

Review

HSP-Molecular Chaperones in Cancer Biogenesis and Tumor Therapy: An Overview

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Abstract. Molecular chaperones, many of which are heat-shock proteins (HSPs), are an important class of molecules with various functions. Pathological conditions in which chaperones become etiological and/or pathogenic factors are called chaperonopathies, and are classified into by defect, by excess, and by 'mistake'. In the latter case, the chaperone is structurally and functionally normal but participates in pathways that favor disease, although in some cases the chaperone may have post-translational modifications that may lead it to change its location and function and, thus, to become pathogenic. For example, HSP-chaperones are involved in carcinogenesis in various ways, so that some forms of cancer may be considered 'chaperonopathies by mistake'. This concept suggests new strategies for anticancer therapy (chaperonotherapy), in which the primary targets or therapeutic agents are chaperones. Chaperonotherapy consists of the utilization of HSP-chaperones for treating chaperonopathies, including cancer. Negative chaperonotherapy is aimed at eliminating or blocking the action of chaperones that favor carcinogenesis or other diseases, whereas positive chaperonotherapy uses chaperones, genes or proteins, to fight against

diseases, such as cancer, by stimulating the immune system or the cellular defenses against stress.

The Chaperoning System

The 'chaperoning system' is a concept proposed in 2008 to encompass all molecular chaperones, co-chaperones, and co-factors of an organism (1). The proposal envisions a physiological system integrated by chaperones and their functionally-related molecules and structures, in all tissues, organs, and biological fluids. This physiological system is essential for the control of protein homeostasis and maintenance of a complete set of proteins in all fluids, cells, and tissues, with the correct and functional conformation (1). The chaperoning system also participates in some other processes, such as antigen presentation, hormone receptor assembly, formation of complexes with a variety of ligands and other cellular functions, not necessarily related to protein homeostasis (2).

The science that deals with the chaperoning system is called chaperonology, a new scientific discipline that also includes the study of the genomic sequences of chaperone genes (e.g. by applying chaperonomics) (3), the pathologies that involve chaperones as etiological-pathogenic factors (i.e. chaperonopathies) (4), and the use of chaperones (molecules and genes) for the treatment of chaperonopathies (i.e. chaperonotherapy) (2).

Chaperones are proteins highly conserved during evolution. As per the classic concept, they assist, namely chaperone, other proteins in their folding and re-folding and, when the proteins are defective or irreversibly misfolded, chaperones direct them to degradation (4). However, during

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Table I. Classification of HSPs according to their molecular mass.

Chaperone family	Molecular weight (kDa)	Main function(s)
Heavy	100 or higher	Prevents protein aggregation, helps protein folding.
HSP90	81-99	Protein folding, cytoprotection; intracellular signaling (<i>e.g.</i> steroid receptor); cell-cycle control.
HSP70	65-80	Prevents protein aggregation, protein folding, cytoprotection and anti-apoptotic function.
HSP60	55-64	Prevention of protein aggregation, protein folding, cytoprotection, macrophage activator possibly through Toll-like receptor.
CCT	55-63	Protein folding.
HSP40	35-54	Protein folding and refolding together with HSP70/HSC70.
Small Hsp	34 or less	Anti-apoptotic function; cytoprotection.
HSP10	10	Protein folding together with HSP60; modulation of immune system.

evolution, they also acquired ‘extrachaperoning’ roles, such as participation in immune system regulation (2, 5-8), cell differentiation (9), gene expression (10), DNA replication (10), signal transduction (10), programmed cell death (11, 12), cellular senescence (2, 13), and carcinogenesis (14-16). In addition, chaperones are present not only in tissues but also in fluids, as products of cell secretion, acting in locations other than those of their origin (1).

