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Chaperone proteins and brain tumors: Potential targets and possible therapeutics[1]

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Chaperone proteins are most notable for the proteo- and cytoprotective capacities they afford during cellular stress. Under conditions of cellular normalcy, chaperones still play integral roles in the folding of nascent polypeptides into functional entities, in assisting in intracellular/intraorganellar transport, in assembly and maintenance of multi-subunit protein complexes, and in aiding and abetting the degradation of senescent proteins. Tumors frequently have relatively enhanced needs for chaperone number and activity because of the stresses of rapid proliferation, increased metabolism, and overall genetic instability. Thus, it may be possible to take advantage of this reliance that tumor cells have on chaperones by pharmacologic and biologic means. Certain chaperones are abundant in the brain, which implies important roles for them. While it is presumed that the requirements of brain tumors for chaperone proteins are similar to those of any other cell type, tumor or otherwise, very little inquiry has been directed at the possibility of using chaperone proteins as therapeutic targets or even as therapeutic agents against central nervous system malignancies. This review highlights some of the research on the functions of chaperone proteins, on what can be done to modify those functions, and on the physiological responses that tumors and organisms can have to chaperone-targeted or chaperone-based therapies. In particular, this review will also underscore areas of research where brain tumors have been part of the field, although in general those instances are few and far between. This relative dearth of research devoted to chaperone protein targets and therapeutics in brain tumors reveals much untrodden turf to explore for potential treatments of these dreadfully refractive diseases. *Neuro-Oncology 7, 260–277, 2005 (Posted to Neuro-Oncology [serial online], Doc. 04-118, May 26, 2005. URL http://neuro-oncology.mc.duke.edu; DOI: 10.1215/S1152851704001188)*

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Chaperone proteins, sometimes called stress proteins and often called heat shock proteins (HSPs),[3] are a family of relatively abundant proteins whose origins date to archaebacter. The high conservation exhibited amongst these entities indicates their importance in life, as all cells must face similar problems in terms of folding nascent proteins in the midst of high concentrations of materials within the intracellular milieu, assembling components of multi-subunit protein complexes, and transporting proteins across organellar or plasma membranes. Related to these functions are the roles played by chaperones when cells face stress conditions, such as heat, hypoxia, starvation, or radiation. As the intracellular environment becomes unfit for native protein conformations, chaperones bind and stabilize sensitive proteins and prevent protein aggregation. Upon release from stress, chaperones disassemble protein

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[3]Abbreviations used: 4-PB/PBA, 4-phenylbutyrate; APC, antigen-presenting cell; CRCL, chaperone-rich cell lysate; CTL, cytotoxic T lymphocyte; DC, dendritic cell; DSG, 15-deoxyspergualin; ER, endoplasmic reticulum; FTI, farnesyltransferase inhibitor; GA, geldanamycin; GRP, glucose-regulated protein; Hip, heat shock protein interacting protein; Hop, heat shock 70/90 organizing protein; HSC, heat shock cognate/constitutive; HSP, heat shock protein; MBP, myelin basic protein; MHC, major histocompatibility complex; NK, natural killer; RNAi, inhibitory RNA; TRA, tumor rejection antigen; TSA, tumor-specific antigen.

aggregates, refold salvageable proteins, and escort denatured polypeptides to their fate of proteolytic recycling.

Many brain cell types express abundant chaperone quantities, and brain tumors presumably have requirements for chaperone activity that are similar to those of any other cells. In fact, tumors overall may exact more of a toll from the chaperone system than nonmalignant cell types. The general hypoxic environment and the demand for rapid cell division put severe strains on the chaperone machinery to produce stable and functional proteins within a tumor cell. The result is that chaperone protein composition and activities are often highly upregulated in tumor cells, which leads to the concept of exploiting tumor chaperones as targets against the malignancy or mediating chaperone activities that will ultimately lead to the demise of the tumor (Lee, 2001; Mosser and Morimoto, 2004). This review attempts to touch on the cell biology of chaperone proteins, both in general and in the context of brain tumors, and speculates on the possibility of manipulating the varied and sundry activities of chaperones in order to eradicate these diseases. Given the high failure rate of most therapies against brain tumors, there is an obvious need for original ideas and fresh approaches to treating malignancies of the central nervous system, and chaperone proteins may provide a critical link to useful, effective therapies.

Chaperone Proteins, a Brief Overview

There has been a vast accumulation of literature on the chaperones even in the past few years alone, and for more detailed reviews the reader is referred elsewhere (Frydman, 2001; Houry, 2001; Naylor and Hartl, 2001). For general purposes, most of the mammalian chaperones and HSPs are grouped into families by apparent molecular weight on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Table 1 briefly summarizes mammalian HSPs of interest, with roles, features, and general references for the listed chaperones, and provides references that cite the chaperones in relation to brain tumors in particular.

Other important mammalian chaperones that do not fit neatly into the above categories include those of the endoplasmic reticulum (ER) such as calreticulin, calnexin, tapaisin, and protein disulfide isomerase. These proteins, along with glucose-regulated proteins (GRPs) GRP94 and GRP78, are important in the folding and maturation of immunologically relevant molecules such as immunoglobulins and major histocompatibility complex (MHC) class I molecules. In more general terms these proteins are critical members of the quality control apparatus of glycoprotein folding in the ER (Argon and Simen, 1999; Ellgaard and Helenius, 2001). Also,

Table 1. Chaperone protein families, roles, and features

Chaperone Family	Chaperone Role	Unique Features	References and Reviews	Brain Tumor Related References
HSP110 GRP170 (large HSPs)	Aggregation deterrent; assist in protein folding	Relatives of HSP70 Can stably bind large proteins in long-lived manner; utilized as anticancer vaccines	Manjili et al., 2002; Wang et al., 2004	Hylander et al., 2000
HSP90 GRP94/gp96	Folding and unfolding; stabilization of client "signaling" proteins; ER quality control; loading of peptides onto MHC I molecules	HSP90 binds HSF-1 to control its own transcription; earliest identified HSPs as anticancer vaccines; roles in stabilizing signal transduction molecules; a buffer against genetic/ mutational variation?	Argon and Simen, 1999; Nicchitta, 1998; Pratt and Toft, 2003; Ullrich et al., 1986	Hermisson et al., 2000; Kato et al., 2001; Lavictoire et al., 2003; Yang et al., 2001; Zagzag et al., 2003;
HSC73, HSP72, mtHSP70/GRP75, GRP78, HSP71t	Folding, unfolding; inter-organellar transport; clathrin uncoating; ER quality control	First HSP to be shown to bind peptides; utilizes ATP hydrolysis/ADP exchange for reaction cycle; roles in apoptosis; utilized as an anticancer vaccine	Flynn et al., 1989; Frydman, 2001	Hermisson et al., 2000; Kato et al., 1993, 2001; Kaul et al., 1997; Lavictoire et al., 2003; Nylandsted et al., 2002; Takano et al., 1997
HSP60 TriC/TCP	Folding, unfolding, refolding; sometimes specialized for particular proteins in cytosol and mitochondria	Forms barrel-like torroids that utilize ATP to unfold/refold proteins; expression on tumor cell surfaces may have immunologic or metastatic consequences	Barazi et al., 2002 ; Feng et al., 2001; Frydman, 2001	Kato et al., 2001
HSP40 DNA-J family	Assist HSP70 activities; involved in HSP70 ATP hydrolysis and nucleotide exchange	May have chaperone function; may require HSP70 cooperation for proper protein folding; important for in vivo function of HSP70	Frydman, 2001	
sHSP, (small HSP; i.e., 20–40 kD)	Stabilization and disassembly of aggregated proteins	Act as multimers; α-crystallin prevents aggregation of proteins in eye lens optical path; cancer status marker	Haslbeck, 2002	Aoyama et al., 1993; Hermisson et al., 2000; Hitotsumatsu et al., 1996; Kato et al., 1993, 2001

Abbreviations: ER, endoplasmic reticulum; GRP, glucose-regulated protein; HSC, heat shock cognate; HSP, heat shock protein; MHC, major histocompatibility complex.

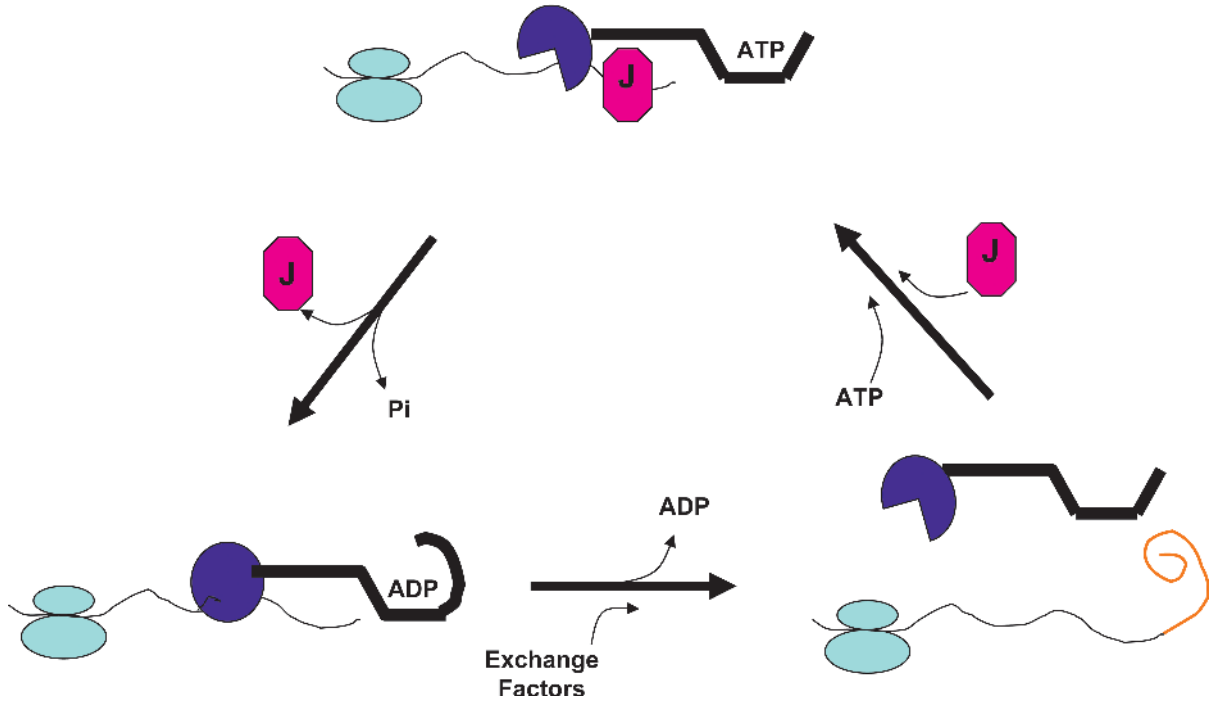

Fig. 1. HSP70 cycle of binding and release of nascent proteins. Shown is a highly simplified version of the binding and release cycle of the HSP70 family members of chaperones and the coupling of that chaperone activity with ATP/ADP binding, hydrolysis, and release. Top. HSP40/DNAJ (magenta octagon, "J") initially binds a nascent polypeptide coming off a ribosome (light blue), and recruits HSC/HSP70 (dark blue sphere, black segments) with ATP bound to the HSP70 nucleotide binding site. Lower Left. Hydrolysis of ATP to ADP (P_i, inorganic phosphate) and the exit of HSP40 results in the closing of the lid domain and sequestration of an 8 to 10 amino acid portion of the nascent polypeptide; ADP is removed with the aid of a host of nucleotide exchange factors, the peptide binding domain of the chaperone opens, and the chaperone is returned to a state capable of binding polypeptide and ATP again. Lower Right. The nascent protein has presumably undergone some appropriate folding toward native state confirmation.

an increasing number of co-chaperones are being identified that appear to modulate the activity or outcome of chaperone-based protein folding. These include Hop (heat shock protein70/90 organizing protein), Hip (heat shock protein interacting protein), and members of the BAG-1 family. A simplified diagram of the function of heat shock cognate (HSC)/HSP70, as an example of how chaperone proteins accomplish the role of assistance in protein folding, is shown in Fig. 1. This sort of binding and release of regions of hydrophobic polypeptide substrates is the essential activity for chaperones in protein folding, unfolding, aggregation stabilization, renaturation, and so forth.

Chaperone Expression in Brain Tumors

Much of the work devoted to chaperones in the context of brain tumors has been descriptive. The types of tumors examined include meningiomas, glioblastomas, (anaplastic) oligodendrogliomas. (anaplastic) ependymomas, (anaplastic) astrocytomas, schwannomas, medulloblastomas, primitive neuroectodermal tumors, leptomeningeal carcinomatosis, craniopharyngiomas, hemangioblastomas, and choriomas. There have been studies devoted to a range of stress proteins (Kato et al., 1993). In particular, there have been extensive studies on the HSP27 content of brain tumors, and it is now considered a marker of poor prognosis (Hitotsumatsu et al., 1996; Kato et al., 1992). Another member of the small HSP family, alpha-B crystallin, also appears to be upregulated in tumors of the CNS (Aoyama et al., 1993; Hitotsumatsu et al., 1996). At least one study has made an attempt to examine and compare HSP expression in glioblastomas histologically and in vitro with glioma cell lines subjected to hyperthermia (Hermisson et al., 2000). However, the functional significance of these highly expressed proteins in brain tumors remains unclear. Recently (Kaul et al., 1997; Takano et al., 1997), it has been proposed that mortalin (GRP75, PRB74, mitochondrial or mtHSP70) expression may be correlated with progressive staging of astrocytomas, in both its prevalence and its subcellular localization. Curiously, the presence of relatively high quantities of mortalin in normal cell types has been associated with the nondividing senescent phenotype of those cells (hence the name "mortalin") (Wadhwa et al., 1993, 2002a). Thus, the unusual amounts and localizations of mortalin in transformed cells, including those derived from brain tumors, may have something to do with mitochondrial responses to transformation. It is also conceivable that mortalin-p53 interactions and sequestration may be involved in the potential for tumor cell growth (Merrick et al., 1996; Wadhwa et al., 2002a). From the brevity of this section it is apparent that while histologic stud-

ies of chaperones in brain tumors have been performed, relatively little is known about the functional nuances of those proteins in such malignancies, and even less is known about the utility of chaperones as an area of therapeutic intervention in brain tumors.

