REVIEW

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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See "Notes" following "References."

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	*E. coli*	ClpA,B,C	Cytosol	Role in stress tolerance; helps the resolubilization of heat-inactivated proteins from insoluble aggregates *(82)*.
	S. cerevisiae	HSP104	Cytosol	
Hsp90	*E. coli*	HtpG	Cytosol	Role in signal transduction (e.g., interaction with steroid hormone receptors, tyrosine kinases, serine/threonine kinases); refolds and maintains proteins *in vitro*; autoregulation of the heat shock response; role in cell cycle and proliferation *(54,55,115–120)*.
	S. cerevisiae	HSP83	Cytosol	
	Mammals	HSP90	Cytosol	
		GRP94	ER	
Hsp70	*E. coli*	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 *in vitro*; role in cell cycle and proliferation; antiapoptotic activity; potential antigen-presenting molecule in tumor cells *(43–46,86–95)*.
	S. cerevisiae	Ssa 1–4	Cytosol	
		Ssb 1,2	Cytosol	
		Kar2	ER	
		Ssc1	Mitochondria	
	Mammals	HSC70	Cytosol/nucleus	
		HSP70	Cytosol/nucleus	
		BIP	ER	
		mHSP70	Mitochondria	
Hsp60	*E. coli*	groEL	Cytosol	Refolds and prevents aggregation of denatured proteins *in vitro*; 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)*.
	S. cerevisiae	HSP60	Mitochondria	
	Plants	Cpn60	Chloroplasts	
	Mammals	HSP60	Mitochondria	
Hsp40	*E. coli*	dnaJ	Cytosol	Essential cochaperone activity with Hsp70 proteins to enhance rate of adenosine triphosphatease activity and substrate release *(43–47)*.
	S. cerevisiae	Ydj1	Cytosol/nucleus	
	Mammals	Hdj1 and Hdj2		
Small Hsps	*E. coli*	lbp A and B	Cytosol	Suppresses aggregation and heat inactivation of proteins *in vitro*; confers thermotolerance through stabilization of microfilaments; antiapoptotic activity *(96,97)*.
	S. cerevisiae	HSP27	Cytosol	
	Mammals	αA and αB 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 proteosome *(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 Hsp90α, Hsp90β, 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 Hsp90β 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 Hsp90α 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 ubiquination 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).* However, 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 reparable 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, H_2O_2, 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.