A number of molecular chaperones are heat-shock proteins (HSPs). The latter were first discovered as a group of proteins that are induced by heat shock (17), as well as by other chemical and physical stressors in a wide range of species (18). A fraction of chaperones are HSPs, *i.e.* they are encoded in genes that are inducible by stressors but many others are not HSPs (4). HSP-chaperones (for the sake of simplicity, ‘HSP’ or ‘chaperone’ we used synonymously in this article) display their canonical functions, *i.e.* those pertaining to protein homeostasis, while others also play other roles more or less unrelated to the maintenance of protein homeostasis (19, 20). The expanding number of members of HSP families in the last few years has made it necessary to revise the nomenclature. HSPs were initially classified according to their molecular mass expressed in kiloDaltons (kDa) (Table I). More recently, guidelines for the nomenclature of the human HSP families have been proposed (21). This nomenclature is based on gene symbols that have been assigned by the HUGO Gene Nomenclature Committee (HGNC) and are used as the primary identifiers in databases such as Entrez Gene and Ensemble. However, in this review, the classic nomenclature appearing in the original publications is used to facilitate reading for those that are not thoroughly familiar with this topic.

Molecular Chaperones: Versatile Molecules with Intra- and Extra-cellular Functions

For many years HSPs were considered typical intracellular, and some organelle-specific, molecules. However, accumulated evidence shows that they also occur in

extracellular sites and in non-canonical sites inside cells (2). For instance, HSP60 was classically considered an intracellular, intra-mitochondrial molecule with specific functions related to mitochondrial protein folding, working together with its co-chaperone HSP10 (14). However, HSP60 has been described in extramitochondrial sites, such as the cytosol (22), intracellular vesicles (23), plasma membrane (24), and the surface of normal and tumor cells (24-26). In the cytosol, HSP60 can have either pro-survival or pro-apoptotic action depending on other cellular factors (22).

HSPs also occur in the extracellular space and in circulation (27, 28), facts that have become firmly established only in the last few years. HSPs may be exported outside cells through microvesicles, *e.g.* exosomes, which derive from endosomes and multivesicular bodies (26, 29). Extracellular vesicles are important for cell-to-cell communication (30). Hence, HSPs are now considered key players in the intercellular cross-talk. For example, microvesicles containing HSP70 on their surface activate macrophages (31) and natural killer cells (32).

HSPs can be released from cells in free, soluble form, possibly *via* Golgi. In this form, HSPs circulate *via* blood throughout the organism and act in an endocrine fashion (2). In addition, HSPs find their targets in cells *viciniori* to those that secreted them, thus acting in a paracrine fashion. For example, extracellular HSP60 has been postulated to have effects on neutrophils (33, 34) and macrophages (33, 34). As far as macrophages are concerned, HSP60 can interact with a number of cell-surface receptors, such as CD14, CD40, and Toll-like-receptors (TLRs), causing in turn either pro- or anti-inflammatory effects (2). For example, HSP60 can induce secretion of cytokines from professional antigen-presenting cells, with consequent activation of T-cells (35, 36).

The Chaperonopathies

Chaperonopathies have been defined as pathological conditions in which molecular chaperone malfunction is an etiological-pathogenic factor (4). The chaperonopathies may

be classified as genetic or acquired. In genetic chaperonopathies, the defective function of a chaperone is due to a mutation of its gene (4, 37, 38). Among genetic chaperonopathies are: i) diseases of the nervous system, such as some distal hereditary motor neuropathies and ataxias, and the Bardet-Biedl and Williams syndromes, to name a few; ii) heart disease, namely the genetic forms of dilated cardiomyopathies; and iii) other rare forms of congenital diseases, such as endoplasmic reticulum pathologies (38). In acquired chaperonopathies, the malfunction of a chaperone may be due to pathological post-translational modifications, such as oxidation, phosphorylation, acetylation, or glycation (4), causing loss or gain of function. Among the acquired chaperonopathies, there are diseases associated with aging, such as cataracts, and with dysregulation of the immune system (2). Chaperonopathies may occur at any age but, as a rule, genetic chaperonopathies have an early onset, while acquired chaperonopathies become manifested in the elderly, sometimes in association with other diseases (38).