Manipulating HSP Levels—Chaperone Proteins as Pharmacologic Targets

It is not inherently obvious that chaperone roles in tumor cells are dissimilar from those in normal cell counterparts. However, the overexpression of these proteins and the known biochemical characteristics of the intracellular milieu in which they reside begs the question: Is there a relationship between the stressed environment of a tumor cell and the inflated presence of chaperones? Furthermore, is it possible that the upregulation of the chaperones can actually enable the stressed but proliferative status of tumor cells? The reliance of tumor cell survival on chaperone proteins makes those proteins attractive as targets for therapeutic intervention, provided one can identify the chaperones to be targeted as well as the means of accomplishing that goal. What follows is a compilation of targets and agents arranged more or less by the chaperone affected by the agent.

HSP90 Binding Agents

The role of chaperones, especially HSP90, in the maintenance of metastable signaling complexes has become an area of great interest. HSP90 has a relatively limited substrate repertoire ("client proteins"), although that list is rapidly expanding (Pratt and Toft, 2003) and is nearly 100 proteins currently. That list includes numerous molecules at key positions in signaling pathways, including several chimeric and mutated proteins known to be involved in tumor initiation or progression (Pratt and Toft, 2003). For the signaling clients, HSP90 and a cohort of other chaperones and co-chaperones maintain and "mature" the client protein in waiting for the arrival of ligand. Thus primed, the ligand binding results in the release of the active substrate and the ensuing signaling cascade or nuclear localization and transcriptional activation. If HSP90/client interactions are disrupted, such signaling molecules are unstable, and they face proteosomal degradation (Mimnaugh et al., 1996; Schulte et al., 1997).

Geldanamycin (GA) is a benzoquinone ansamycin antibiotic that binds with high specificity to the N-terminal nucleotide binding pocket of HSP90 (Prodromou et al., 1997; Stebbins et al., 1997; Whitesell et al., 1994). Blocking of the nucleotide binding pocket inhibits critical HSP90 ATPase activity, preventing its stabilizing interactions with essential proteins necessary for proliferative signaling or cell cycle progression, and thus preventing continued growth of tumor cells. While GA affects HSP90 of normal cells in a similar fashion, with the resultant downstream effects on signaling, the drug clearly has a more dramatic impact on tumor cells. This is presumably because higher proportions—in some cases, virtually all—of HSP90 molecules in tumor cells are found in complexed states with client proteins (Kamal et al., 2003). This complexed state is apparently a high binding conformation of HSP90 for GA, resulting in a higher accumulation of GA in tumor tissue than in normal tissue, and thus a more detrimental impingement on HSP90-mediated signaling events. Figure 2 shows an elementary scheme of HSP90 function and GA-induced dysfunction in the context of signaling or transcriptional activator client ligands.

HSP90 client proteins of interest to those studying brain tumors include EGFR and a mutated variant EGFRvIII, PDGFR, FAK, AKT, hTERT, p53, cdk4, MAPK, PI3K, EF-2 kinase, and HIF-1α. (For a review of potential and current molecular targets of malignant gliomas, see Rich and Bigner [2004].) In separate studies, GA and the clinically relevant 17-aminoallylgeldanamycin have been demonstrated to target HSP90 as a chaperone of EF-2 kinase (Yang et al., 2001), of HIF-1α (Zagzag et al., 2003), and of the mutant EGFR variant III (Lavictoire et al., 2003). The GA-induced disturbance of chaperone/EF-2 kinase resulted in reduced clonogenicity of glioma cells in culture and inhibited growth of glioma xenografts in nude mice (Yang et al., 2001). Disruption of HSP90/HIF-1α complexes decreased migration of a number of glioma cell lines with a concomitant decrease in FAK phosphorylation (Zagzag et al., 2003), although it is not clear that FAK levels themselves were not reduced.

Frequently in high-grade glioblastoma there are amplifications of EGFR, and in at least half of the tumors there is an in-frame deletion resulting in a mutant receptor, EGFRvIII (Humphrey et al., 1990; Wikstrand et al., 1995; Wong et al., 1992). As shown in the aforementioned recent study by Lavictoire et al. (2003), HSP90 interacts with the nascent mutant receptor in EGFRvIII-FLAG-tag transfected glioma cell lines, while HSP90's putative interactions with the wild-type receptor are conflicting (Park et al., 2003; Sakagami et al., 1999; Supino-Rosin et al., 2000; Xu et al., 2001). By co-immunoprecipitation, other chaperones appeared associated with the chaperone-mutant receptor complex, including HSP70 and the ER residents GRP78 (BiP) and GRP94. GA treatment of cells expressing the construct decreased the expression of EGFRvIII, and also cdk4, part of another signaling pathway that is likely involved in glioblastoma formation and progression. While neither the cellular in vitro phenotype nor the in vivo effects of GA treatment on EGFRvIII-expressing cells have been examined, the results of the aforementioned Lavictoire et al. (2003) report certainly call for those studies to be done.

Survivin is an antiapoptotic protein shown to be overexpressed in brain tumors that may be a target for immunotherapy (Katoh et al., 2003). Inhibition of HSP90 activity leads to survivin degradation and cell cycle arrest (Fortugno et al., 2003), and thus another potential sensitive brain tumor target is added to the cache of HSP90 clients.

Given HSP90's critical role in the nexus of signaling that cancer cells frequently utilize, it is not surprising

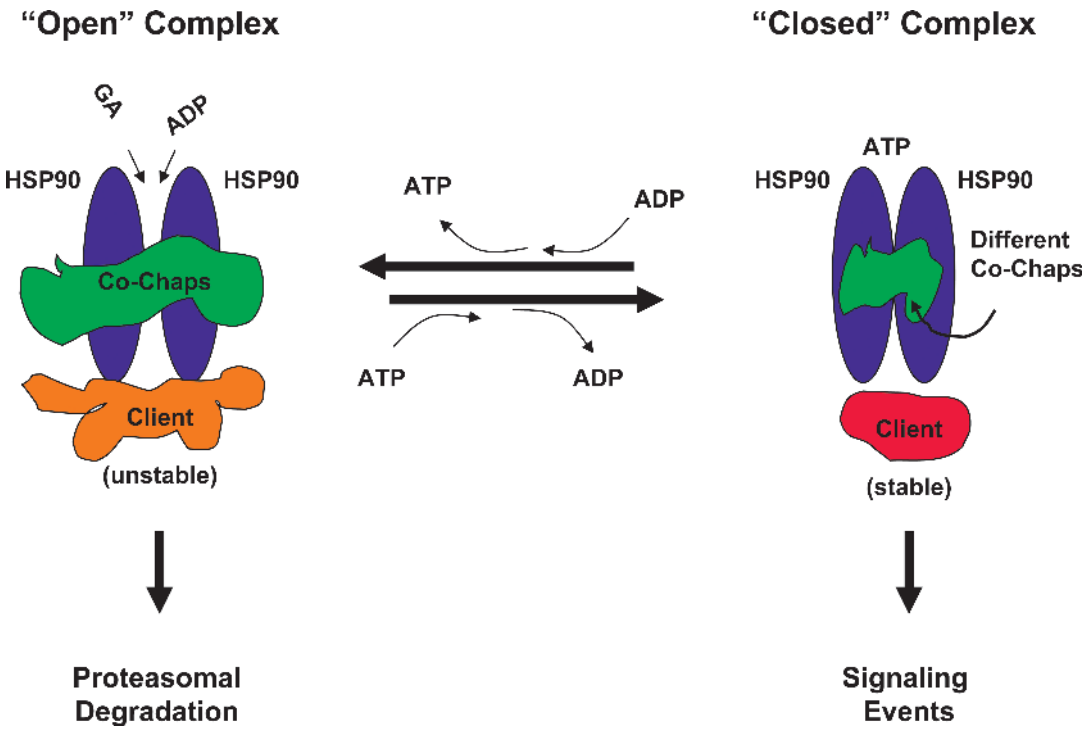

Fig. 2. HSP90 "chaperone machine" in open and closed complexes. HSP90 dimers (blue ovals) interact with a host of other and co-chaperones (co-chaps) such as HSP70, HSP40, Hip, Hop, immunophilins, Cdc37, and p23 (represented in green) in determining the maturation state of client proteins (orange or red shapes). The fate of the client protein depends on the co-chaperone cohort and the nucleotide status of the complex. Binding of the client occurs in the open state, and maturation of the client proceeds with subsequent binding and hydrolysis of ATP and a change in co-chaperones in the closed state. In the open state, the client is unstable and subject to proteasomal degradation, particularly in the presence of HSP90 inhibitors such as geldanamycin (GA), which maintains the complex in the open conformation. In the closed state, the client protein is able to assume the proper confirmation for downstream activities such as hormone binding or signal transduction.

that several other HSP90 inhibitors are known, such as another anamycin, herbimycin A, and radicicol, which also bind to the N-terminal nucleotide binding pocket, as well as novobiocin, which appears to target a recently identified C-terminal ATP-binding site in HSP90 (Sreedhar et al., 2004; Workman, 2004). Other pharmaceutical searches are under way (Le Brazidec et al., 2004; Soga et al., 2003).

Other Chaperones, Other Pharmacologics

Pharmacologic agents that react with HSP family members other than HSP90 are rarer and less well understood. Indeed, the immunosuppressant 15-deoxyspergualin (DSG) and some of its analogs have been shown to bind to the constitutively expressed HSC70 as well as HSP90 (Nadler et al., 1992, 1995), but the molecular consequences of HSC70 binding that lead to immunosuppression are currently unknown. DSG has nanomolar effective ranges on cells, despite near micromolar amounts of HSC70 in cells. This situation is reminiscent of the relationship of GA activity/cytotoxicity to the amount of HSP90 in the cell, which is similar to the amount of HSC70 (i.e., GA is cytotoxic to tumor cells at nanomolar concentrations). There is no clear parallel for DSG and HSC70, in that it is unknown which, if any, HSC70 conformations are particular for high-affinity DSG binding. One might predict that a specific or critical population of HSC70 may be the drug target, but what makes that population critical is undefined.

4-Phenylbutyrate (4-PB, also termed PBA) has also been shown to decrease activities of HSC70, particularly studied in the context of modifying the chaperone's interactions with the cystic fibrosis transmembrane conductance regulator (Rubenstein and Zeitlin, 2000). At the same time, 4-PB treatment of IB3-1 cells increases the expression of the inducible HSP70 protein. However, 4-PB works as a histone deacetylase inhibitor and does not directly interact with the chaperones. While the relationship between presumed transcriptional regulation by 4-PB and its effect on HSC70 mRNA is murky, 4-PB does have pleiotropic effects on gene expression, including induction of some stress response genes, such as the HSP70 and HSP110 family members (Wright et al., 2004). There have been several reports of 4-PB treatments and outcomes using glioma cell lines, including proliferative inhibition and reduced invasive phenotype with decreased expression of c-myc and urokinase (Engelhard et al., 1998), enhanced gap junction communication with alterations in glial fibrillary acid protein expression (Asklund et al., 2004), and reduced GAPDH mRNA expression (Appelskog et al., 2004). There is

also a report of a 4-PB-treated patient who had had a recurrent glioma achieving a complete clinical response (Baker et al., 2002), demonstrating the drug's clinical effectiveness in at least an isolated case. The roles of chaperone proteins that may be affected by 4-PB were not examined in these studies, but it may be of interest to explore the upshot of the drug on chaperones in brain tumor models.

The lypophilic rhodocyanine dye MKT-077 has been utilized in clinical trials, demonstrating anticarcinoma activity, with accumulation and damage selectively to tumor cell mitochondria (Koya et al., 1996; Modica-Napolitano et al., 1996). MKT-077 binds to the mitochondrial HSP70 mortalin/GRP75/mtHSP70 (Wadhwa et al., 2002b), where it seems to have effects on p53, telomerase, Ras, and actin. The pleiotropic effects of the drug could quite possibly be related to its interactions with mortalin, particularly in relation to mitochondrial toxicity and p53-mediated senescence events (such as telomerase activity), but the Ras/actin connection to mortalin has not been elucidated.

Drugs or other small molecule inhibitors of other HSPs/chaperones are even fewer in number and have thus far had less impact on oncotherapy. Mizoribine (bredinin) is regarded as an immunosuppressive inhibitor of purine biosynthesis that has been demonstrated to bind HSP60 (Itoh et al., 1999). As an inhibitor of HSP60 function, it was shown to reduce integrin-mediated tumor cell (breast carcinoma) migration on cultured substrates (Barazi et al., 2002). The complicated roles played by HSP60 in protein-folding complexes (particularly in mitochondria) versus its unknown roles and complexation states in other subcellular locations warrant further study from the perspective of cell biology, as well as from the perspective of a therapeutic target.

Since the discovery that mutant oncogenic Ras has transforming activity, there has been a search for Ras inhibitors. For Ras to be active oncogenically, it must be bound to the inner leaf of the plasma membrane, and that membrane retention requires prenylation of specific amino acids. Prenyltransferase inhibitors (often called farnesyltransferase inhibitors, or FTIs) have been designed either to inhibit the substrate modification via pseudosubstrate FTIs or to prevent enzyme activity with farnesyl group mimics. There have been reports of altered (and unaltered) farnesylation of the co-chaperone HSP40 (HDJ-2) in tumor cells following treatment with FTIs (Karp et al., 2001; Kelland et al., 2001; Sun et al., 2003). There have been no straightforward associations made between that phenomenon and increased expression of HSP70 seen in at least some tumor cell types upon FTI exposure (Hu et al., 2002, 2003). Nonetheless, it is tempting to speculate that HSP40/HSP70 may be involved in the activities of FTIs, which, it turns out, do not rely on mutant Ras status to show antitumor efficacy (Brunner et al., 2003; Cox and Der, 1997).