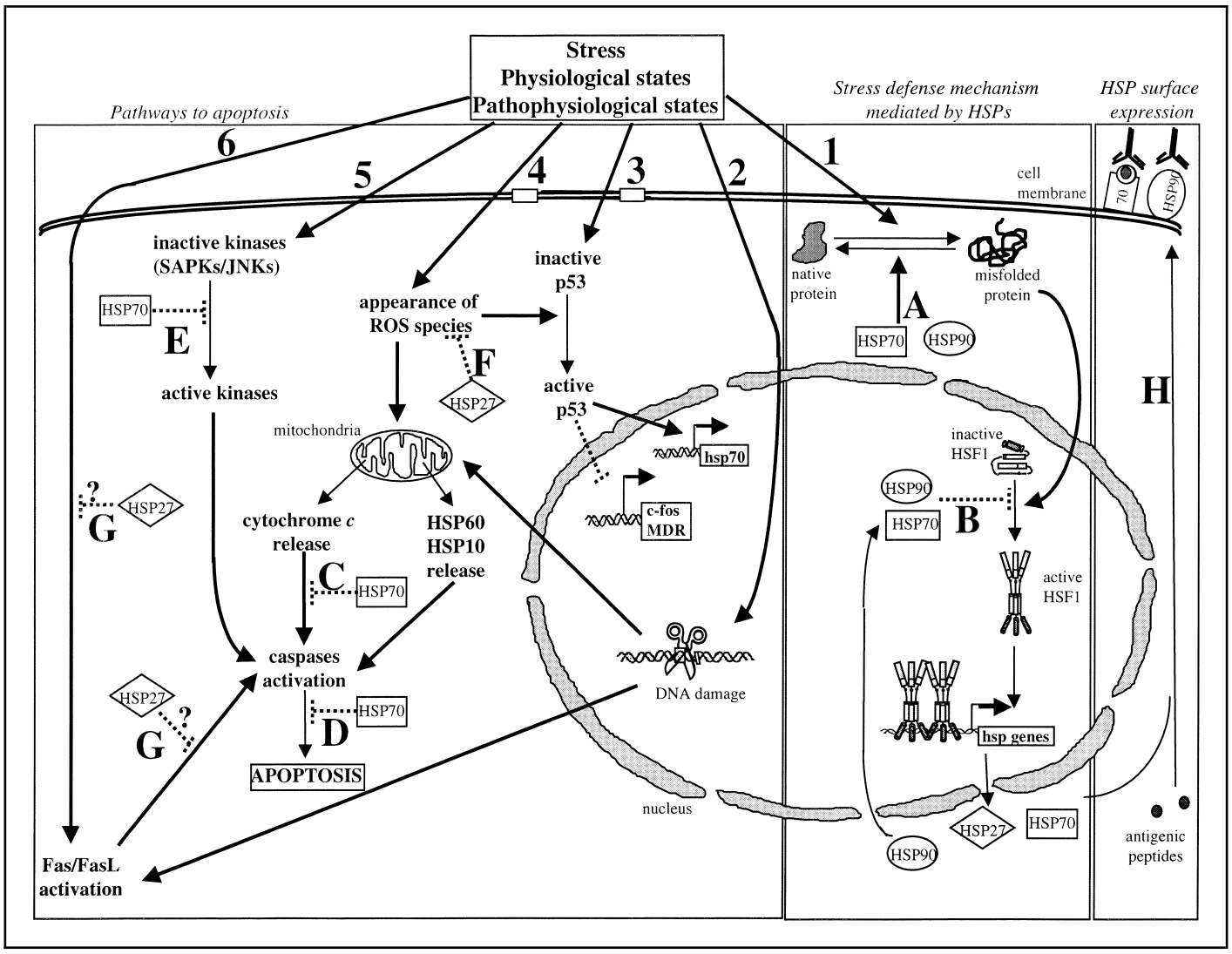

Fig. 1. Schematic of the major cellular pathways activated by stress. Exposure of cells to various stresses leads to the coordinate or separate activation of several major signaling pathways. **Solid arrows** = activation events, **dotted arrows** = inhibitory events, and HSP = heat shock protein. Environmental 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 accumulation 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 transformation *(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).* Radicicol, 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.

REFERENCES

(1) Ritossa F. A new puffing pattern induced by temperature shock and DNP in *Drosophila.* Experientia 1962;18:571–3.

(2) Lindquist S. The heat-shock response. Annu Rev Biochem 1986;55: 1151–91.

(3) Morimoto RI. Cells in stress: transcriptional activation of heat shock genes. Science 1993;259:1409–10.

(4) Ellis RJ. The general concept of molecular chaperones. Philos Trans R Soc Lond B Biol Sci 1993;339:257–61.

(5) Georgopoulos C, Welch WJ. Role of the major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 1993;9:601–34.

(6) Welch WJ. Heat shock proteins functioning as molecular chaperones: their roles in normal and stressed cells. Philos Trans R Soc Lond B Biol Sci 1993;339:327–33.

(7) Wu C. Heat shock transcription factors: structure and regulation. Annu Rev Cell Dev Biol 1995;11:441–69.

(8) Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 1998;12:3788–96.

(9) Milarski KL, Morimoto RI. Expression of human HSP70 during the synthetic phase of the cell cycle. Proc Natl Acad Sci U S A 1986;83:9517–21.

(10) Jerome V, Vourc'h C, Baulieu EE, Catelli MG. Cell cycle regulation of the chicken hsp90 alpha expression. Exp Cell Res 1993;205:44–51.