An important group is represented by the 'chaperonopathies by mistake' (or 'by collaborationism') in which a molecular chaperone may be normal, but participates in a pathway that favors disease development, rather than the opposite (38). In this case, the pathology can be due to two types of pathogenic pathways: i) the pathway is normal and includes the participation of one or more chaperones but it becomes part of a pathological process; and ii) the pathway, including chaperones, is not part of a physiological process of the cell but becomes active and functional in pathological cases (38). In both conditions, the chaperones contribute to disease pathogenesis 'by mistake' (38).

In some cases, autoimmune diseases can be elicited by a molecular chaperone acting as an autoantigen 'by mistake' (39-41), or due to molecular mimicry between human and/or bacterial HSP60 and other human proteins (42, 43).

In some types of neoplasms, chaperones can favor growth and proliferation of malignant cells, thus 'collaborating' with the tumor (38). In the following paragraphs we will give some examples on how a normal chaperone may contribute to carcinogenesis. This is an important concept since it points to novel anticancer strategies.

Cancer Can Be a Chaperonopathy by Mistake

In the last two decades, many reports support the notion that chaperones are implicated in the pathogenesis of a range of human cancer types, being involved in various metabolic and molecular mechanisms of cancerous cells, such as cell proliferation (14, 15), invasiveness (44), induction of neoangiogenesis (45), metastasization (46) and induction of immune tolerance (40, 47). Hence, molecular chaperones are beneficial for the cancerous cell and, thus are pathogenic for the organism (38). For example, HSP60 favors the survival

of certain types of tumors (15), and in some cases, it may even be essential for tumor-cell growth. For instance, elevated levels of this protein in tumor cells have been linked to: a) the ability to survive apoptotic stimuli (16); b) loss of replicative senescence (13, 15); and c), uncontrolled proliferation and neoplastic transformation (15, 16, 48). Likewise, HSP90 regulates late-stage maturation, activation, and stability of a range of 'client' proteins, such as Human Epidermal Growth Factor Receptor 2 (HER2), Anaplastic Lymphoma Receptor Tyrosine Kinase (ALK), Epidermal Growth Factor Receptor (EGFR) and V-Raf Murine Sarcoma Viral Oncogene Homolog B1 (BRAF), some of which are involved in signal transduction and other key pathways that are important for malignancy (49).

The degree of expression and levels of HSPs have been found to be altered in cancer cells, as exemplified in Table II, in which it can be seen that more often than not, the levels of HSPs are elevated in tumors by comparison with the normal tissue/cell counterparts. This pattern is also illustrated in Figure 1. For example, higher levels of HSP60 were found in the 'adenoma-to-carcinoma sequence' of the large bowel (50, 51), in the 'dysplasia-to-carcinoma sequence' of the uterine exocervix (52), and in the prostate carcinogenesis (53). In contrast, lower levels were detected in tongue (54), bronchial (55, 56) and urinary bladder (57, 58) cancer. HSP27, HSP70, and HSP90 have also been found increased in several types of cancer in which they may favor tumorigenesis by inhibiting programmed cell death and senescence (59). For example, HSP70 and HSP90 bind tumor suppressor proteins, such as p53 and HER2, and thus allow unlimited cellular growth or increased resistance to chemotherapy in breast cancer (60).

Tumor cells that overexpress HSPs may show an increased tendency to invade their microenvironment and to spread to distant organs, producing metastasis. For example, a positive correlation was found between increased expression of HSP27 and HSP70 and tumor-invasiveness (61). HSPs may also influence tumor neoangiogenesis. For instance, HSP90 stabilizes vascular endothelial growth factor and nitric oxide synthetase in endothelial cells (62, 63), and HSP27 mediates endothelial cell mobility and proliferation (64).

