91); however, JNK-independent apoptosis induced by Fas cannot be suppressed by Hsp70 (91). This result suggests that JNK itself or regulators of JNK activity could also be targets of Hsp70. In the case of tumor necrosis factor-induced apoptosis, Hsp70 rescues cells from apoptosis downstream of JNK activation, suggesting that the Hsp70 may also prevent the effector step of apoptotic cell death (86,93).

How does Hsp70 protect cells against apoptosis? A possible mechanism of action could be through binding of Hsp70 to proapoptotic proteins, such as p53 and c-myc (57,59). Hsp70 also interacts with and is repressed by Bag11, an antiapoptotic protein that enhances the activities of Bcl2 and Raf-1 (93,94). Complementary observations have been made for Hsp27, when overexpressed Hsp27 has been shown to block apoptosis induced by heat, Fas ligand, H₂O₂, and anticancer drugs (96,97). However, Hsp27 does not confer other stresses, such as resistance against lymphokine-activated killer cells or UV radiation (97); these results reveal that different chaperones are not redundant in protection against stress-induced cell death.

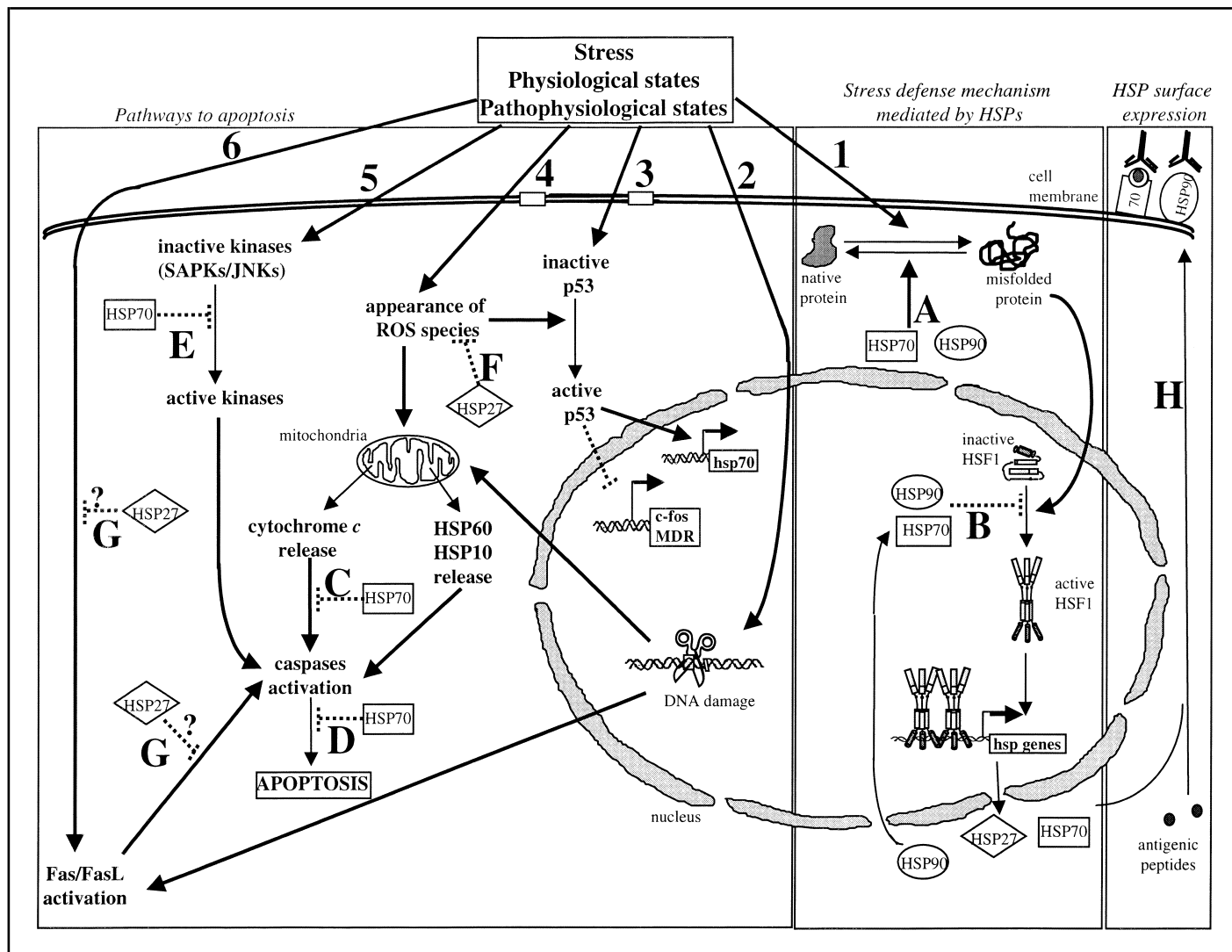


Fig. 1. Schematic of the major cellular pathways activated by stress. Exposure of cells to various stresses leads to the coordinate or separate activation of several major signaling pathways. **Solid arrows** = activation events, **dotted arrows** = inhibitory events, and HSP = heat shock protein. Environmental stresses (heat shock, UV irradiation, and toxic chemicals), physiologic stresses (oxygen levels, metabolic state, and pH), and pathophysiologic stresses (fever, inflammation, tissue injury, infection, ischemia/reperfusion, and cancer) are associated with the appearance of misfolded proteins (pathway 1), whose accumulation results in the activation of the heat shock transcription factor in the nucleus, which, in turn, activates the transcription of hsp genes (2,3,7,8). Stress such as UV irradiation also induces DNA damage (pathway 2), which can result in apoptotic cell death mediated by the release of cytochrome *c* from mitochondria and subsequent activation of caspases (78–81). The mitochondrial release of HSP60/HSP10, which also occurs during induction of apoptosis, may accelerate activation of caspases. The tumor suppressor protein p53 can also be activated as a consequence of stress exposure (pathway 3), resulting in the increased transcription of some p53 target genes, such as Hsp70, and the decreased transcription of other genes, such as *c-fos* or multidrug resistance genes (66–69). Stress also results in increased levels of reactive oxygen species (ROS) (pathway 4), whose accumu-

lation causes the release of cytochrome *c* from the mitochondria, leading to the activation of caspases and eventually apoptosis. Stress-activated protein kinases become activated on stress (SAPKs-stress activated protein kinases or JNKs-c-jun n-terminal kinase) (pathway 5), and this event also results in the caspase-dependent apoptotic pathway. Finally, stress, such as DNA damage, can increase the Fas/FasL ligand (pathway 6), leading to caspase-dependent apoptotic cell death independently of mitochondrial involvement (88–90). HSPs are proposed to function at different levels of these interrelated pathways. First, HSPs will assist folded intermediates and misfolded proteins to acquire a native state (4–6) (pathway A). HSPs have also been shown to play a role in the negative regulation of their own synthesis by autoregulation (8) (pathway B). Hsp70 is also able to block the apoptotic pathway at several levels: It can either inhibit caspase activation by cytochrome *c* (pathway C), block metabolic events downstream of caspases activation (pathway D), or inhibit SAPKs/JNKs activation (17,85–93) (pathway E). Hsp27 can also inhibit the appearance of ROS species (pathway F) and can also block Fas-induced apoptosis (96,97) (pathway G). Finally, at least in some tumor cells, HSPs can be expressed at the cell surface where they may play a role either as antigen or more likely as antigen-presenting molecules (100,103,104) (pathway H).