The flavonoids are a class of biocompounds of which some members are capable of suppressing heat shock or other stress response in cells (Hosokawa et al., 1990). At least one of these compounds, quercetin (or a derivative), has been utilized in clinical trials (Ferry et al., 1996; Mulholland et al., 2001). Quercetin has complicated effects on the ability of cells to induce a stress response (Elia and Santoro, 1994), believed initially to be due to a "decline" in the ability of the major HSP transcription factor (HSF-1) to bind to heat shock elements. Other studies have shown that the dosage, scheduling, cell type, and culture conditions and the extent, type, and duration of the stress all affect the outcome of quercetin treatments of cells (Jakubowicz-Gil et al., 2002; Rong et al., 2000). Quercetin has been used as a sensitizer of cells to hyperthermia (Asea et al., 2001; Wachsberger et al., 2003), and it is likely that it will find further therapeutic portals as we increase our understanding of the physiology of the stress response in cancer cells.

Recently there has been the discovery of a novel benzylidene lactam inhibitor of thermotolerance, KNK437, which appears to inhibit the expression of various HSPs (Yokota et al., 2000), which may have implications in generating heat- or stress-intolerant tumor cells (Nonaka et al., 2003). Although the mechanism of action of KNK437 is unknown currently, one might predict that the compound will take a place alongside agents such as quercetin in terms of discerning the aspects of stress response that might prove therapeutically useful.

From the information described above, it is apparent that there is a paucity of publications involving small-molecule inhibitors of chaperone proteins in brain tumor preclinical and clinical studies. With our increasing appreciation of the activities of chaperones as players in the maintenance and performance of proteins involved in (or even critical to) the tumorigenicity of brain tumors, this is clearly an area ripe for basic and applied research.

Manipulating HSP Levels Nonpharmacologically—"Gene Therapy" and Hyperthermia

Genetic Manipulations

Antisense DNA constructs, and more recently, inhibitory RNA (RNAi) sequences, are means of downregulating expression of a particular protein by targeting its translation (Pardridge, 2004). In the setting of preclinical brain tumor models, only one group (Nylandsted et al., 2002) has published results on pursuing inhibition of HSP expression. The results indicated that depletion of the inducible HSP70 via adenovirus-delivered antisense constructs in glioma cell lines resulted in apoptotic cell death. Glioblastoma xenografts in nude mice were greatly reduced in size (with concomitant improvement in survival), and the results hinted that an immune mechanism might be involved. Since the presence of chaperones, and the inducible HSP70 in particular, seems to predispose cells away from apoptosis (Garrido et al., 2001; Jaattela, 1999a, b; Takayama et al., 2003), it was reasonable that depletion of HSP70 resulted in apoptosis of the tumor cells. While there is considerable debate about the effect of apoptosis in immune responses (Manfredi et al., 2002; Melero et al., 2000; Navratil et

al., 2004; Proskuryakov et al., 2003), it has been often shown that apoptotic tumor cell death does not result in viable antitumor immune responses, ranging from simply bland to anergic (e.g., Basu et al. [2000] and Feng et al. [2001]; however, see also the studies of Bellone et al. [1997] and Scheffer et al. [2003]). Thus, in the in vivo portion of the work, Nylandsted et al. found macrophage-like cells (presumably microglia in the brain tumor model) infiltrating the apoptotic tumors. Further in vitro work showed that human macrophage effectively phagocytosed apoptotic tumor cell corpses but remained unsuppressed to activation stimuli, unlike previous reports (Reiter et al., 1999). As mentioned, this is one of the few reports describing an attempt to use antisense techniques to reduce HSP expression as a potential therapeutic for brain tumors.

"Gene therapy" upregulation of chaperone expression may be beneficial in terms of extracting an immune response against tumors, provided the tumor cells somehow expose the chaperones to the immune system by either display of chaperones on the cell surface (see below) or via cell lysis and release of chaperone proteins. Huang et al. (2003) have utilized oncolytic viruses, engineered to overexpress HSP70, that were capable of infecting tumor cells as a means to achieve both ends: After a period of HSP70 overexpression, the viruses lyse the tumor cells. Following the release of antigenic tumor contents, HSP70 presumably serves as a transporter of tumor antigen peptides to responder antigen-presenting cells (APCs), as well as a danger signal to engender a stronger immune response than tumor cell lysis alone. (See below for more on HSPs and the immune response.) Viral delivery of therapeutic genes to brain tumors has long been a goal (Vecil and Lang, 2003), so the utility of adding HSP genes to the repertoire should be apparent.

On a note related to neurodegenerative disorders featuring polyglutamine (poly-Q) aggregation, transgenic overexpression of chaperones has had varied effects on outcomes for animal models of such diseases. For instance, Kazemi-Esfarjani and Benzer (2000) found that a DNA-J (HSP40)-like gene could suppress poly-Q disorders in a *Drosophila* model, as does HSP70 (Bonini, 2002). However, Hansson et al. (2003) saw little impact of HSP70 overexpression in Huntington's disease mice. Such models provide an invaluable setting for dissecting the roles of chaperones in the etiology, the prognosis, and perhaps the remedy for these disorders.

Hyperthermia

An obvious means of manipulating the HSP expression of tumor cells is to subject the cells to hyperthermia, but it is important to bear in mind that hyperthermia aims to provoke a complex systemic response beyond solely heat shock induction. There have been more than a dozen reported clinical trials involving hyperthermia as a treatment modality for brain tumors (Borok et al., 1988; Kobayashi et al., 1990; Moran et al., 1995; Nakajima et al., 1993; Roberts et al., 1986; Tanaka et al., 1987; Thrall et al., 1999). The trials differ in the types of technology employed to generate heat within the tumor, combinations with brachy- or chemotherapy, target temperatures, and treatment frequencies and durations. Obviously, with such a diverse number of variables built into the different trials, it is difficult to draw reasonable conclusions from the whole lot, and the brain tumor experience in total seems diminutive when compared with more than a thousand reports on clinical hyperthermia already in the literature. While there appears to be much promise for this therapeutic mode, clearly, more research is needed in the area, particularly in some standardized means of conducting clinical trials.

Manipulating Exogenous Chaperone Proteins: Chaperone Proteins in the Immune Response

Antigens and Activation

There has been an increasing interest in the use of chaperone proteins as a means of modifying the immune response, mostly from a cancer vaccine perspective (reviewed in Castelli et al. [2004], Graner and Katsanis [2004], and Srivastava [2002]). The basis for such vaccines derives from early experiments (Srivastava et al., 1986; Ullrich et al., 1986) demonstrating that certain purified proteins from tumors could be used as tumor rejection antigens (TRAs), while also seeking to answer whether or not TRAs were tumor-specific antigens (TSAs) or if tumors had shared antigens in common. From the results of those two groups, it certainly appeared that at least for the TRAs they had discovered, the antigens were indeed TSAs. The conundrum that faced the researchers was that the TSAs were not at all tumor specific, in that those proteins turned out to be members of the HSP90 family, which were widely expressed and highly conserved entities across cells, tissues, organisms, and species. The specificity seemed to lie only in the fact that the TSA purified from one tumor type would not protect against a challenge from a different tumor type, nor vice-versa, despite the apparent identity of each of the different "TSAs." Purified HSPs in and of themselves (e.g., from normal tissue) did not engender antitumor immunity either, so chaperones per se were not antigenic. Wherein lay the real antigens?

The discovery that chaperone proteins of the HSP70 family could bind peptides (Flynn et al., 1989) provided a plausible mechanism for the vaccine phenomena in that the chaperones purified from a given tumor could have bound with them, as cargo, peptides derived from proteolytic processing of the tumor proteome. The specificity was immunologically implicit, which meant that each tumor had an individual peptide repertoire, a "fingerprint" recognizable in the currency of the cellular immune system. Such a scenario explains how chaperone proteins that are fundamentally identical from tumor to tumor, or even to those in normal tissues, could provide specific immunity against a particular tumor but fail to protect against a challenge from a different tumor type. It has been proposed that chaperones have access to intracellular peptides, likely resulting from protea-

somal degradation of proteins (Srivastava et al., 1994). This places the chaperones essentially in contact with the "peptidome" of the tumor, and purifying the tumor-derived chaperones provides a snapshot of potential antigens to the immune system upon vaccination with the purified chaperones. One of the chief tenets of chaperone protein vaccines is that one does not need to know the identities of the antigens within a given tumor; the immune response selects the appropriate antigens for action as provided by the tumor-derived chaperones.

Aside from a duty of peptide transport, chaperones appear to provide a surprising stimulus to the immune system, acting essentially as proinflammatory cytokines (Prohaszka and Fust, 2004; Srivastava, 2002; van Eden et al., 2003). This spurring of innate immune responses leads to activation of APCs such as macrophage cells and dendritic cells (DCs) and presumably of their neurological relatives, microglia. These now-activated APCs are capable of effectively prompting T cells into action by direct stimulation and by cytokine secretion. When coupled with the presence of tumor-derived antigenic peptides, the T cell response should be maximized. Also, the proinflammatory cytokine environment leads to the activation of innate immune response cells such as natural killer (NK) cells. Aspects of the intracellular peptide binding roles for chaperone proteins have come into question of late (Nicchitta, 2003). Nonetheless, it appears that peptides become associated in some fashion with chaperones prior to or upon extraction from cells (M.W. Graner, unpublished data; Menoret et al., 1999; Zeng et al., 2005), and the effects of exogenous chaperones on the innate immune cells are certainly not denied (Nicchitta, 2003). Thus, chaperone proteins are at a nexus of the innate and adaptive immune responses.

Chaperone Protein Vaccines

Chaperone proteins that have been demonstrated to be effective as vaccines against tumor challenges in either prophylactic or therapeutic settings include GRP170, HSP110, GRP94/gp96, HSP90, HSP/HSC70, and calreticulin (reviewed in Graner and Katsanis [2004] and Srivastava [2002]). Some of the vaccines included multiple chaperones enriched from tumor lysate (collectively referred to as chaperone-rich cell lysate, or CRCL [Graner et al., 2000, 2003; Zeng et al., 2003, 2004]). Chaperone protein immunizations invariably have been more potent than vaccinating with tumor lysates, even when those lysates have been pulsed onto "professional" APCs such as DCs in the form of a cellular vaccine (Graner et al., 2003; Zeng et al., 2003). One of the more unusual chaperones, which appears to be capable of binding peptides and delivering them successfully to the immune system, is α2-macroglobulin (Binder et al., 2001). The impact of this form of adjuvant/antigen package on antitumor immune responses is just beginning to be realized.

The chaperone vaccines have all shown exquisite specificity in generating immune responses against the tumor of origin of the chaperones; however, it is also possible to complex peptides of choice to chaperones such as GRP94/gp96 and HSP/HSC70 and to complex even large polypeptides/proteins to HSP110 (Blachere et al., 1997; Kumaraguru et al., 2003; Manjili et al., 2002; Wang et al., 2003; Wearsch and Nicchitta, 1997). Peptides can also be incorporated with ease into CRCL (M.W. Graner and A. Romanoski, unpublished data; Kislin et al., 2004). As mentioned above, vaccination or in vitro assays with such "designer" chaperone-(poly)peptide complexes result in immune responses against the complexed peptide (or protein), but not against the chaperones themselves.

Chaperone Interactions with Antigen-Presenting Cells

The chaperones appear to interact with professional APCs via specific receptors, although that area of research is emerging as somewhat contentious. Srivastava et al. (1994) predicted the existence of such receptors on APCs as a conduit for exogenous peptide delivery into the antigen-processing pathway. Binder et al. (2000) reported that CD91 (also known as the α2 macroglobulin receptor and the low-density lipoprotein receptor–related protein) appeared to be a receptor on macrophage for GRP94/gp96; in a subsequent study, Basu et al. (2001) indicated that on both macrophage cells and DCs, CD91 was a common receptor for GRP94/gp96, HSP70, HSP90, and calreticulin. The Toll-like receptors 2 and 4 have been demonstrated to be receptors for HSP60 (Ohashi et al., 2000) and for GRP94/gp96 (Vabulas et al., 2002), while HSP60 has been shown to bind to monocyte CD14 (Kol et al., 2000). HSP70 has been described as a chaperone cytokine, or "chaperokine" (Asea et al., 2000), that also utilized CD14 as a "co-receptor," and LOX-1 (Delneste et al., 2002) and CD-40 (Becker et al., 2002) are reported as cell surface receptors for HSP70. To further confuse the issue, Berwin et al. (2002) provided recent evidence in macrophage cells that GRP94/gp96 can mediate specific immunity independent of CD91, which was followed by identification of scavenger receptor A as a receptor for GRP94/gp96 and calreticulin (Berwin et al., 2003). Scavenger receptor CD36 had previously been demonstrated to be a receptor for GRP94/gp96 (Panjwani et al., 2000), and the large chaperones GRP170 and HSP110 appear to interact with macrophage cells via scavenger receptors, also (J.R. Subject, personal communication, 2004). The sequence of events going from cell lysis and chaperone release into the extracellular space (or purification of the chaperones), followed by APC uptake, activation, and re-presentation of peptide cargo, with subsequent stimulation of specific T cell responses, is shown in Fig. 3.