High levels of HSPs may correlate with prognosis of several types of cancer. For example, high levels of HSP27 are correlated with poor prognosis in ovarian cancer (65) and HSP60 overexpression is correlated with tumor progression and with a poor prognosis in large bowel (51) and in prostate (53) carcinomas. Elevated levels of HSP70 are associated with poor prognosis in breast (66) and endometrial (67) cancer; high HSP90 expression is associated with poor prognosis in invasive ductal breast carcinoma (68), but with good prognosis in endometrial cancer (67).

Here, we have given only few examples of studies that suggest direct involvement of HSPs in human tumorigenesis

Table II. Tumors in which the HSPs levels were found to be different from those in their normal tissue counterparts.

System	Tumor	Hsp	Level (compared to the normal tissue counterpart)	References
Digestive	Squamous cell carcinoma of the tongue	HSP27	Higher	54
		HSP90	Higher	54
	Adenocarcinoma of large bowel	HSP60	Higher	50, 110, 111
		HSP10	Higher	109
		CCTβ	Higher	115
		HSP70	Higher	116
		HSP27, HSP90β	Higher	113, 114
	Gastric adenocarcinoma	CCTβ	Higher	115
	Hepatocellular carcinoma	HSP90 α	Higher	117
	Pancreatic carcinoma	HSP60	Higher	126
Oesophageal squamous cell carcinoma	HSP60	Higher	127	
Female reproductive	Ovarian carcinoma	HSP10, HSP27, HSP72	Higher	118, 65, 119
		HSP60, HSP10	Higher	52, 109
	Exocervical carcinoma	HSP60	Higher	131
Male reproductive	Breast ductal carcinoma	HSP60, HSP10	Higher	53, 112
		HSP27-HSP70	Lower	120
Respiratory	Prostate carcinoma	HSP27	Lower	121
		HSP60, HSP10	Lower	55, 56
	Testis tumors (seminomas and non-seminomas)	HSP70, GRP94	Higher	122, 134
Urinary	Bronchial carcinoma	HSP27, αB crystallin	Higher	121
		HSP60	Lower	57, 58
Hemo-lymphopoietic	Transitional cell carcinoma of bladder	HSP90	Higher	57
		HSP60	Lower	128
		HSP60-HSP70, HSP90	Higher	123, 124
Nervous	Carcinosarcoma of bladder	HSP60	Higher	125
		HSP60	Higher	129
Endocrine	Astrogloma	HSP60, HSP27	Lower; higher	130, 132
		HSP60	Higher	133
Skeletal	Glioblastoma	HSP60	Higher	135
		HSP72	Lower	136
	Adrenal Cushing tumor	HSP60	Higher	133
	Osteosarcoma	HSP60	Higher	135
	Chondrosarcoma	HSP72	Lower	136

MALT: Mucose-associated lymphoid tissue.

and their correlation with clinical outcome. These studies should prompt a consistent effort to study HSPs as targets for modern anticancer chaperonotherapies.

HSPs as Anti-apoptotic Proteins with Tumorigenic Properties

A number of studies showed that elevated levels of HSPs can protect malignant cells against apoptosis generated by therapy (69). The apoptotic mechanism follows two pathways, the intrinsic and the extrinsic. For both pathways, the final outcome is the activation of proteases, called caspases, which are normally expressed as latent zymogens, pro-caspases, and which are enzymatically cleaved and thus activated in response to an apoptotic stimulus (70).

In the intrinsic pathway, mitochondria are involved in the formation of the apoptosome. Cell death signals induce the release of cytochrome *c* from mitochondria to the cytosol and its interaction with the cytosolic apoptosis protease-

activating factor-1 (APAF-1) and pro-caspase-9 to form the apoptosome, an active complex that induces the apoptotic protease cascade by activation of pro-caspase-3 (71). HSP27 and HSP70 interfere with apoptosis through inhibition of apoptosome formation. HSP27 interferes with apoptosis by binding and sequestering cytosolic cytochrome *c* while HSP70 does so by binding directly to APAF-1, in turn inhibiting its oligomerization and blocking the assembly of the apoptosome (72). HSP90 inhibits cytochrome *c*-mediated activation of pro-caspase-9 (73).