(11) Hang H, He L, Fox MH. Cell cycle variation of Hsp70 levels in HeLa cells at 37°C and after a heat shock. J Cell Physiol 1995;165:367–75.

(12) Knauf U, Bielka H, Gaestel M. Over-expression of the small heat-shock protein, hsp25, inhibits growth of Ehrlich ascites tumor cells. FEBS Lett 1992;309:297–302.

(13) Spector NL, Samson W, Ryan C, Gribben J, Urba W, Welch WJ, et al. Growth arrest of human B lymphocytes is accompanied by induction of the low molecular weight mammalian heat shock protein (Hsp28). J Immunol 1992;148:1668–73.

(14) Aligue R, Akhavan-Niak H, Russell P. A role for Hsp90 in cell cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90. EMBO J 1994;13:6099–106.

(15) Galea-Lauri J, Latchman DS, Katz DR. The role of the 90-kDa heat shock protein in cell cycle control and differentiation of the monoblastoid cell line U937. Exp Cell Res 1996;226:243–54.

(16) Samali A, Orrenius S. Heat shock proteins: regulators of stress response and apoptosis. Cell Stress Chaperones 1998;3:228–36.

(17) Vayssier M, Polla BS. Heat shock proteins chaperoning life and death. Cell Stress Chaperones 1998;3:221–7.

(18) Jaattela M. Escaping cell death: survival proteins in cancer. Exp Cell Res 1999;248:30–43.

(19) Jaattela M. Heat shock proteins as cellular lifeguards. Ann Med 1999;31: 261–71.

(20) Morano KA, Thiele DJ. Heat shock function and regulation in response to cellular stress, growth, and differentiation signals. Gene Expr 1999;7: 271–82.

(21) Morimoto RI. Heat shock: the role of transient inducible responses in cell damage, transformation, and differentiation. Cancer Cells 1991;3: 295–301.

(22) Fuller KJ, Issels RD, Slosman DO, Guillet JG, Soussi T, Polla BS. Cancer and the heat shock response. Eur J Cancer 1994;30A:1884–91.

(23) Conroy SE, Latchman DS. Do heat shock proteins have a role in breast cancer? Br J Cancer 1996;74:717–21.

(24) Wong HR, Wispe JR. The stress response and the lung. Am J Physiol 1997;273:L1–L9.

(25) Polla BS, Bachelet M, Elia G, Santoro MG. Stress proteins in inflammation. Ann N Y Acad Sci 1998;851:75–85.

(26) Ciocca DR, Oesterreich S, Chamness GC, McGuire WL, Fuqua SA. Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. J Natl Cancer Inst 1993;85:1558–70.

(27) Oesterreich S, Lee AV, Sullivan TM, Samuel SK, Davie JR, Fuqua SA. Novel nuclear matrix protein HET binds to and influences activity of the HSP27 promoter in human breast cancer cells. J Cell Biochem 1997;67: 275–86.

(28) Ciocca DR, Clark GM, Tandon AK, Fuqua SA, Welch WJ, McGuire WL. Heat shock protein hsp70 in patients with axillary lymph node-negative breast cancer: prognostic implications. J Natl Cancer Inst 1993;85: 570–4.

(29) Kaur J, Ralhan R. Differential expression of 70-kDa heat shock-protein in human oral tumorigenesis. Int J Cancer 1995;63:774–9.

(30) Ralhan R, Kaur J. Differential expression of Mr 70,000 heat shock protein in normal, premalignant, and malignant human uterine cervix. Clin Cancer Res 1995;1:1217–22.

(31) Santarosa M, Favaro D, Quaia M, Galligioni E. Expression of heat shock protein 72 in renal cell carcinoma: possible role and prognostic implications in cancer patients. Eur J Cancer 1997;33:873–7.