Since the chaperones bind with some specificity on DCs and can subsequently enhance activation and antigen presentation, pulsing DCs with tumor chaperones and with heat-treated cells or cellular material should provide for a powerful vaccine. That is indeed the case in animal studies where DC/tumor chaperone vaccines have been used in both prophylactic and therapeutic antitumor settings (Graner et al., 2003; Zeng et al., 2003, 2004). In addition, Feng et al. have shown that chaperones derived from nontumor tissue (i.e., normal

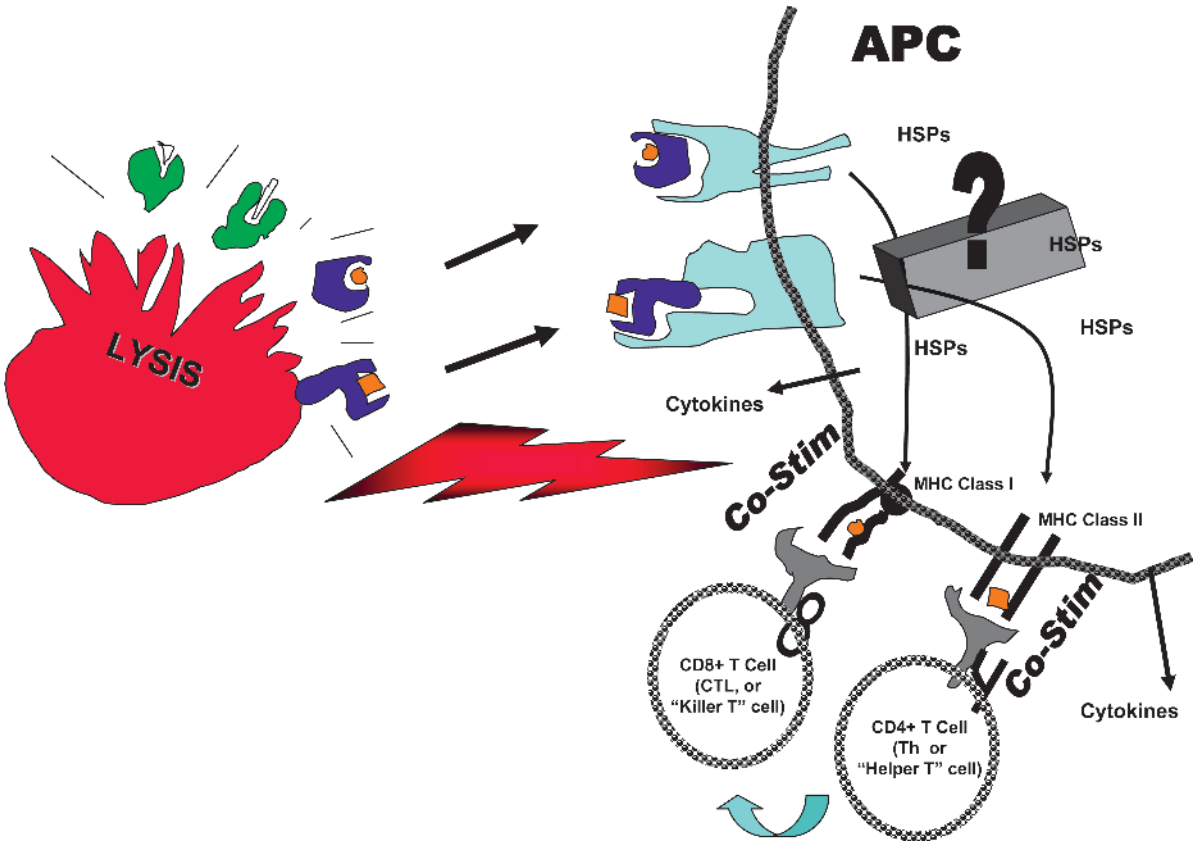

Fig. 3. Chaperones and the immune response. Chaperone proteins (green and dark blue shapes) are released upon cell death (lysis), with associated peptides (small white or orange shapes). Chaperones from the extracellular environment are bound by specific receptors (light blue shapes) on professional antigen-presenting cells (APCs). In a process that is far from understood (black box with "?"), peptides are transferred from the chaperone/receptor complex into the antigen presentation pathways that end with peptide display on the APC surface restricted by MHC class I and II molecules. Such transfer may involve intracellular HSPs of the APC. Presumably, similar intracellular, HSP-based mechanisms exist in typical cells (i.e., non-APCs) for the processing and display of peptides by MHC I molecules. Assuming that the lysed cell is a tumor cell, some of the peptides transferred into the presentation pathways may be antigenic. These peptides, when presented by MHC class I or II molecules, could stimulate CD8+ and CD4+ T cell responses, respectively, and would do so in the context of upregulated expression of inflammatory cytokines (TNF-α, ILs-1β, -6, -12, etc.) and costimulatory molecules (CD40, CD80/86). Thus activated, the CD8+ (cytotoxic) T cell repertoire would be further stimulated by the CD4+ (T helper) T cells to seek out and destroy tumor cells that presented the same peptide that was originally delivered to the APC via released chaperones or chaperones purified from the tumor and utilized as a vaccine. The reader is advised to bear in mind that much of this information is currently controversial and mechanistically unproven. The overall vaccine effects, however, are well established.

tissue) can act as exogenous "danger signals" to stimulate DCs into effectively processing apoptotic tumor cells and generating potent antitumor immunity involving both CD4+ and CD8+ T cells (Feng et al., 2002, 2003). While it would appear obvious that combining the best APCs with a vaccine that possesses its own adjuvant properties (and antigens, if tumor derived) would be efficacious, relatively few research groups aside from those mentioned above have studied DC/chaperone vaccines in vivo. It is apparent from the use of DCs as cellular vaccines in animal models of brain tumors and in human clinical trials (reviewed in Fecci and Sampson [2002] and Yang et al. [2003]) that DC vaccination is safe and that the "immune privilege" of the blood-brain barrier does not prevent DC-driven immune responses by T cells against tumors of the CNS.

Chaperone Vaccines and Brain Tumors

Despite the attention given chaperone protein vaccines, as well as publications of promising clinical trial results (Belli et al., 2002; Janetzki et al., 2000; Mazzaferro et al., 2003; Younes, 2003), to date there have been no preclinical or clinical applications of this type of immunotherapy against brain tumors. This is perhaps due to the relative novelty of chaperone proteins as vaccines and the comparatively small size of typical brain tumors (i.e., difficulty in obtaining sufficient biochemical material for chaperone purification). A significant advantage of CRCL vaccines is the high yield of vaccine per unit amount of starting material (generally 1 mg of vaccine per gram of solid tumor), which is some 20 to 50 times more material than can be obtained for any routine

attempt to purify a single chaperone such as GRP94 or HSP70. Such an approach may be able to overcome the lack of biochemical starting material. Another concern in the use of brain-tumor-derived vaccines is that of mounting autoimmune responses against normal brain tissue antigens (e.g., myelin basic protein [MBP]) that would have catastrophic consequences. However, there have been no reports of autoimmunity in brain tumor vaccine trials using tumor lysates, tumor peptides, or tumor RNA pulsed onto DCs, the most potent of APCs (Kobayashi et al., 2003; Yamanaka et al., 2003; Yu et al., 2001, 2004; reviewed in Soling and Rainov [2001]), but there is justifiable trepidation that tumor-derived, chaperone-based vaccines may break immunological tolerance. The relationship between chaperone proteins and MBP has been explored before (Aquino et al., 1998), and other neuronal proteins appear to associate with chaperones, particularly HSP70, including myelin proteolipid protein and myelin oligodendrocyte protein (Cwiklinska et al., 2003). HSP70 seems to have an especially apt role in the generation of autoimmunity to MBP via MHC class II presentation (Mycko et al., 2004). The use of chaperone vaccines against brain tumors clearly must be carefully tested in in vitro settings and in animal models before cautious attempts in human immunotherapy trials.

One potential means of employing the immune stimulatory power of chaperone-based vaccines would be to make "designer" vaccines, where the chaperones are generated recombinantly or from presumably immunologically innocuous material. Then, (poly)peptide antigens of choice may be bound by the chaperones by in vitro methodologies, as mentioned above. This allows for specific immunological targeting of tumor antigens, presumably with an increase in antigen density of the vaccine as compared with chaperone purification or extraction from tumors themselves, and with a concomitant decrease in the amount of potentially autoimmune antigens that may be present in the tumor. The list of potential antigens that are believed to have relevance for brain tumor immunotherapy includes EGFRvIII (Ashley et al., 1997; Heimberger et al., 2003; Moscatello et al, 1997), SART-1 and SART-3 (Imaizumi et al., 1999; Murayama et al., 2000), TRP-2 (Liu et al., 2003), HER2/Neu, gp100, MAGE-1 (Liu et al., 2004a), and AIM-2 (Liu et al., 2004b), as well as GAGE, TRP-1, p97, SSX-1, -2, -4, SCP-1, and TS85 (reviewed in Walker et al. [2003]). This is clearly a small number of antigens overall, and more research in this area will undoubtedly be beneficial.

While there is general agreement that there are relatively few known antigens for most brain tumors, more are being discovered through monoclonal antibody screens, serial analysis of gene expression, rapid expression screening, and database mining algorithms (Loging et al., 2000; Wikstrand et al., 1999). Examples of new targets emerging from those techniques include the somatostatin receptor type 2 (C.J. Wikstrand et al., manuscript submitted), a member of the multidrug resistance transporter family, MRP3, and a novel membrane glycoprotein, GPNMB. As for MRP3, previous work (Yamada et al., 2001) has demonstrated numerous HLA-A2402 restricted epitopes within the protein that are recognized by cytotoxic T lymphocytes (CTLs), including CTLs generated from cancer patients. One could conceive of a personalized vaccination strategy whereby chaperone proteins are complexed with a cocktail of peptides that have been chosen for vaccine incorporation on the basis of molecular characterization or phenotyping of a patient's brain tumor.

Chaperone Protein Cell Surface Expression

A phenomenon that is gaining increasing interest in the area of chaperones in the immune response is that of cell surface expression of chaperones, particularly by tumor cells (Multhoff and Hightower, 1996). One of the original publications that indicated tumor-derived chaperones could be used as vaccines also described the TRA as abundant in the cytosol and in addition as a cell surface protein (Ullrich et al., 1986), while Srivastava and Old (1989) also referred to GRP94/gp96 as a cell surface molecule. Cell surface HSP60 was shown to be a target for γδ T cells (Kaur et al., 1993), and human leukemia T cell virus-infected lines expressed sufficient cell surface HSP70 to elicit specific HSP70 antibodies in rabbits (Chouchane et al., 1994). Calreticulin was identified as a cell surface protein for the first time on a melanoma cell line as having lectin-like activity involved in cell spreading (White et al., 1995), and the Epstein-Barr virus–transformed lymphocyte cell surface protein BE2 was determined to be GRP78 (Berger et al., 1997). Since virtually all of the chaperone proteins were believed to be intracellularly localized, some of the accounts of chaperone cell surface expression were met with skepticism, but it is now being verified by a variety of techniques that many tumor cells very likely have chaperone proteins present in some context on their cell surfaces (Shin et al., 2003).

Involvement of cell surface HSP90 in immune phenomena was also suggested by Ferrarini et al. (1992), but it is not clear how thorough the searches for HSP90 expression have been until recently (Becker et al., 2004). For neuro-oncologists it is of interest to note the description of calreticulin on the cell surface of a hybrid murine neuroblastoma/rat glioma cell line NG108-15 (Xiao et al., 1999). In a similar vein it has been shown that chaperones (in particular, HSP70, HSP110, and GRP78) can be released from stressed cells, including glia (Guzhova et al., 2001; Hightower and Guidon, 1989; Tytell et al., 1986). However, most attention that has been directed to HSP70 family members as cell surface immune mediators has been for their role as targets of NK cells (reviewed in Multhoff [2002] and Multhoff and Hightower [1996]) and possibly as targets for tumor-infiltrating T cells (Trieb et al., 2000). A specific 14-mer peptide of the inducible HSP70 appears to be the ligand for NK cells, perhaps via interactions with CD94 and CD56 (Gross et al., 2003a; Multhoff et al., 2001). Recognition of cell surface HSP70 on tumor cells by NK cells results in the apoptotic lysis of the tumor cells by release of granzyme B by NK cells and its uptake by tumor

cells through HSP70 (Gross et al., 2003b). Results of a phase 1 trial using ex vivo HSP70 peptide stimulation and reinfusion of autologous NK cells have been recently reported (Krause et al., 2004), showing an excellent toxicity profile and some clinical response in patients with advanced diseases. While HSPs have been identified at the cell surface of heat-shocked cells and immune consequences have been noted in studies by Feng (2001) and Wu et al. (1999) as well as all of the aforementioned studies with HSP70, only recently has a mechanism been proposed for how HSP70 may be displayed on (and possibly released from) the cell surface via lipid rafts (Broquet et al., 2003).

The chaperone-mediated immune recognition of tumor cells has led to the use of tumor cells that express chaperones on their cell surfaces, or as secreted proteins, either by purposefully transfected means with targeted constructs or by stimulus that results in such expression. Injection of mice with tumor cells containing a construct expressing membrane-bound HSP70 (with a signal sequence and transmembrane domain) transfected into P815 cells resulted in both CTL and innate immune responses against both the wild-type tumor cells and the transfectants (Chen et al., 2002). Transient and stable overexpression of HSP70 increased HSP70 secretion from a murine cell line that already tended to secrete HSP70 in culture and led to reduced tumorigenicity of wild-type cells if the irradiated transfectants were used as a vaccine (Wang et al., 2004). In a tumor model where HSP70 was genetically engineered to be secreted, it was found that tumorigenicity was generally reduced in correlation with the amount of HSP70 secreted by the various cell lines as well as with the mode of inoculation (Massa et al., 2004). The differential cell surface expression of GRP94/gp96 on murine tumor cells rather than on normal cells, which was noted by Altmeyer et al. (1996), may be related to the inducibility of the *grp94* promoter in transformed cells, apparently independent of glucose starvation or activation of hypoxia response elements (Reddy et al., 2002). Tumor cells transfected with constructs encoding secretable GRP94/gp96, much like those secreting HSP70, displayed dramatically reduced tumorigenicity and were effective vaccines against untransfected tumor cell challenge in an animal model (Yamazaki et al., 1999). Likewise, other murine tumor cells engineered to express GRP94/gp96 as transmembrane cell surface proteins were also less tumorigenic and primed APCs for T cell responses (Zheng et al., 2001). Implied within these studies was the role of the chaperone in providing antigen to APCs. As intoned above, this implication was questioned in work from Baker-LePain et al. (2002), whereby syngeneic murine fibroblasts (i.e., presumably encoding no tumor antigens) were transfected with secretable GRP94/gp96 constructs (lacking KDEL retention signals), including truncated constructs whose translation products lacked the presumptive peptide binding domain of the chaperone. Those fibroblasts were used as prophylactic vaccines against an aggressive tumor model, which resulted in significant delays in tumor growth, as well as upregulation of DC maturation markers. Again, these immune responses occurred seemingly in the absence of any vaccinating antigen, which highlights the role of the innate immune response to the presence of GRP94 in immune activation. However, it is not clear that in combination with a potent immune activator such as GRP94, fetal calf serum products may have been influential antigens common to both the vaccine and the tumor inoculum (Mackensen et al., 2000; Sulit et al., 1976; Toldbod et al., 2003).