The extrinsic pathway of apoptosis involves activation of 'death' receptors, such as Tumor Necrosis Factor (TNF) receptor 1 and TNF receptor superfamily, member 6 (FAS)/CD95/ apoptosis antigen-1 (APO-1), by binding their respective ligands, which leads to the formation of the death-inducing signaling complex at the plasma membrane level and to the activation of pro-caspase-8 that can activate caspases directly or indirectly, the latter by inducing the release of mitochondrial cytochrome *c* (74, 75). HSP27

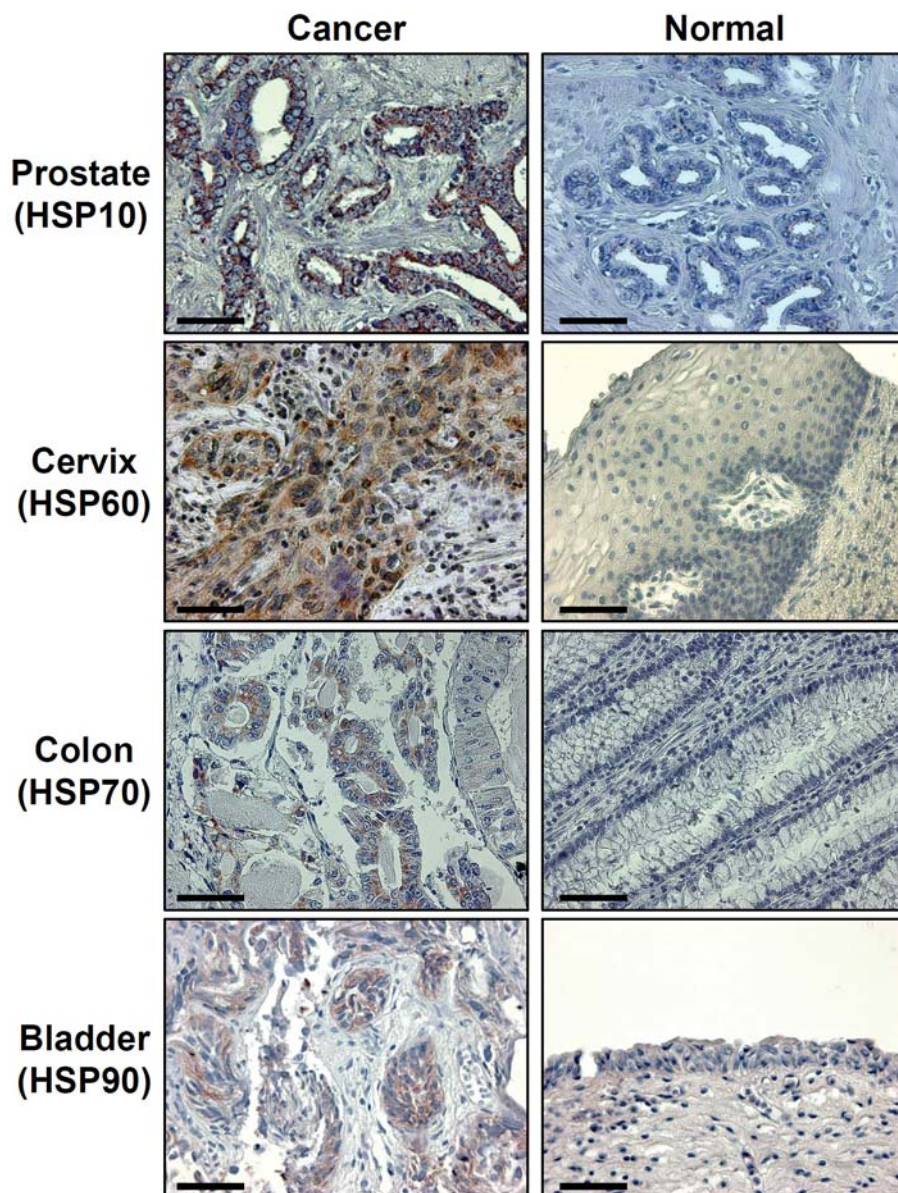


Figure 1. Representative images of immunohistochemical demonstration of HSPs with specific antibodies in histological sections from cancer tissues and their normal counterparts. The images demonstrate increased levels HSPs in different types of cancer. Immunostaining was performed as described in reference 137. Bars, 100 μ m.

interferes with the FAS-mediated apoptosis pathway to prevent cell death; phosphorylated dimers of HSP27 interact with a protein, named Daxx, which is a mediator of FAS-induced apoptosis, preventing the interaction between the same protein Daxx and FAS, blocking the FAS-mediated apoptosis pathway (76). HSP90 has a role in modulating TNF receptor-1 signaling and stabilizing the receptor interacting protein of TNF receptor-1 (77).

The antiapoptotic action of HSP90 has also been demonstrated by its interaction with proteins able to generate

a survival signal in response to growth factor stimulation. For example, HSP90 can bind AKT1, an antiapoptotic protein, member of the protein kinase family, in turn preventing its inactivation, thus favoring cell survival (78).

There is evidence that HSP60 may have both, pro- and anti-apoptotic roles. Some studies reported experiments, using *in vitro* models, which demonstrated that HSP60 has a pro-apoptotic role (79, 80). HSP60 in combination with the co-chaperone HSP10 may regulate caspase-3 activation by acting as a chaperone to promote maintenance of pro-