(32) Liu FF, Miller N, Levin W, Zanke B, Cooper B, Henry M, et al. The potential role of HSP70 as an indicator of response to radiation and hyperthermia treatments for recurrent breast cancer. Int J Hyperthermia 1996;12:197–208; discussion 209–10.

(33) Vargas-Roig LM, Gago FE, Tello O, Aznar JC, Ciocca DR. Heat shock protein expression and drug resistance in breast cancer patients treated with induction chemotherapy. Int J Cancer 1998;79:468–75.

(34) Jameel A, Skilton RA, Campbell TA, Chander SK, Coombes RC, Luqmani YA. Clinical and biological significance of HSP89 alpha in human breast cancer. Int J Cancer 1992;50:409–15.

(35) Yufu Y, Nishimura J, Nawata H. High constitutive expression of heat shock protein 90 alpha in human acute leukemia cells. Leuk Res 1992; 16:597–605.

(36) Hsu PL, Hsu SM. Abundance of heat shock proteins (hsp89, hsp60, and hsp27) in malignant cells of Hodgkin's disease. Cancer Res 1998;58: 5507–13.

(37) Nevins JR. Induction of the synthesis of a 70,000 dalton mammalian heat shock protein by the adenovirus E1A gene product. Cell 1982;29:913–9.

(38) Wu BJ, Hurst HC, Jones NC, Morimoto RI. The E1A 13S product of adenovirus 5 activates transcription of the cellular human HSP70 gene. Mol Cell Biol 1986;6:2994–9.

(39) Williams GT, McClanahan TK, Morimoto RI. E1a transactivation of the human HSP70 promoter is mediated through the basal transcriptional complex. Mol Cell Biol 1989;9:2574–87.

(40) Jolly C, Michelland S, Rocchi M, Robert-Nicoud M, Vourc'h C. Analysis of the transcriptional activity of amplified genes in tumour cells by fluorescence *in situ* hybridization. Hum Genet 1997;101:81–7.

(41) Simon MC, Kitchener K, Kao HT, Hickey E, Weber L, Voellmy R, et al. Selective induction of human heat shock gene transcription by the adenovirus E1A gene products, including the 12S E1A product. Mol Cell Biol 1987;7:2884–90.

(42) Phillips B, Abravaya K, Morimoto RI. Analysis of the specificity and mechanism of transcriptional activation of the human hsp70 gene during infection by DNA viruses. J Virol 1991;65:5680–92.

(43) Liberek K, Georgopoulos C, Zylicz M. Role of the *Escherichia coli* DnaK and Dnaj heat shock proteins in the initiation of bacteriophage lambda DNA replication. Proc Natl Acad Sci U S A 1988;85:6632–6.