The efficacy of cellular vaccines that have been engineered to express chaperones on the cell surface, or to secrete them extracellularly, has clearly been demonstrated in the animal models described above. However, such vaccines as clinically useful therapeutics face the same problems that any genetically modified cellular therapies encounter, namely, the substantial efforts of tumor cell harvest, propagation, and manipulation in vitro; slow turnaround time; and the safety issues in preparation, validation, and administration. Such drawbacks may place substantial limitations on this type of therapy. Nonetheless, the principle of "relocating" the chaperones within or outside a tumor certainly appears to be an effective means of sparking an immune response against the cancerous cells. Perhaps a goal of biologic and/or pharmacologic targeting of chaperones should be to find a means that induces tumors themselves to perform this alteration of chaperone protein expression patterns.

Another novel approach that might exploit cell surface chaperones would be to target them directly with agents that react specifically with chaperones, perhaps with the drugs described above in "Manipulating HSP Levels—Chaperone Proteins as Pharmacologic Targets," or with antibodies or other protein reagents that can bind with high specificity to chaperones. Current evidence indicates that normal tissue does not express or display cell surface chaperones and that tumor targeting would thus be relatively specific. The chaperone-specific reagents may need to be armed with radioisotopes or toxins, and this would depend on whether or not the cell surface chaperones are internalized, among other things. Since such tactics have a high potential for autoimmunity, and since they are clearly in their "pre-infancy," they would have to be evaluated rigorously and carefully. However, such approaches have been employed in at least two murine tumor model systems with successful results, one using anti-HSP60 antibodies conjugated to the ribotoxin Saporin-6 (Piselli et al., 1995) and one using a viral apoptosis-inducing polypeptide fused to peptide ligand motifs for binding GRP78 (Arap et al., 2004).

Concluding Remarks and a View to the Future

Chaperone proteins are essential to cellular activities because of the involvement of chaperones in the births, lives, and deaths of the rest of the proteins expressed by the cell. Those cellular proteins must be properly folded, appropriately targeted intracellularly, perhaps assimi-

lated into multi-subunit structures, and removed and recycled upon senescence. Much of this cycle requires chaperone activity for the cycle to function and to ensure homeostasis. Deviations from homeostasis during stress also engage chaperone activity, which is set in motion to protect cellular proteins (and thus the cell) during the stress and to repair damage following the release from the stress. Tumors often survive in a stressful environment; it is generally hypoxic, with high metabolic rates and genetic instability leading to production of mutated proteins, and possibly under the threat of an immune response. Tumors therefore tend to upregulate their chaperone repertoire in numbers and activities as a "stress management" strategy.

Thus, while tumors may indeed overexpress chaperone proteins, it is not inherently obvious whether this is beneficial to the tumor (part of the antiapoptotic pathways/survival mechanism) or to the potential immune response against the tumor (Jaattela, 1995; Jolly and Morimoto, 2000; Mosser and Morimoto, 2004). Ostensibly, the very existence of tumors implies that benefit to the tumor overrides the detriment of immune attack. However, it is likely that the context in which the chaperones are expressed, which is perhaps related to their expression levels, may be the determining factor between tumor cell survival and continued proliferation or recognition and assault by the immune system. The intertwining of chaperones in so many different aspects of fundamental cell activities certainly implies that these proteins could be good targets for therapeutic agents or could themselves be therapeutic agents. While there have now been a couple of decades' worth of work poured into understanding chaperone (immuno)biology, biochemistry, and genetics, it would seem that the more we have learned, the less we feel we truly understand in the overall biological picture of chaperones. This is particularly true in the setting of brain tumors and their therapies, where failure of the best and most aggressive therapies to prevent recurrence or even to prolong survival is routine. Clearly, specific efforts need to be devoted to exploring the effects of chaperone targets/therapeutics/vaccines against brain tumors, first in animal models and then in clinical settings. One should particularly examine combinations of therapies that would strike targets at multiple levels, since chaperones may be seen both as targets and as modulators of targets, both intracellularly and exogenously. Some examples of combination modalities are listed below; the reader is encouraged to add freely to this list.

- Vaccines in the context of hyperthermia
- Vaccines combined with chemotherapy
- RNAi coupled with apoptosis-inducing chemotherapy
- Adjuvant chaperone proteins as "danger signals" combined with RNAi
- Adjuvant chaperones as "danger signals" in the context of apoptotic agents
- Chaperone protein suppression combined with radiotherapy
- Chaperone protein suppression combined with chemotherapy
- HSP90 inhibitors plus kinase inhibitors
- Targeted immunotherapy (i.e., antibodies) plus HSP90 inhibitors
- Targeted immunotherapy plus vaccines

Novel brain tumor therapies and therapeutic strategies are desperately needed. Continued and directed research into chaperone proteins may provide just such an avenue leading to improved treatments with the ultimate goal of eradicating these malignancies.

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References

Altmeyer, A., Maki, R.G., Feldweg, A.M., Heike, M., Protopopov, V.P., Masur, S.K., and Srivastava, P.K. (1996) Tumor-specific cell surface expression of the-KDEL containing, endoplasmic reticular heat shock protein gp96. *Int. J. Cancer* **69**, 340–349.

Aoyama, A., Steiger, R.H., Frohli, E., Schafer, R., von Deimling, A., Wiestler, O.D., and Klemenz, R. (1993) Expression of alpha B-crystallin in human brain tumors. *Int. J. Cancer* **55**, 760–764.

Appelskog, I.B., Ammerpohl, O., Svechnikova, I.G., Lui, W.O., Almqvist, P.M., and Ekstrom, T.J. (2004) Histone deacetylase inhibitor 4-phenylbutyrate suppresses GAPDH mRNA expression in glioma cells. *Int. J. Oncol.* **24**, 1419–1425.

Aquino, D.A., Peng, D., Lopez, C., and Farooq, M. (1998) The constitutive heat shock protein-70 is required for optimal expression of myelin basic protein during differentiation of oligodendrocytes. *Neurochem. Res.* **23**, 413–420.

Arap, M.A., Lahdenranta, J., Mintz, P.J., Hajitou, A., Sarkis, A.S., Arap, W., and Pasqualini, R. (2004) Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. *Cancer Cell* **6**, 275–284.

Argon, Y., and Simen, B.B. (1999) GRP94, an ER chaperone with protein and peptide binding properties. *Semin. Cell Dev. Biol.* **10**, 495–505.

Asea, A., Kraeft, S.K., Kurt-Jones, E.A., Stevenson, M.A., Chen, L.B., Finberg, R.W., Koo, G.C., and Calderwood, S.K. (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat. Med.* **6**, 435–442.

Asea, A., Ara, G., Teicher, B.A., Stevenson, M.A., and Calderwood, S.K. (2001) Effects of the flavonoid drug quercetin on the response of human prostate tumours to hyperthermia in vitro and in vivo. *Int. J. Hyperthermia* **17**, 347–356.

Ashley, D.M., Sampson, J.H., Archer, G.E., Batra, S.K., Bigner, D.D., and Hale, L.P. (1997) A genetically modified allogeneic cellular vaccine generates MHC class I-restricted cytotoxic responses against tumor-associated antigens and protects against CNS tumors in vivo. *J. Neuroimmunol.* **78**, 34–46.

Asklund, T., Appelskog, I.B., Ammerpohl, O., Ekstrom, T.J., and Almqvist, P.M. (2004) Histone deacetylase inhibitor 4-phenylbutyrate modulates glial fibrillary acidic protein and connexin 43 expression, and enhances gap-junction communication, in human glioblastoma cells. *Eur. J. Cancer* **40**, 1073–1081.

Baker, M.J., Brem, S., Daniels, S., Sherman, B., and Phuphanich, S. (2002) Complete response of a recurrent, multicentric malignant glioma in a patient treated with phenylbutyrate. *J. Neurooncol.* **59**, 239–242.

Baker-LePain, J.C., Sarzotti, M., Fields, T.A., Li, C.Y., and Nicchitta, C.V. (2002) GRP94 (gp96) and GRP94 N-terminal geldanamycin binding domain elicit tissue nonrestricted tumor suppression. *J. Exp. Med.* **196**, 1447–1459.

Barazi, H.O., Zhou, L., Templeton, N.S., Krutzsch, H.C., and Roberts, D.D. (2002) Identification of heat shock protein 60 as a molecular mediator of alpha 3 beta 1 integrin activation. *Cancer Res.* **62**, 1541–1548.

Basu, S., Binder, R.J., Suto, R., Anderson, K.M., and Srivastava, P.K. (2000) Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int. Immunol.* **12**, 1539–1546.

Basu, S., Binder, R.J., Ramalingam, T., and Srivastava, P.K. (2001) CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* **14**, 303–313.

Becker, T., Hartl, F.U., and Wieland, F. (2002) CD40, an extracellular receptor for binding and uptake of Hsp70-peptide complexes. *J. Cell Biol.* **158**, 1277–1285.

Becker, B., Multhoff, G., Farkas, B., Wild, P.J., Landthaler, M., Stolz, W., and Vogt, T. (2004) Induction of Hsp90 protein expression in malignant melanomas and melanoma metastases. *Exp. Dermatol.* **13**, 27–32.

Belli, F., Testori, A., Rivoltini, L., Maio, M., Andreola, G., Sertoli, M.R., Gallino, G., Piris, A., Cattelan, A., Lazzari, I., Carrabba, M., Scita, G., Santantonio, C., Pilla, L., Tragni, G., Lombardo, C., Arienti, F., Marchiano, A., Queirolo, P., Bertolini, F., Cova, A., Lamaj, E., Ascani, L., Camerini, R., Corsi, M., Cascinelli, N., Lewis, J.J., Srivastava, P., and Parmiani, G. (2002) Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: Clinical and immunologic findings. *J. Clin. Oncol.* **20**, 4169–4180.

Bellone, M., Iezzi, G., Martin-Fontecha, A., Rivolta, L., Manfredi, A.A., Protti, M.P., Freschi, M., Dellabona, P., Casorati, G., and Rugarli, C. (1997) Rejection of a nonimmunogenic melanoma by vaccination with natural melanoma peptides on engineered antigen-presenting cells. *J. Immunol.* **158**, 783–789.

Berger, C.L., Dong, Z., Hanlon, D., Bisaccia, E., and Edelson, R.L. (1997) A lymphocyte cell surface heat shock protein homologous to the endoplasmic reticulum chaperone, immunoglobulin heavy chain binding protein BIP. *Int. J. Cancer* **71**, 1077–1085.

Berwin, B., Hart, J.P., Pizzo, S.V., and Nicchitta, C.V. (2002) Cutting edge: CD91-independent cross-presentation of GRP94(gp96)-associated peptides. *J. Immunol.* **168**, 4282–4286.

Berwin, B., Hart, J.P., Rice, S., Gass, C., Pizzo, S.V., Post, S.R., and Nicchitta, C.V. (2003) Scavenger receptor-A mediates gp96/GRP94 and calreticulin internalization by antigen-presenting cells. *EMBO J.* **22**, 6127–6136.

Binder, R.J., Han, D.K., and Srivastava, P.K. (2000) CD91: A receptor for heat shock protein gp96. *Nat. Immunol.* **1**, 151–155.

Binder, R.J., Karimeddini, D., and Srivastava, P.K. (2001) Adjuvanticity of alpha 2-macroglobulin, an independent ligand for the heat shock protein receptor CD91. *J. Immunol.* **166**, 4968–4972.

Blachere, N.E., Li, Z., Chandawarkar, R.Y., Suto, R., Jaikaria, N.S., Basu, S., Udono, H., and Srivastava, P.K. (1997) Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. *J. Exp. Med.* **186**, 1315–1322.

Bonini, N.M. (2002) Chaperoning brain degeneration. *Proc. Natl. Acad. Sci. USA* **10** (Suppl. 4), 16407–16411.

Borok, T.L., Winter, A., Laing, J., Paglione, R., Sterzer, F., Sinclair, I., and Plafker, J. (1988) Microwave hyperthermia radiosensitized iridium-192 for recurrent brain malignancy. *Med. Dosim.* **13**, 29–36.

Broquet, A.H., Thomas, G., Masliah, J., Trugnan, G., and Bachelet, M. (2003) Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. *J. Biol. Chem.* **278**, 21601–21606.

Brunner, T.B., Hahn, S.M., Gupta, A.K., Muschel, R.J., McKenna, W.G., and Bernhard, E.J. (2003) Farnesyltransferase inhibitors: An overview of the results of preclinical and clinical investigations. *Cancer Res.* **63**, 5656–5668.

Castelli, C., Rivoltini, L., Rini, F., Belli, F., Testori, A., Maio, M., Mazzaferro, V., Coppa, J., Srivastava, P.K., and Parmiani, G. (2004) Heat shock proteins: Biological functions and clinical application as personalized vaccines for human cancer. *Cancer Immunol. Immunother.* **53**, 227–233.

Chen, X., Tao, Q., Yu, H., Zhang, L., and Cao, X. (2002) Tumor cell membrane-bound heat shock protein 70 elicits antitumor immunity. *Immunol. Lett.* **84**, 81–87.