caspase-3 in a protease-sensitive state (79, 80). In contrast, in a cancer model system, other experiments have shown that the molecular chaperone HSP60 orchestrates a cytoprotective pathway centered on stabilization of levels of survivin, a protein member of the Inhibitors of apoptosis (IAP) family, as well as inhibition of p53 function (81). In addition, HSP60 may form a stable complex with pro-caspase-3 (82), with the consequent anti-apoptotic effect.

Localization and Secretion of HSPs in Tumor Cells

The notion that HSPs are actively involved in the carcinogenic process has led several researchers to study the intracellular and extracellular localization of these chaperones. The localization of HSP60 to the cell membrane seems uncommon in normal cells but frequent in tumor cells (26, 40, 83). HSP60 is present in lipid rafts (26, 84), which are subdomains of the plasma membrane, containing high concentrations of cholesterol and glycosphingolipids. HSP60 also occurs in exosomes produced and released by human tumor cells through an active secretion mechanism, independently of cell death, namely unrelated to necrosis or apoptosis (84). Interestingly, the Golgi apparatus also, participates in exosome formation and HSP60 secretion in tumor cells (26). These data may reflect a general physiological phenomenon, occurring in many tumors.

Other groups have demonstrated that HSP70 is frequently also localized to the cell membrane in a variety of human tumors, but not in the corresponding normal tissues (85). These data are potentially important for therapeutic antitumor immunity, since membrane HSPs are easily reachable targets for specific antibodies (86).

Anticancer Chaperonotherapy: Reality or Utopia?

Current anticancer drug-development strategies involve identifying, and blocking, novel molecular targets crucial for tumor progression. However, cancer cells have several defense mechanisms against cytotoxic drugs and, in order to help the organism in the fight against tumors, it is necessary to consider novel methods of therapy (61).

As discussed above, HSPs are involved in the pathogenesis of some types of cancer in various ways, so that these types of cancer may be considered chaperonopathies 'by mistake'. This concept may open new frontiers in cancer treatment involving chaperonotherapy (87).