HSPS AS TARGETS FOR CANCER THERAPY

Hsps as Tumor Antigens or Antigen-Presenting Molecules: Hsp-Based Vaccines Against Cancer?

The rationale for using Hsp-based vaccines against tumor antigens follows from observations that a subset of the Hsps is detected antigenically on the surface of certain tumor cells,

where they can activate the immune response resulting in elimination by the immune system (98–101). In a chemically induced murine tumor, the two Hsp90 isoforms were detected in the cytoplasm and on the surface of tumor cells, where they were accessible to the host immune system (102). The cell-surface expression of intracellular members of the Hsp70 family (103,104) and of the endoplasmic reticulum-resident heat shock

protein Grp94 (105) in tumor cells has also been reported. The appearance of Hsp70 on the plasma membrane can be induced by heat or chemical stress in sarcoma and leukemic cells but not in normal untransformed cells (104). While these observations are provocative, it is unclear how Hsps appear on the cell surface, since Hsps are cytosolic or nuclear-localized proteins that are not known to be membrane associated or membrane anchored. A trivial explanation is that Hsps are released by neighboring dead cells and adsorbed on the surface of the cell, although this would not explain differences among normal and transformed cells (98–101). The appearance of cytosolic chaperones on the cell surface, induced by stress, perhaps associated with the transformed state, could suggest a novel stress-induced transport mechanism to signal that the cell is stressed and damaged for detection and removal by the immune system.

Another intriguing feature of these observations is the proposal that Hsps are antigenic *per se* or that they could function as accessory molecules in antigen presentation. Stress proteins are known to be the major antigens recognized by the immune system in a number of pathologic states, including bacterial infection, autoimmune diseases, inflammation, and neurodegenerative diseases (101,106,107). However, while the initial observations have indicated that Hsps or fragments of Hsps could function as antigens and be recognized by the immune system, more recent animal studies (98,99,108) suggest a role for Hsps in Hsp-peptide complexes, in which the associated peptide elicits an immune response. Indeed, examination of preparations of Hsp70 and Grp96 (Grp94) used in these studies on Hsps as adjuvants in vaccines (109,110) has revealed the presence of chaperone-associated peptides. Furthermore, Hsps depleted of these associated peptides were not immunogenic (109,110). As a practical approach, vaccination of mice with preparations of Hsps derived from autologous tumors elicited resistance against the tumors from which they were isolated (98–101,110), although suppression of subsequent metastases was variable (111). It is noteworthy that autoimmunity has not been observed in mice or in clinical trials for progressive malignancies, perhaps because the immune response is targeted toward the Hsp-associated peptides and not against the carrier Hsps (99,100,108).