Chouchane, L., Bowers, F.S., Sawasdikosol, S., Simpson, R.M., and Kindt, T.J. (1994) Heat-shock proteins expressed on the surface of human T cell leukemia virus type I-infected cell lines induce autoantibodies in rabbits. *J. Infect. Dis.* **169**, 253–259.

Cox, A.D., and Der, C.J. (1997) Farnesyltransferase inhibitors and cancer treatment: Targeting simply Ras? *Biochim. Biophys. Acta* **1333**, F51–F71.

Cwiklinska, H., Mycko, M.P., Luvsannorov, O., Walkowiak, B., Brosnan, C.F., Raine, C.S., and Selmaj, K.W. (2003) Heat shock protein 70 associations with myelin basic protein and proteolipid protein in multiple sclerosis brains. *Int. Immunol.* **15**, 241–249.

Delneste, Y., Magistrelli, G., Gauchat, J., Haeuw, J., Aubry, J., Nakamura, K., Kawakami-Honda, N., Goetsch, L., Sawamura, T., Bonnefoy, J., and Jeannin, P. (2002) Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. *Immunity* **17**, 353–362.

Elia, G., and Santoro, M.G. (1994) Regulation of heat shock protein synthesis by quercetin in human erythroleukaemia cells. *Biochem. J.* **300** (Pt. 1), 201–209.

Ellgaard, L., and Helenius, A. (2001) ER quality control: Towards an understanding at the molecular level. *Curr. Opin. Cell Biol.* **13**, 431–437.

Engelhard, H.H., Homer, R.J., Duncan, H.A., and Rozental, J. (1998) Inhibitory effects of phenylbutyrate on the proliferation, morphology, migration and invasiveness of malignant glioma cells. *J. Neurooncol.* **37**, 97–108.

Fecci, P.E., and Sampson, J.H. (2002) Clinical immunotherapy for brain tumors. *Neuroimaging Clin. N. Am.* **12**, 641–664.

Feng, H., Zeng, Y., Whitesell, L., and Katsanis, E. (2001) Stressed apoptotic tumor cells express heat shock proteins and elicit tumor-specific immunity. *Blood* **97**, 3505–3512.

Feng, H., Zeng, Y., Graner, M.W., and Katsanis, E. (2002) Stressed apoptotic tumor cells stimulate dendritic cells and induce specific cytotoxic T cells. *Blood* **100**, 4108–4115.

Feng, H., Zeng, Y., Graner, M.W., Likhacheva, A., and Katsanis, E. (2003) Exogenous stress proteins enhance the immunogenicity of apoptotic tumor cells and stimulate antitumor immunity. *Blood* **101**, 245–252.

Ferrarini, M., Heltai, S., Zocchi, M.R., and Rugarli, C. (1992) Unusual expression and localization of heat-shock proteins in human tumor cells. *Int. J. Cancer* **51**, 613–619.

Ferry, D.R., Smith, A., Malkhandi, J., Fyfe, D.W., deTakats, P.G., Anderson, D., Baker, J., and Kerr, D.J. (1996) Phase I clinical trial of the flavonoid

quercetin: Pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clin. Cancer Res.* **2**, 659–668.

Flynn, G.C., Chappell, T.G., and Rothman, J.E. (1989) Peptide binding and release by proteins implicated as catalysts of protein assembly. *Science* **245**, 385–390.

Fortugno, P., Beltrami, E., Plescia, J., Fontana, J., Pradhan, D., Marchisio, P.C., Sessa, W.C., and Altieri, D.C. (2003) Regulation of survivin function by Hsp90. *Proc Natl. Acad. Sci. USA* **100**, 13791–13796.

Frydman, J. (2001) Folding of newly translated proteins in vivo: The role of molecular chaperones. *Annu. Rev. Biochem.* **70**, 603–647.

Garrido, C., Gurbuxani, S., Ravagnan, L., and Kroemer, G. (2001) Heat shock proteins: Endogenous modulators of apoptotic cell death. *Biochem. Biophys. Res. Commun.* **286**, 433–442.

Graner, M., and Katsanis, E. (2004) Chaperone proteins/heat shock proteins as anti-cancer vaccines. In: Morse, M.A., Clay, T.M., and Lyerly, H.K. (Eds.), *Handbook of Cancer Vaccines*. Totowa, N.J.: Humana Press, pp. 297–316.

Graner, M., Raymond, A., Akporiaye, E., and Katsanis, E. (2000) Tumor-derived multiple chaperone enrichment by free-solution isoelectric focusing yields potent antitumor vaccines. *Cancer Immunol. Immunother.* **49**, 476–484.

Graner, M.W., Zeng, Y., Feng, H., and Katsanis, E. (2003) Tumor-derived chaperone-rich cell lysates are effective therapeutic vaccines against a variety of cancers. *Cancer Immunol. Immunother.* **52**, 226–234.

Gross, C., Hansch, D., Gastpar, R., and Multhoff, G. (2003a) Interaction of heat shock protein 70 peptide with NK cells involves the NK receptor CD94. *Biol. Chem.* **384**, 267–279.

Gross, C., Koelch, W., DeMaio, A., Arispe, N., and Multhoff, G. (2003b) Cell surface-bound heat shock protein 70 (Hsp70) mediates perforin-independent apoptosis by specific binding and uptake of granzyme B. *J. Biol. Chem.* **278**, 41173–41181.

Guzhova, I., Kislyakova, K., Moskaliova, O., Fridlanskaya, I., Tytell, M., Cheetham, M., and Margulis, B. (2001) In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. *Brain Res.* **914**, 66–73.

Hansson, O., Nylandsted, J., Castilho, R.F., Leist, M., Jaattela, M., and Brundin, P. (2003) Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. *Brain Res.* **970**, 47–57.

Haslbeck, M. (2002) sHSPs and their role in the chaperone network. *Cell. Mol. Life Sci.* **59**, 1649–1657.

Heimberger, A.B., Crotty, L.E., Archer, G.E., Hess, K.R., Wikstrand, C.J., Friedman, A.H., Friedman, H.S., Bigner, D.D., and Sampson, J. H.(2003) Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. *Clin. Cancer Res.* **9**, 4247–4254.

Hermisson, M., Strik, H., Rieger, J., Dichgans, J., Meyermann, R., and Weller, M. (2000) Expression and functional activity of heat shock proteins in human glioblastoma multiforme. *Neurology* **54**, 1357–1365.

Hightower, L.E., and Guidon, P.T., Jr. (1989) Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *J. Cell Physiol.* **138**, 257–266.

Hitotsumatsu, T., Iwaki, T., Fukui, M., and Tateishi, J. (1996) Distinctive immunohistochemical profiles of small heat shock proteins (heat shock protein 27 and alpha B-crystallin) in human brain tumors. *Cancer* **77**, 352–361.

Hosokawa, N., Hirayoshi, K., Nakai, A., Hosokawa, Y., Marui, N., Yoshida, M., Sakai, T., Nishino, H., Aoike, A., Kawai, K., and Nagata, K. (1990) Flavonoids inhibit the expression of heat shock proteins. *Cell Struct. Funct.* **15**, 393–401.

Houry, W.A. (2001) Chaperone-assisted protein folding in the cell cytoplasm. *Curr. Protein Pept. Sci.* **2**, 227–244.

Hu, W., Wu, W., Yeung, S.C., Freedman, R.S., Kavanagh, J.J., and Verschraegen, C.F. (2002) Increased expression of heat shock protein 70 in adherent ovarian cancer and mesothelioma following treatment with manumycin, a farnesyl transferase inhibitor. *Anticancer Res.* **22**, 665–672.

Hu, W., Wu, W., Verschraegen, C.F., Chen, L., Mao, L., Yeung, S.C., Kudelka, A.P., Freedman, R.S., and Kavanagh, J.J. (2003) Proteomic identification of heat shock protein 70 as a candidate target for enhancing apoptosis induced by farnesyl transferase inhibitor. *Proteomics* **3**, 1904–1911.

Huang, X.F., Ren, W., Rollins, L., Pittman, P., Shah, M., Shen, L., Gu, Q., Strube, R., Hu, F., and Chen, S.Y. (2003) A broadly applicable, personalized heat shock protein-mediated oncolytic tumor vaccine. *Cancer Res.* **63**, 7321–7329.

Humphrey, P.A., Wong, A.J., Vogelstein, B., Zalutsky, M.R., Fuller, G.N., Archer, G.E., Friedman, H.S., Kwatra, M.M., Bigner, S.H., and Bigner, D.D. (1990) Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma. *Proc. Natl. Acad. Sci. USA* **87**, 4207–4211.

Hylander, B.L., Chen, X., Graf, P.C., and Subjeck, J.R. (2000) The distribution and localization of hsp110 in brain. *Brain Res.* **869**, 49–55.

Imaizumi, T., Kuramoto, T., Matsunaga, K., Shichijo, S., Yutani, S., Shigemori, M., Oizumi, K., and Itoh, K. (1999) Expression of the tumor-rejection antigen SART1 in brain tumors. *Int. J. Cancer* **83**, 760–764.

Itoh, H., Komatsuda, A., Wakui, H., Miura, A.B., and Tashima, Y. (1999) Mammalian HSP60 is a major target for an immunosuppressant mizoribine. *J. Biol. Chem.* **274**, 35147–35151.

Jaattela, M. (1995) Over-expression of hsp70 confers tumorigenicity to mouse fibrosarcoma cells. *Int. J. Cancer* **60**, 689–693.

Jaattela, M. (1999a) Escaping cell death: Survival proteins in cancer. *Exp. Cell Res.* **248,** 30–43.

Jaattela, M. (1999b) Heat shock proteins as cellular lifeguards. *Ann. Med.* **31**, 261–271.

Jakubowicz-Gil, J., Paduch, R., Gawron, A., and Kandefer-Szerszen, M. (2002) The effect of heat shock, cisplatin, etoposide and quercetin on Hsp27 expression in human normal and tumour cells. *Folia Histochem. Cytobiol.* **40**, 31–35.

Janetzki, S., Palla, D., Rosenhauer, V., Lochs, H., Lewis, J.J., and Srivastava, P.K. (2000) Immunization of cancer patients with autologous cancer-derived heat shock protein gp96 preparations: A pilot study. *Int. J. Cancer* **88**, 232–238.

Jolly, C., and Morimoto, R.I. (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. *J. Natl. Cancer Inst.* **92**, 1564–1572.

Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M.F., Fritz, L.C., and Burrows, F.J. (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* **425**, 407–410.

Karp, J.E., Lancet, J.E., Kaufmann, S.H., End, D.W., Wright, J.J., Bol, K., Horak, I., Tidwell, M.L., Liesveld, J., Kottke, T.J., Ange, D., Buddharaju, L., Gojo, I., Highsmith, W.E., Belly, R.T., Hohl, R.J., Rybak, M.E., Thibault, A., and Rosenblatt, J. (2001) Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: A phase 1 clinical-laboratory correlative trial. *Blood* **97**, 3361–3369.

Kato, M., Katsumoto, T., Ohno, K., Kato, S., Herz, F., and Takeshita, K. (1992) Expression of glial fibrillary acidic protein (GFAP) by cultured angiofibroma stroma cells from patients with tuberous sclerosis. *Neuropathol. Appl. Neurobiol.* **18**, 559–565.

Kato, S., Hirano, A., Kato, M., Herz, F., and Ohama, E. (1993) Comparative study on the expression of stress-response protein (srp) 72, srp 27, alpha B-crystallin and ubiquitin in brain tumours. An immunohistochemical investigation. *Neuropathol. Appl. Neurobiol.* **19**, 436–442.

Kato, S., Kato, M., Hirano, A., Takikawa, M., and Ohama, E. (2001) The immunohistochemical expression of stress-response protein (srp) 60 in human brain tumours: Relationship of srp 60 to the other five srps, proliferating cell nuclear antigen and p53 protein. *Histol. Histopathol.* **16**, 809–820.

Katoh, M., Wilmotte, R., Belkouch, M.C., de Tribolet, N., Pizzolato, G., and Dietrich, P.Y. (2003) Survivin in brain tumors: An attractive target for immunotherapy. *J. Neurooncol.* **64**, 71–76.

Kaul, S.C., Matsui, M., Takano, S., Sugihara, T., Mitsui, Y., and Wadhwa, R. (1997) Expression analysis of mortalin, a unique member of the Hsp70 family of proteins, in rat tissues. *Exp. Cell Res.* **232**, 56–63.

Kaur, I., Voss, S.D., Gupta, R.S., Schell, K., Fisch, P., and Sondel, P.M. (1993) Human peripheral gamma delta T cells recognize hsp60 molecules on Daudi Burkitt's lymphoma cells. *J. Immunol.* **150**, 2046–2055.

Kazemi-Esfarjani, P., and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in Drosophila. *Science* **287**, 1837–1840.

Kelland, L.R., Smith, V., Valenti, M., Patterson, L., Clarke, P.A., Detre, S., End, D., Howes, A.J., Dowsett, M., Workman, P., and Johnston, S.R. (2001) Preclinical antitumor activity and pharmacodynamic studies with the farnesyl protein transferase inhibitor R115777 in human breast cancer. *Clin. Cancer Res.* **7**, 3544–3550.

Kislin, K., Graner, M.W., and Katsoanis, E. (2004) Peptide-loaded chaperone-rich cell lysatses may enhance the effectiveness of anti-cancer vaccines. Presented at AACR National Meeting, Orlando, Fla. (abstract).

Kobayashi, T., Kida, Y., Matsui, M., and Amemiya, Y. (1990) [Interstitial hyperthermia of malignant brain tumors using implant heating system (IHS)]. *No Shinkei Geka* **18**, 247–252.

Kobayashi, T., Yamanaka, R., Homma, J., Tsuchiya, N., Yajima, N., Yoshida, S., and Tanaka, R. (2003) Tumor mRNA-loaded dendritic cells elicit tumor-specific CD8(+) cytotoxic T cells in patients with malignant glioma. *Cancer Immunol. Immunother.* **52**, 632–637.

Kol, A., Lichtman, A.H., Finberg, R.W., Libby, P., and Kurt-Jones, E.A. (2000) Cutting edge: Heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J. Immunol.* **164**, 13–17.