(44) Alfano C, McCracken R. Heat shock protein-mediated disassembly of

nucleoprotein structures is required for the initiation of bacteriophage lambda DNA replication. J Biol Chem 1989;264:10709–18.
(45) Hoffmann HJ, Lyman SK, Lu C, Petit MA, Echols H. Activity of the Hsp70 chaperone complex—DnaK, DnaJ, and GrpE—in initiating phage lambda DNA replication by sequestering and releasing lambda P protein. Proc Natl Acad Sci U S A 1992;89:12108–11.
(46) Campbell KS, Mullane KP, Aksoy IA, Studbal H, Zalvide J, Pipas JM, et al. DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. Genes Dev 1997;11:1098–110.
(47) Kelley WL, Georgopoulos C. The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. Proc Natl Acad Sci U S A 1997;94:3679–84.
(48) Srinivasan A, McClellan AJ, Vartikar J, Marks I, Cantalupo P, Li Y, et al. The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. Mol Cell Biol 1997;17:4761–73.
(49) Jaattela M. Over-expression of hsp70 confers tumorigenicity to mouse fibrosarcoma cells. Int J Cancer 1995;60:689–93.
(50) Garrido C, Fromentin A, Bonnotte B, Favre N, Moutet M, Arrigo AP, et al. Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. Cancer Res 1998;58:5495–9.
(51) Volloch VZ, Sherman MY. Oncogenic potential of Hsp72. Oncogene 1999;18:3648–51.
(52) Seo JS, Park YM, Kim JI, Shim EH, Kim CW, Jang JJ, et al. T cell lymphoma in transgenic mice expressing the human hsp70 gene. Biochem Biophys Res Commun 1996;218:582–7.
(53) Ziemiecki A, Catelli MG, Joab I, Moncharmont B. Association of the heat shock protein hsp90 with steroid hormone receptors and tyrosine kinase oncogene products. Biochem Biophys Res Commun 1986;138:1298–307.
(54) Xu Y, Lindquist S. Heat-shock protein hsp90 governs the activity of pp60v-src kinase. Proc Natl Acad Sci U S A 1993;90:7074–8.
(55) Xu Y, Singer MA, Lindquist S. Maturation of the tyrosine kinase s-src as a kinase and as a substrate depends on the molecular chaperone Hsp90. Proc Natl Acad Sci U S A 1999;96:109–14.
(56) Whitesell L, Shifrin SD, Schwab G, Neckers LM. Benzoquinonoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res 1992;52:1721–8.
(57) Pinhasi-Kimhi O, Michalovitz D, Ben-Zeev A, Oren M. Specific interaction between the p53 cellular tumour antigen and major heat shock proteins. Nature 1986;320:182–4.
(58) Sawai ET, Butel JS. Association of a cellular heat shock protein with simian virus 40 large T antigen in transformed cells. J Virol 1989;63:3961–73.
(59) Koskinen PJ, Sistonen L, Evan G, Morimoto R, Alitalo K. Nuclear colocalization of cellular and viral myc proteins with HSP70 in myc-overexpressing cells. J Virol 1991;65:842–51.
(60) Kingston RE, Baldwin AS Jr, Sharp PA. Regulation of heat shock protein 70 gene expression by c-myc. Nature 1984;312:280–2.
(61) Kaddurah-Daouk R, Greene JM, Baldwin AS Jr, Kingston RE. Activation and repression of mammalian gene expression by the c-myc protein. Genes Dev 1987;1:347–57.
(62) Lane DP, Midgley C, Hupp T. Tumour suppressor genes and molecular chaperones. Phil Trans R Soc Lond B Biol Sci 1993;339:369–72; discussion 372–3.
(63) Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, et al. Identification of p53 as a sequence-specific DNA-binding protein. Science 1991;252:1708–11.
(64) Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J Natl Cancer Inst 1996;88:1442–55.
(65) Adams PD, Kaelin WG Jr. Negative control elements of the cell cycle in human tumors. Curr Opin Cell Biol 1998;10:791–7.
(66) Amundson SA, Myers TG, Fornace AJ Jr. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 1998;17:3287–99.
(67) Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995;80:293–9.