A particularly attractive aspect of vaccination with Hsp-peptide complexes is the elevated immunogenicity per unit of immunogen. Indeed, several immunizations of mice with low concentrations of Grp96 appear sufficient to render animals resistant to tumor growth (100,111). Since patients with metastasis have lower rates of disease-free survival than patients with a primary tumor, the use of Hsp-based vaccines could provide a valuable adjuvant to other current forms of treatment to prevent disease recurrence and to increase patient survival. The search for specific tumor antigens continues to be a major objective for cancer therapy; therefore, the use of Hsp-peptide complexes offers a potentially safe alternative source of tumor-specific antigens that could be used to target tumor cells for drug delivery or for elimination by the host immune system. As a potentially general application, the use of Hsps as adjuvants could, as well, be valuable in the treatment of diseases other than cancer.

Hsp90 as an Anticancer Drug Target

The search for new anticancer drugs identified the benzoquinone ansamycin family of antibiotics (112,113) that act by inhibiting cell proliferation and reversing oncogenic transforma-

tion (56,114). Examination of the mechanism of action of geldanamycin, a well-characterized member of the ansamycins, identified the amino-terminal adenosine triphosphate (ATP)/adenosine diphosphate-binding domain of Hsp90 as the target; binding of geldanamycin to Hsp90 results in the competitive inhibition of ATPase activity (115,116). Consequently, exposure of cells to geldanamycin has pleiotropic effects on Hsp90-substrate interactions and results in the decreased activity of essential key signal transduction proteins, including steroid receptors, cell cycle kinases, transcription factors, and p53 (117–120). Radicol, an antifungal agent that also binds to the N-terminal domain of Hsp90, inhibits chaperone activity and suppresses transformation by the Src and Ras oncogenes (121). On the basis of these two promising drugs, Hsp90 has recently emerged as a promising target for anticancer therapy (120); however, one might expect that drugs targeting Hsp90 will have side effects on normal cellular function given the role of Hsp90 in diverse biologic processes.

Hsps and Hyperthermia in Cancer Treatment

As an adjuvant to radiation therapy and chemotherapy in clinical trials, hyperthermia has proven to be successful as a therapy to kill or weaken tumor cells in a wide range of cancers (122–124). The use of hyperthermia in the treatment of cancer was first demonstrated by Crile (125), who observed the heat-induced regression of melanomas implanted in the feet of mice. Hyperthermia is particularly effective in combination with radiotherapy and chemotherapy, perhaps by increasing the toxicity of the drug or effectiveness of radiation exposure to the tumor cells, while decreasing the dose and associated side effects. Two complexities associated with hyperthermia in cancer treatment are the induction of thermotolerance, the state in which cells transiently acquire resistance to multiple stress conditions by prior exposure to a sublethal heat shock (82), and multidrug resistance in which cells treated with one chemotherapeutic agent can acquire a drug-resistant state (126). Either the thermotolerant state or the multidrug-resistant state can counteract the therapeutic effects of hyperthermia or render the heat-treated tumors more resistant to further chemical or radiation treatments (122–124,127), thereby limiting the potential use of hyperthermia in cancer treatment.

Despite the success of hyperthermia in cancer therapy and our substantial understanding of the molecular biologic events associated separately with hyperthermia, chemical, or radiation therapy, what remains unclear are the events that occur at a molecular level, *in vivo*, during and after various combinations of treatment modalities.

CONCLUSIONS

Studies on the regulation of the heat shock response and the function of molecular chaperones have provided numerous insights into the dominant effects of the environment and physiologic stress on cell growth and cell-signaling pathways that initiate repair, allow adaptation, and ensure survival. The fundamental nature of the strategies employed by the cell to detect and respond to stress and the principles that govern the decisions by which the cell executes the choice to live or die support our hypothesis that the cell stress response and cell death pathways are intimately related. As we obtain more information, we will have a better understanding of which proteins in a particular pathway utilize chaperone activity or chaperone interactions to

modify protein structure and function directly. For example, the identification of protein–protein interactions between specific chaperones and components of the apoptotic machinery—such as steroid aporeceptors, Bag1, the apoptosome, kinases, or caspases—offers intriguing possibilities for measuring heat shock proteins as markers of the intensity and duration of cell stress. The identification and characterization of specific protein complexes that are targeted for such stress-dependent interactions should uncover molecules and complexes that offer novel strategies for investigations of small molecule anticancer agents. In addition, the appearance of Hsp70 and other chaperones at tumor surfaces offers new directions to be exploited and enhanced to increase the sensitivity for or optimize the detection of the tumor antigen or antigen-presenting cell for elimination by the immune system. Similarly, the initial success in rodent model systems of vaccination with Hsp–peptide complexes from autologous tumor samples that confers resistance against tumor recurrence suggests a protective role for Hsps against cancer.

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NOTES

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