Koya, K., Li, Y., Wang, H., Ukai, T., Tatsuta, N., Kawakami, M., and Chen, L.B. (1996) MKT-077, a novel rhodacyanine dye in clinical trials, exhibits anticarcinoma activity in preclinical studies based on selective mitochondrial accumulation. *Cancer Res.* **56**, 538–543.

Krause, S.W., Gastpar, R., Andreesen, R., Gross, C., Ullrich, H., Thonigs, G., Pfister, K., and Multhoff, G. (2004) Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: A clinical phase 1 trial. *Clin. Cancer Res.* **10**, 3699–3707.

Kumaraguru, U., Gouffon, C.A., Jr., Ivey, R.A., 3rd, Rouse, B.T., and Bruce, B.D. (2003) Antigenic peptides complexed to phylogenically diverse Hsp70s induce differential immune responses. *Cell Stress Chaperones* **8**, 134–143.

Lavictoire, S.J., Parolin, D.A., Klimowicz, A.C., Kelly, J.F., and Lorimer, I.A. (2003) Interaction of Hsp90 with the nascent form of the mutant epidermal growth factor receptor EGFRvIII. *J. Biol. Chem.* **278**, 5292–5299.

Le Brazidec, J.Y., Kamal, A., Busch, D., Thao, L., Zhang, L., Timony, G., Grecko, R., Trent, K., Lough, R., Salazar, T., Khan, S., Burrows, F., and Boehm, M.F. (2004) Synthesis and biological evaluation of a new class of geldanamycin derivatives as potent inhibitors of Hsp90. *J. Med. Chem.* **47**, 3865–3873.

Lee, A.S. (2001) The glucose-regulated proteins: Stress induction and clinical applications. *Trends Biochem. Sci.* **26**, 504–510.

Liu, G., Khong, H.T., Wheeler, C.J., Yu, J.S., Black, K.L., and Ying, H. (2003) Molecular and functional analysis of tyrosinase-related protein (TRP)-2 as a cytotoxic T lymphocyte target in patients with malignant glioma. *J. Immunother.* **26**, 301–312.

Liu, G., Ying, H., Zeng, G., Wheeler, C.J., Black, K.L., and Yu, J.S. (2004a) HER-2, gp100, and MAGE-1 are expressed in human glioblastoma and recognized by cytotoxic T cells. *Cancer Res.* **64**, 4980–4986.

Liu, G., Yu, J.S., Zeng, G., Yin, D., Xie, D., Black, K.L., and Ying, H. (2004b) AIM-2: A novel tumor antigen is expressed and presented by human glioma cells. *J. Immunother.* **27**, 220–226.

Loging, W.T., Lal, A., Siu, I.M., Loney, T.L., Wikstrand, C.J., Marra, M.A., Prange, C., Bigner, D.D., Strausberg, R.L., and Riggins, G.J. (2000) Identifying potential tumor markers and antigens by database mining and rapid expression screening. *Genome Res.* **10**, 1393–1402.

Mackensen, A., Drager, R., Schlesier, M., Mertelsmann, R., and Lindemann, A. (2000) Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after vaccination with human peptide-pulsed dendritic cells. *Cancer Immunol. Immunother.* **49**, 152–156.

Manfredi, A.A., Iannacone, M., D'Auria, F., and Rovere-Querini, P. (2002) The disposal of dying cells in living tissues. *Apoptosis* **7**, 153–161.

Manjili, M.H., Henderson, R., Wang, X.Y., Chen, X., Li, Y., Repasky, E., Kazim, L., and Subjeck, J.R. (2002) Development of a recombinant HSP110-HER-2/neu vaccine using the chaperoning properties of HSP110. *Cancer Res.* **62**, 1737–1742.

Massa, C., Guiducci, C., Arioli, I., Parenza, M., Colombo, M.P., and Melani, C. (2004) Enhanced efficacy of tumor cell vaccines transfected with secretable hsp70. *Cancer Res.* **64**, 1502–1508.

Mazzaferro, V., Coppa, J., Carrabba, M.G., Rivoltini, L., Schiavo, M., Regalia, E., Mariani, L., Camerini, T., Marchiano, A., Andreola, S., Camerini, R., Corsi, M., Lewis, J.J., Srivastava, P.K., and Parmiani, G. (2003) Vaccination with autologous tumor-derived heat-shock protein gp96 after liver resection for metastatic colorectal cancer. *Clin. Cancer Res.* **9**, 3235–3245.

Melero, I., Vile, R.G., and Colombo, M.P. (2000) Feeding dendritic cells with tumor antigens: Self-service buffet or a la carte? *Gene Ther.* **7**, 1167–1170.

Menoret, A., Peng, P., and Srivastava, P.K. (1999) Association of peptides with heat shock protein gp96 occurs in vivo and not after cell lysis. *Biochem. Biophys. Res. Commun.* **262**, 813–818.

Merrick, B.A., He, C., Witcher, L.L., Patterson, R.M., Reid, J.J., Pence-Pawlowski, P.M., and Selkirk, J.K. (1996) HSP binding and mitochondrial localization of p53 protein in human HT1080 and mouse C3H10T1/2 cell lines. *Biochim. Biophys. Acta* **1297**, 57–68.

Mimnaugh, E.G., Chavany, C., and Neckers, L. (1996) Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. *J. Biol. Chem.* **271**, 22796–22801.

Modica-Napolitano, J.S., Koya, K., Weisberg, E., Brunelli, B.T., Li, Y., and Chen, L.B. (1996) Selective damage to carcinoma mitochondria by the rhodacyanine MKT-077. *Cancer Res.* **56**, 544–550.

Moran, C.J., Marchosky, J.A., Wippold, F.J., 2nd, DeFord, J.A., and Fearnot, N.E. (1995) Conductive interstitial hyperthermia in the treatment of intracranial metastatic disease. *J. Neurooncol.* **26**, 53–63.

Moscatello, D.K., Ramirez, G., and Wong, A.J. (1997) A naturally occurring mutant human epidermal growth factor receptor as a target for peptide vaccine immunotherapy of tumors. *Cancer Res.* **57**, 1419–1424.

Mosser, D.D., and Morimoto, R.I. (2004) Molecular chaperones and the stress of oncogenesis. *Oncogene* **23**, 2907–2918.

Mulholland, P.J., Ferry, D.R., Anderson, D., Hussain, S.A., Young, A.M., Cook, J.E., Hodgkin, E., Seymour, L.W., and Kerr, D.J. (2001) Pre-clinical and clinical study of QC12, a water-soluble, pro-drug of quercetin. *Ann. Oncol.* **12**, 245–248.

Multhoff, G. (2002) Activation of natural killer cells by heat shock protein 70. *Int. J. Hyperthermia* **18**, 576–585.

Multhoff, G., and Hightower, L.E. (1996) Cell surface expression of heat shock proteins and the immune response. *Cell Stress Chaperones* **1**, 167–176.

Multhoff, G., Pfister, K., Gehrmann, M., Hantschel, M., Gross, C., Hafner, M., and Hiddemann, W. (2001) A 14-mer Hsp70 peptide stimulates natural killer (NK) cell activity. *Cell Stress Chaperones* **6**, 337–344.

Murayama, K., Kobayashi, T., Imaizumi, T., Matsunaga, K., Kuramoto, T., Shigemori, M., Shichijo, S., and Itoh, K. (2000) Expression of the SART3 tumor-rejection antigen in brain tumors and induction of cytotoxic T lymphocytes by its peptides. *J. Immunother.* **23**, 511–518.

Mycko, M.P., Cwiklinska, H., Szymanski, J., Szymanska, B., Kudla, G., Kilianek, L., Odyniec, A., Brosnan, C.F., and Selmaj, K.W. (2004) Inducible heat shock protein 70 promotes myelin autoantigen presentation by the HLA class II. *J. Immunol.* **172**, 202–213.

Nadler, S.G., Tepper, M.A., Schacter, B., and Mazzucco, C.E. (1992) Interaction of the immunosuppressant deoxyspergualin with a member of the Hsp70 family of heat shock proteins. *Science* **258**, 484–486.

Nadler, S.G., Eversole, A.C., Tepper, M.A., and Cleaveland, J.S. (1995) Elucidating the mechanism of action of the immunosuppressant 15-deoxyspergualin. *Ther. Drug Monit.* **17**, 700–703.

Nakajima, T., Roberts, D.W., Ryan, T.P., Hoopes, P.J., Coughlin, C.T., Trembly, B.S., and Strohbehn, J.W. (1993) Pattern of response to interstitial hyperthermia and brachytherapy for malignant intracranial tumour: A CT analysis. *Int. J. Hyperthermia* **9**, 491–502.

Navratil, J.S., Sabatine, J.M., and Ahearn, J.M. (2004) Apoptosis and immune responses to self. *Rheum. Dis. Clin. North Am.* **30**, 193–212.

Naylor, D.J., and Hartl, F.U. (2001) Contribution of molecular chaperones to protein folding in the cytoplasm of prokaryotic and eukaryotic cells. *Biochem. Soc. Symp.* 45–68.

Nicchitta, C.V. (1998) Biochemical, cell biological and immunological issues surrounding the endoplasmic reticulum chaperone GRP94/gp96. *Curr. Opin. Immunol.* **10**, 103–109.

Nicchitta, C.V. (2003) Re-evaluating the role of heat-shock protein-peptide interactions in tumour immunity. *Nat. Rev. Immunol.* **3**, 427–432.

Nonaka, T., Akimoto, T., Mitsuhashi, N., Tamaki, Y., Yokota, S., and Nakano, T. (2003) Changes in the localization of heat shock protein 72 correlated with development of thermotolerance in human esophageal cancer cell line. *Anticancer Res.* **23**, 4677–4687.

Nylandsted, J., Wick, W., Hirt, U.A., Brand, K., Rohde, M., Leist, M., Weller, M., and Jaattela, M. (2002) Eradication of glioblastoma, and breast and colon carcinoma xenografts by Hsp70 depletion. *Cancer Res.* **62**, 7139–7142.

Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000) Cutting edge: Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J. Immunol.* **164**, 558–561.

Panjwani, N., Popova, L., Febbraio, M., and Srivastava, P.K. (2000) The CD36 scavenger receptor as a receptor for Gp96. *Cell Stress Chaperones* **5**, 391 (abstract).

Pardridge, W.M. (2004) Intravenous, non-viral RNAi gene therapy of brain cancer. *Expert Opin. Biol. Ther.* **4**, 1103–1113.

Park, J.W., Yeh, M.W., Wong, M.G., Lobo, M., Hyun, W.C., Duh, Q.Y., and Clark, O.H. (2003) The heat shock protein 90-binding geldanamycin inhibits cancer cell proliferation, down-regulates oncoproteins, and inhibits epidermal growth factor-induced invasion in thyroid cancer cell lines. *J. Clin. Endocrinol. Metab.* **88**, 3346–3353.

Piselli, P., Vendetti, S., Poccia, F., Cicconi, R., Mattei, M., Bolognesi, A., Stirpe, F., and Colizzi, V. (1995) In vitro and in vivo efficacy of heat shock protein specific immunotoxins on human tumor cells. *J. Biol. Regul. Homeost. Agents* **9**, 55–62.

Pratt, W.B., and Toft, D.O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med.* **228**, 111–133.

Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., and Pearl, L.H. (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90**, 65–75.

Prohaszka, Z., and Fust, G. (2004) Immunological aspects of heat-shock proteins the optimum stress of life. *Mol. Immunol.* **41**, 29–44.

Proskuryakov, S.Y., Konoplyannikov, A.G., and Gabai, V.L. (2003) Necrosis: A specific form of programmed cell death? *Exp. Cell Res.* **283**, 1–16.

Reddy, R.K., Dubeau, L., Kleiner, H., Parr, T., Nichols, P., Ko, B., Dong, D., Ko, H., Mao, C., DiGiovanni, J., and Lee, A.S. (2002) Cancer-inducible transgene expression by the Grp94 promoter: Spontaneous activation in tumors of various origins and cancer-associated macrophages. *Cancer Res.* **62**, 7207–7212.

Reiter, I., Krammer, B., and Schwamberger, G. (1999) Cutting edge: Differential effect of apoptotic versus necrotic tumor cells on macrophage antitumor activities. *J. Immunol.* **163**, 1730–1732.

Rich, J.N., and Bigner, D.D. (2004) Development of novel targeted therapies in the treatment of malignant glioma. *Nat. Rev. Drug Discov.* **3**, 430–446.

Roberts, D.W., Coughlin, C.T., Wong, T.Z., Fratkin, J.D., Douple, E.B., and Strohbehn, J.W. (1986) Interstitial hyperthermia and iridium brachytherapy in treatment of malignant glioma. A phase I clinical trial. *J. Neurosurg.* **64**, 581–587.

Rong, Y., Yang, E.B., Zhang, K., and Mack, P. (2000) Quercetin-induced apoptosis in the monoblastoid cell line U937 in vitro and the regulation of heat shock proteins expression. *Anticancer Res.* **20,** 4339–4345.

Rubenstein, R.C., and Zeitlin, P.L. (2000) Sodium 4-phenylbutyrate down-regulates Hsc70: Implications for intracellular trafficking of DeltaF508-CFTR. *Am. J. Physiol. Cell Physiol.* **278**, C259–C267.

Sakagami, M., Morrison, P., and Welch, W.J. (1999) Benzoquinoid ansamycins (herbimycin A and geldanamycin) interfere with the maturation of growth factor receptor tyrosine kinases. *Cell Stress Chaperones* **4**, 19–28.

Scheffer, S.R., Nave, H., Korangy, F., Schlote, K., Pabst, R., Jaffee, E.M., Manns, M.P., and Greten, T.F. (2003) Apoptotic, but not necrotic, tumor cell vaccines induce a potent immune response in vivo. *Int. J. Cancer* **103**, 205–211.

Schulte, T.W., An, W.G., and Neckers, L.M. (1997) Geldanamycin-induced destabilization of Raf-1 involves the proteasome. *Biochem. Biophys. Res. Commun.* **239**, 655–659.