(68) Agoff SN, Hou J, Linzer DI, Wu B. Regulation of the human hsp70 promoter by p53. Science 1993;259:84–7.
(69) Elkeles A, Juven-Gershon T, Israeli D, Wilder S, Zalcenstein A, Oren M. The c-fos proto-oncogene is a target for transactivation by the p53 tumor suppressor. Mol Cell Biol 1999;19:2594–600.
(70) Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991;253:49–53.
(71) Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature 1991;351:453–6.
(72) Thor AD, Moore DH II, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, et al. Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. J Natl Cancer Inst 1992;84:845–55.
(73) Martin HM, Filipe MI, Morris RW, Lane DP, Silvestre F. p53 expression and prognosis in gastric carcinoma. Int J Cancer 1992;50:859–62.
(74) Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc70–p53 complex with an altered half-life. Mol Cell Biol 1988;8:531–9.
(75) Yehiely F, Oren M. The gene for the rat heat-shock cognate, hsc70, can suppress oncogene-mediated transformation. Cell Growth Differ 1992;3:803–9.
(76) Lane DP. Exploiting the p53 pathway for cancer diagnosis and therapy. Br J Cancer 1999;80(Suppl 1):1–5.
(77) Davidoff AM, Iglehart JD, Marks JR. Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. Proc Natl Acad Sci U S A 1992;89:3439–42.
(78) Stewart BW. Mechanisms of apoptosis: integration of genetic, biochemical, and cellular indicators. J Natl Cancer Inst 1994;86:1286–96.
(79) Steller H. Mechanisms and genes of cellular suicide. Science 1995;267:1445–9.
(80) Evan G, Littlewood T. A matter of life and cell death. Science 1998;81:1317–22.
(81) Barry MA, Behnke CA, Eastman A. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. Biochem Pharmacol 1990;40:2353–62.
(82) Parsell DA, Taulien J, Lindquist S. The role of heat-shock proteins in thermotolerance. Philos Trans R Soc Lond B Biol Sci 1993;339:279–85; discussion 285–6.
(83) Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998;281:1322–6.
(84) LaCasse EC, Baird S, Korneluk RG, MacKenzie AE. The inhibitors of apoptosis (IAPs) and their emerging role in cancer. Oncogene 1998;17:3247–59.
(85) Wei YQ, Zhao X, Kariya Y, Teshigawara K, Uchida A. Inhibition of proliferation and induction of apoptosis by abrogation of heat-shock protein (HSP) 70 expression in tumor cells. Cancer Immunol Immunother 1995;40:73–8.
(86) Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol Cell Biol 1997;17:5317–27.
(87) Buzzard KA, Giaccia AJ, Killender M, Anderson RL. Heat shock protein 72 modulates pathways of stress-induced apoptosis. J Biol Chem 1998;273:17147–53.
(88) Beere H, Wolf B, Mosser R, Klein K Kuwana T, Morimoto R, et al. Heat shock protein 70 (Hsp70) inhibits apoptosis by preventing recruitment of procaspase-9 to aggregated Apaf-1. Nature Cell Biol 2000;2:469–75.
(89) Meriin AB, Yaglom JA, Gabai VL, Zon L, Ganiatsas S, Mosser DD, et al. Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72. Mol Cell Biol 1999;19:2547–55.
(90) Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, et al. Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. J Biol Chem 1997;272:18033–37.
(91) Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, Sherman MY. Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. FEBS Lett 1998;438:1–4.
(92) Liossis SN, Ding XZ, Kiang JG, Tsokos GC. Overexpression of the heat shock protein 70 enhances the TCR/CD3- and Fas/Apo-1/CD95-mediated apoptotic cell death in Jurkat T cells. J Immunol 1997;158:5668–75.
(93) Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M. Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. EMBO J 1998;17:6124–34.