Treatment of cancer as chaperonopathies 'by mistake' may follow four strategies: i) intracellularly, with drugs able to modify HSPs levels and/or activity; ii) at the plasma membrane level, with monoclonal antibodies able to recognize surface HSPs present in tumor but not in normal cells; iii) at the immune system level, using HSPs as adjuvant for eliciting an immune response against the tumor; and iv) also at the immune system level, using HSPs as vaccines.

Inhibition of HSP90 by drugs in breast cancer has given good results in pre-clinical and clinical studies (88, 89). The molecular chaperone HSP90 is important in maintaining the conformation, stability, and function of key oncogenic client proteins involved in signal transduction pathways leading to proliferation, and cell-cycle progression, as well as other features of the malignant phenotype, such as invasion, angiogenesis, and metastasis (89). HSP90 can be inhibited by the benzoquinone ansamycin antibiotic geldanamycin and its analogue 17-allylamino-17-deemethoxygeldanamycin (17-AAG) (88). These drugs selectively kill cancer cells because these cells, as compared to their normal counterparts, exhibit a stressed phenotype, with an increased dependency on the cytoprotective action of HSP90 (88-90). The use of HSP90 inhibitors has the potential to provide a simultaneous attack on multiple oncogenic pathways of tumor cells (88, 89). HSP90 inhibitors such as 17-AAG have been shown to have an important side-effect, namely the induction of HSP70 expression, thus reducing therapeutic efficacy (91). To solve this problem, inhibitors of HSP70 might be used, such as a benzylidene lactam KNK437; however, this compound is non-specific and, therefore, further studies are necessary (91, 92).

HSP70 could be a promising therapeutic target in Breakpoint Cluster Region-Abelson (BCR/ABL)-positive human leukemia cells where it plays a mechanistic role in mediating the anti-apoptotic effects of BCR/ABL (93). HSP70 contributes to BCR-ABL-mediated resistance to apoptosis caused by anti-leukemia agents, for example, Ara-C, etoposide, and APO-2L/TNF-Related Apoptosis-Inducing Ligand (TRAIL) (93). Neutralization of HSP70 functions could be achieved with peptides that mimic a domain of the apoptosis inducing factor (AIF) that is required for HSP70 binding. These peptides bind HSP70 and block its function (94, 95). Experiments *in vitro* carried out on different cell lines, such as leukemia, colon and breast cancer lines, demonstrated that several of these peptides increase sensitivity to chemotherapy (95). Experiments *in vivo*, carried on syngeneic rat colon cancer and mouse melanoma models, demonstrated that the use of one HSP70 inhibitor, called AIF-derived decoy for HSP70 (ADD70), reduced tumor size and metastatic potential, and led to a complete and permanent cure after treatment with cisplatin (96). Studies *in vitro* with HSP27, carried out on BG1 ovarian cells and HeLa uterine cancer cells, demonstrated that inhibition of HSP27 expression by a tubulin de-polymerization inhibitor (paclitaxel) reduced resistance to some chemotherapy drugs (97). However, it is not yet clear how HSP27 is related to tubulin. These data point to a new possibility, namely the use of HSPs inhibitors in combination with other antitumor drugs, which may result in a better therapeutic outcome, as compared with their use separately.

The levels of HSP60 can be manipulated with therapeutic purposes in various ways. For example, *in vitro* studies have

Table III. *Clinical trials on HSPs as anticancer vaccines.*

Condition	Intervention	Phase	Status	Identifier ^a
Brain and central nervous system tumors	HSPPC-96	II	Active, not recruiting	NCT00905060
Chronic myeloid leukemia	HSP70	I	Completed	NCT00027144
Breast neoplasm	HSP70	I & II	Terminated	NCT00027131
Melanoma of skin	Recombinant HSP70	I	Completed	NCT00005633
Follicular lymphoma; small lymphocytic lymphoma	HSPPC-96	II	Completed	NCT00081809
Cervical cancer pre-cancerous condition	HSPE7	II	Completed	NCT00054041
Pancreatic cancer	HSPPC-96	I	Completed	NCT00003025
Sarcoma	HSPPC-96	II	Completed	NCT00005628

^aFrom ClicalTrials.gov

indicated that flavonoids can lower the levels of HSP60 in a number of human tumor cell lines (98). However, the effect of this strategy *in vivo* has yet to be evaluated.