Shin, B.K., Wang, H., Yim, A.M., Le Naour, F., Brichory, F., Jang, J.H., Zhao, R., Puravs, E., Tra, J., Michael, C.W., Misek, D.E., and Hanash, S.M. (2003) Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. *J. Biol. Chem.* **278**, 7607–7616.

Soga, S., Shiotsu, Y., Akinaga, S., and Sharma, S.V. (2003) Development of radicicol analogues. *Curr. Cancer Drug Targets* **3**, 359–369.

Soling, A., and Rainov, N.G. (2001) Dendritic cell therapy of primary brain tumors. *Mol. Med.* **7**, 659–667.

Sreedhar, A.S., Soti, C., and Csermely, P. (2004) Inhibition of Hsp90: A new strategy for inhibiting protein kinases. *Biochim. Biophys. Acta* **1697**, 233–242.

Srivastava, P. (2002) Interaction of heat shock proteins with peptides and antigen presenting cells: Chaperoning of the innate and adaptive immune responses. *Annu. Rev. Immunol.* **20**, 395–425.

Srivastava, P.K., and Old, L.J. (1989) Identification of a human homologue of the murine tumor rejection antigen GP96. *Cancer Res.* **49**, 1341–1343.

Srivastava, P.K., DeLeo, A.B., and Old, L.J. (1986) Tumor rejection antigens of chemically induced sarcomas of inbred mice. *Proc. Natl. Acad. Sci. USA* **83**, 3407–3411.

Srivastava, P.K., Udono, H., Blachere, N.E., and Li, Z. (1994) Heat shock proteins transfer peptides during antigen processing and CTL priming. *Immunogenetics* **39**, 93–98.

Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U., and Pavletich, N.P. (1997) Crystal structure of an Hsp90-geldanamycin complex: Targeting of a protein chaperone by an antitumor agent. *Cell* **89**, 239–250.

Sulit, H.L., Golub, S.H., Irie, R.F., Gupta, R.K., Grooms, G.A., and Morton, D.L. (1976) Human tumor cells grown in fetal calf serum and human serum: Influences on the tests for lymphocyte cytotoxicity, serum blocking and serum arming effects. *Int. J. Cancer* **17**, 461–468.

Sun, J., Ohkanda, J., Coppola, D., Yin, H., Kothare, M., Busciglio, B., Hamilton, A.D., and Sebti, S.M. (2003) Geranylgeranyltransferase I inhibitor GGTI-2154 induces breast carcinoma apoptosis and tumor regression in H-Ras transgenic mice. *Cancer Res.* **63**, 8922–8929.

Supino-Rosin, L., Yoshimura, A., Yarden, Y., Elazar, Z., and Neumann, D. (2000) Intracellular retention and degradation of the epidermal growth factor receptor, two distinct processes mediated by benzoquinone ansamycins. *J. Biol. Chem.* **275**, 21850–21855.

Takano, S., Wadhwa, R., Yoshii, Y., Nose, T., Kaul, S.C., and Mitsui, Y. (1997) Elevated levels of mortalin expression in human brain tumors. *Exp. Cell Res.* **237**, 38–45.

Takayama, S., Reed, J.C., and Homma, S. (2003) Heat-shock proteins as regulators of apoptosis. *Oncogene* **22**, 9041–9047.

Tanaka, R., Kim, C.H., Yamada, N., and Saito, Y. (1987) Radiofrequency hyperthermia for malignant brain tumors: Preliminary results of clinical trials. *Neurosurgery* **21**, 478–483.

Thrall, D.E., Larue, S.M., Powers, B.E., Page, R.L., Johnson, J., George, S.L., Kornegay, J.N., McEntee, M.C., Levesque, D.C., Smith, M., Case, B.C., Dewhirst, M.W., and Gillette, E.L. (1999) Use of whole body hyperthermia as a method to heat inaccessible tumours uniformly: A phase III trial in canine brain masses. *Int. J. Hyperthermia* **15**, 383–398.

Toldbod, H.E., Agger, R., Bolund, L., and Hokland, M. (2003) Potent influence of bovine serum proteins in experimental dendritic cell-based vaccination protocols. *Scand. J. Immunol.* **58**, 43–50.

Trieb, K., Lang, S., and Kotz, R. (2000) Heat-shock protein 72 in human osteosarcoma: T-lymphocyte reactivity and cytotoxicity. *Pediatr. Hematol. Oncol.* **17**, 355–364.

Tytell, M., Greenberg, S.G., and Lasek, R.J. (1986) Heat shock-like protein is transferred from glia to axon. *Brain Res.* **363**, 161–164.

Ullrich, S.J., Robinson, E.A., Law, L.W., Willingham, M., and Appella, E. (1986) A mouse tumor-specific transplantation antigen is a heat shock-related protein. *Proc. Natl. Acad. Sci. USA* **83**, 3121–3125.

Vabulas, R.M., Braedel, S., Hilf, N., Singh-Jasuja, H., Herter, S., Ahmad-Nejad, P., Kirschning, C.J., Da Costa, C., Rammensee, H.G., Wagner, H., and Schild, H. (2002) The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway. *J. Biol. Chem.* **277**, 20847–20853.

van Eden, W., Koets, A., van Kooten, P., Prakken, B., and van der Zee, R. (2003) Immunopotentiating heat shock proteins: Negotiators between innate danger and control of autoimmunity. *Vaccine* **21**, 897–901.

Vecil, G.G., and Lang, F.F. (2003) Clinical trials of adenoviruses in brain tumors: A review of Ad-p53 and oncolytic adenoviruses. *J. Neuro-oncol.* **65**, 237–246.

Wachsberger, P.R., Burd, R., Bhala, A., Bobyock, S.B., Wahl, M.L., Owen, C.S., Rifat, S.B., and Leeper, D.B. (2003) Quercetin sensitizes cells in a tumour-like low pH environment to hyperthermia. *Int. J. Hyperthermia* **19**, 507–519.

Wadhwa, R., Kaul, S.C., Ikawa, Y., and Sugimoto, Y. (1993) Identification of a novel member of mouse hsp70 family. Its association with cellular mortal phenotype. *J. Biol. Chem.* **268**, 6615–6621.

Wadhwa, R., Taira, K., and Kaul, S.C. (2002a) An Hsp70 family chaperone, mortalin/mthsp70/PBP74/Grp75: What, when, and where? *Cell Stress Chaperones* **7**, 309–316.

Wadhwa, R., Colgin, L., Yaguchi, T., Taira, K., Reddel, R.R., and Kaul, S.C. (2002b) Rhodacyanine dye MKT-077 inhibits in vitro telomerase assay but has no detectable effects on telomerase activity in vivo. *Cancer Res.* **62**, 4434–4438.

Walker, P.R., Calzascia, T., de Tribolet, N., and Dietrich, P.Y. (2003) T-cell immune responses in the brain and their relevance for cerebral malignancies. *Brain Res. Brain Res. Rev.* **42**, 97–122.

Wang, M.H., Grossmann, M.E., and Young, C.Y. (2004) Forced expression of heat-shock protein 70 increases the secretion of Hsp70 and provides protection against tumour growth. *Br. J. Cancer* **90**, 926–931.

Wang, X.Y., Chen, X., Manjili, M.H., Repasky, E., Henderson, R., and Subjeck, J.R. (2003) Targeted immunotherapy using reconstituted chaperone complexes of heat shock protein 110 and melanoma-associated antigen gp100. *Cancer Res.* **63**, 2553–2560.

Wang, X.Y, Manjili, M.H., Park J., Chen, X., Repasky, E., and Subjeck, J.R. (2004) Development of cancer vaccines using autologous and recombinant high molecular weight stress proteins. *Methods* **32**, 13–20.

Wearsch, P.A., and Nicchitta, C.V. (1997) Interaction of endoplasmic reticulum chaperone GRP94 with peptide substrates is adenine nucleotide-independent. *J. Biol. Chem.* **272**, 5152–5156.

White, T.K., Zhu, Q., and Tanzer, M.L. (1995) Cell surface calreticulin is a putative mannoside lectin which triggers mouse melanoma cell spreading. *J. Biol. Chem.* **270**, 15926–15929.

Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E., and Neckers, L.M. (1994) 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. USA* **91**, 8324–8328.

Wikstrand, C.J., Hale, L.P., Batra, S.K., Hill, M.L., Humphrey, P.A., Kurpad, S.N., McLendon, R.E., Moscatello, D., Pegram, C.N., Reist, C.J., Traweek, S.T., Wong, A.J., Zalutsky, M.R., and Bigner, D.D. (1995) Monoclonal antibodies against EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. *Cancer Res.* **55**, 3140–3148.

Wikstrand, C.J., Cokgor, I., Sampson, J.H., and Bigner, D.D. (1999) Monoclonal antibody therapy of human gliomas: Current status and future approaches. *Cancer Metastasis Rev.* **18**, 451–464.

Wong, A.J., Ruppert, J.M., Bigner, S.H., Grzeschik, C.H., Humphrey, P.A., Bigner, D.D., and Vogelstein, B. (1992) Structural alterations of the epidermal growth factor receptor gene in human gliomas. *Proc. Natl. Acad. Sci. USA* **89**, 2965–2969.

Workman, P. (2004) Combinatorial attack on multistep oncogenesis by inhibiting the Hsp90 molecular chaperone. *Cancer Lett.* **206**, 149–157.

Wright, J.M., Zeitlin, P.L., Cebotaru, L., Guggino, S.E., and Guggino, W.B. (2004) Gene expression profile analysis of 4-phenylbutyrate treatment of IB3-1 bronchial epithelial cell line demonstrates a major influence on heat-shock proteins. *Physiol. Genomics* **16**, 204–211.

Wu, W.Z., Liu, K.D., Xie, Q., and Wu, H.S. (1999) The study on the expression of membrane HSP70 protein in H22 cell and its immunoprotective mechanism against carcinoma. *J. Exp. Clin. Cancer Res.* **18**, 543–548.

Xiao, G., Chung, T.F., Fine, R.E., and Johnson, R.J. (1999) Calreticulin is transported to the surface of NG108-15 cells where it forms surface patches and is partially degraded in an acidic compartment. *J. Neurosci. Res.* **58**, 652–662.

Xu, W., Mimnaugh, E., Rosser, M.F., Nicchitta, C., Marcu, M., Yarden, Y., and Neckers, L. (2001) Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J. Biol. Chem.* **276**, 3702–3708.

Yamada, A., Kawano, K., Koga, M., Matsumoto, T., and Itoh, K. (2001) Multidrug resistance-associated protein 3 is a tumor rejection antigen recognized by HLA-A2402-restricted cytotoxic T lymphocytes. *Cancer Res.* **61**, 6459–6466.

Yamanaka, R., Abe, T., Yajima, N., Tsuchiya, N., Homma, J., Kobayashi, T., Narita, M., Takahashi, M., and Tanaka, R. (2003) Vaccination of recurrent glioma patients with tumour lysate-pulsed dendritic cells elicits immune responses: Results of a clinical phase I/II trial. *Br. J. Cancer* **89**, 1172–1179.

Yamazaki, K., Nguyen, T., and Podack, E.R. (1999) Cutting edge: Tumor secreted heat shock-fusion protein elicits CD8 cells for rejection. *J. Immunol.* **163**, 5178–5182.

Yang, J., Yang, J.M., Iannone, M., Shih, W.J., Lin, Y., and Hait, W.N. (2001) Disruption of the EF-2 kinase/Hsp90 protein complex: A possible mechanism to inhibit glioblastoma by geldanamycin. *Cancer Res.* **61**, 4010–4016.

Yang, L., Ng, K.Y., and Lillehei, K.O. (2003) Cell-mediated immunotherapy: A new approach to the treatment of malignant glioma. *Cancer Control* **10**, 138–147.

Yokota, S., Kitahara, M., and Nagata, K. (2000) Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells. *Cancer Res.* **60**, 2942–2948.

Younes, A. (2003) A phase II study of heat shock protein-peptide complex-96 vaccine therapy in patients with indolent non-Hodgkin's lymphoma. *Clin. Lymphoma* **4**, 183–185.

Yu, J.S., Wheeler, C.J., Zeltzer, P.M., Ying, H., Finger, D.N., Lee, P.K., Yong, W.H., Incardona, F., Thompson, R.C., Riedinger, M.S., Zhang, W., Prins, R.M., and Black, K.L. (2001) Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res.* **61**, 842–847.

Yu, J.S., Liu, G., Ying, H., Yong, W.H., Black, K.L., and Wheeler, C.J. (2004) Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res.* **64**, 4973–4979.

Zagzag, D., Nomura, M., Friedlander, D.R., Blanco, C.Y., Gagner, J.P., Nomura, N., and Newcomb, E.W. (2003) Geldanamycin inhibits migration of glioma cells in vitro: A potential role for hypoxia-inducible factor (HIF-1α) in glioma cell invasion. *J. Cell Physiol.* **196**, 394–402.

Zeng, Y., Feng, H., Graner, M.W., and Katsanis, E. (2003) Tumor-derived, chaperone-rich cell lysate activates dendritic cells and elicits potent antitumor immunity. *Blood* **101**, 4485–4491.

Zeng, Y., Graner, M.W., Feng, H., Li, G., and Katsanis, E. (2004) Imatinib mesylate effectively combines with chaperone-rich cell lysate-loaded dendritic cells to treat bcr-abl+ murine leukemia. *Int. J. Cancer* **110**, 251–259.

Zeng, Y., Graner, M.W., Thompson, S., Marron, M., and Katsanis, E. (2005) Induction of BCR-ABL-specific immunity following vaccination with chaperone-rich cell lysates derived from BCR-ABL+ tumor cells. *Blood* **105**, 2016–2022.

Zheng, H., Dai, J., Stoilova, D., and Li, Z. (2001) Cell surface targeting of heat shock protein gp96 induces dendritic cell maturation and antitumor immunity. *J. Immunol.* **167**, 6731–6735.