(94) Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, et al. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J 1997;16:4887–96.
(95) Zeiner M, Gebauer M, Gehring U. Mammalian protein RAP46: an interaction partner and modulator of 70 kDa heat shock proteins. EMBO J 1997;16:5483–90.
(96) Richards EH, Hickey E, Weber L, 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 1996;56:2446–51.
(97) Trautinger F, Kokesch C, Herbacek I, Knobler RM, 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 B 1997;39:90–5.
(98) Srivastava PK, Maki RG. Stress-induced proteins in immune response to cancer. Curr Top Microbiol Immunol 1991;167:109–23.
(99) Srivastava PK, Udono H. Heat shock protein–peptide complexes in cancer immunotherapy. Curr Opin Immunol 1994;6:728–32.
(100) Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. Science 1995;269:1585–8.
(101) Multhoff G, Hightower LE. Cell surface expression of heat shock proteins and the immune response. Cell Stress Chaperones 1996;1:167–76.
(102) Ullrich SJ, Robinson EA, Law LW, Willingham M, Appella E. A mouse tumor-specific transplantation antigen is a heat shock-related protein. Proc Natl Acad Sci U S A 1986;83:3121–5.
(103) Ferrarini M, Heltai S, Zocchi MR, Rugarli C. Unusual expression and localization of heat-shock proteins in human tumor cells. Int J Cancer 1992;51:613–9.
(104) Multhoff G, Botzler C, Wiesnet M, Muller E, Meier T, Wilmanns W, et al. A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells. Int J Cancer 1995;61:272–9.
(105) Altmeyer A, Maki RG, Feldweg AM, Heike M, Protopopov VP, Masur SK, et al. Tumor-specific cell surface expression of the KDEL-containing, endoplasmic reticular heat shock protein gp96. Int J Cancer 1996;69:340–9.
(106) Young RA, Elliott TJ. Stress proteins, infection, and immune surveillance. Cell 1989;59:5–8.
(107) Young D, Roman E, Moreno C, O'Brien R, Born W. Molecular chaperones and the immune response. Philos Trans R Soc Lond B Biol Sci 1993;339:363–7; discussion 367–8.
(108) Srivastava PK. Heat shock proteins in immune response to cancer: the Fourth Paradigm. Experientia 1994;50:1054–60.
(109) Udono H, Srivastava PK. Heat shock protein 70-associated peptides elicit specific cancer immunity. J Exp Med 1993;178:1391–6.
(110) Udono H, Srivastava PK. Comparison of tumor-specific immunogenicities of stress-induced proteins gp96, hsp90, and hsp70. J Immunol 1994;152:5398–403.
(111) Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 1997;278:117–20.
(112) Li LH, Clark TD, Cowie CH, Rinehart KL Jr. Effects of geldanamycin and its derivatives on RNA-directed DNA polymerase and infectivity of Rauscher leukemia virus. Cancer Treat Rep 1977;61:815–24.
(113) Price PJ, Suk WA, Skeen PC, Spahn GJ, Chirigos MA. Geldanamycin inhibition of 3-methylcholanthrene-induced rat embryo cell transformation. Proc Soc Exp Biol Med 1977;155:461–3.
(114) Uehara Y, Hori M, Takeuchi T, Umezawa H. Phenotypic change from transformed to normal induced by benzoquinonoid ansamycins accompanies inactivation of p60src in rat kidney cells infected with Rous sarcoma virus. Mol Cell Biol 1986;6:2198–206.
(115) Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 1997;90:65–75.
(116) Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90–geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 1997;89:239–50.
(117) Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A 1994;91:8324–8.
(118) Smith DF, Whitesell L, Nair SC, Chen S, Prapapanich V, Rimerman RA. Progesterone receptor structure and function altered by geldanamycin, an hsp90-binding agent. Mol Cell Biol 1995;15:6804–12.
(119) Whitesell L, Cook P. Stable and specific binding of heat shock protein 90 by geldanamycin disrupts glucocorticoid receptor function in intact cells. Mol Endocrinol 1996;10:705–12.
(120) Scheibel T, Buchner J. The Hsp90 complex—a super-chaperone machine as a novel drug target. Biochem Pharmacol 1998;56:675–82.
(121) Sharma SV, Agatsuma T, Nakano H. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. Oncogene 1998;16:2639–45.
(122) Abe M, Hiraoka M. Hyperthermia in combination with radiation in the treatment of cancers. In: Morimoto RI, Tissieres A, Georgopoulos C, editors. Stress proteins in biology and medicine. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1990. p. 117–29.
(123) Issels RD. Hyperthermia and thermochemotherapy. Cancer Treat Res 1993;67:143–60.
(124) Dewhirst MW, Prosnitz L, Thrall D, Prescott D, Clegg S, Charles C, et al. Hyperthermic treatment of malignant diseases: current status and view toward the future. Semin Oncol 1997;24:616–25.
(125) Crile G. The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res 1963;23:372–380.
(126) Moscow JA, Cowan KH. Multidrug resistance. J Natl Cancer Inst 1988;80:14–20.
(127) Wallner K, Li GC. Adriamycin resistance, heat resistance and radiation response in Chinese hamster fibroblasts. Int J Radiat Oncol Biol Phys 1986;12:829–33.

NOTES

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