The use of monoclonal antibodies directed against specific membrane antigens in anticancer therapy has achieved excellent results. Some researchers have demonstrated that injections of a monoclonal antibody against a form of membranous HSP70 into mice with colon cancer significantly inhibited tumor growth, enhanced the overall survival and increased the infiltration of immune cells (natural killer cells, macrophages and granulocytes) within the tumor (86). These data, although still initial, are of great promise.

Another interesting therapeutic strategy is the use of HSPs as adjuvant for eliciting a specific antitumor immune response. HSPs can be effective as carriers of tumor antigens (99). Therefore, HSPs could be used as adjuvant to present tumor molecules (*e.g.* short peptides) to the immune system of the host. In this case, the immunization is mediated by HSPs linked to specific tumor epitopes that interact with receptors on the antigen-presenting cells (*e.g.* dendritic cells or macrophages) and, thus, induce a specific immune response (*i.e.* a cytotoxic T-lymphocyte response) with production of pro-inflammatory cytokines (61, 100).

HSPs can also be used as vaccines. For instance, in mice, the HSP110-HER2 chaperone complex used as a vaccine induces protective immunity, eliciting interferon (IFN)-gamma-producing T-cells against spontaneous mammary tumors (101, 102). Likewise, DNA vaccines, encoding HSP60 linked to HPV16 E6 and E7 for human papillomaviruses-associated cervical cancer, had a more potent immunotherapeutic effect than the tumor antigen without the chaperone (103).

In another experimental model, HSP60-containing exosomes derived from heat-shocked mouse B-lymphoma cells, induced a substantial antitumor CD8(+) T-cell response (104). This vaccine seemingly was not toxic for normal cells and specific for tumor cells. So far, pre-clinical and clinical studies have been carried out on renal cancer and melanoma with promising results (105-107).

Other trials on several types of cancer have been completed or are in progress (Table III). However, it should be borne in mind that repeated HSP administration could induce cross-tolerance to other pro-inflammatory stimuli, thus potentially limiting the applications of HSPs as an adjuvant or enhancer of the immune response against tumors (108).

Conclusion

HSP-chaperones are implicated in the pathogenesis and progression of a range of human cancer types. They are involved in vital mechanisms of cancerous cells, such as cell proliferation, differentiation, invasiveness, neoangiogenesis, metastasis, and immune system recognition. Various HSPs are nowadays considered biomarkers of carcinogenesis, and their expression is correlated with the degree of differentiation and aggressiveness of certain tumors. For these reasons, chaperones may also have potential as predictors of response to therapy. In addition, their use as anticancer agents is growing, for example as inducers of antitumor immune responses. When HSP-chaperones are essential to tumor growth and survival, they can act as specific targets for anti-chaperone compounds. Since expression of one or several HSPs is simultaneously elevated in tumors, one would think that this increase is just a manifestation of an augmented need for chaperones in the tumor cell, which grows and proliferates at a high rate and, therefore, it synthesizes more protein than its normal counterpart. But, even if HSPs do not have any specific pro-tumor effect, the mere fact that they assist in protein synthesis and augment their concentration and activity to allow the tumor to produce more protein seems reason enough to qualify the HSPs as mistakenly helping the enemy. In this situation, negative chaperonotherapy would be indicated, as much as if the chaperones actually had a specific pro-tumor action, such as that of preventing apoptosis. This is a field which although it represents a challenging endeavor with potential risks, offers very promising alternatives for treating cancer.

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