# Heat Shock Proteins in Glioblastomas

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**KEYWORDS**

• Immunotherapy • Major histocompatibility complex
• Antigens • T cell

Glioblastoma multiforme (GBM) is the most common primary central nervous system tumor, affecting as many as 17,000 patients every year just in the United States.[1] The prognosis for these malignant brain tumors is poor, with a median survival of 14 months and a 5-year survival rate below 2%. Development of novel treatments such as immunotherapy is essential to improving survival and quality of life for these patients.

## VACCINES AND IMMUNOTHERAPY

Advances in cancer immunology have led to the development of various therapies using tumor-specific T cells to generate antitumor activity. Current approaches to immunotherapy are based on the principle that tumor-specific antigens are capable of inducing cytotoxic T lymphocytes (CTLs) to specifically target the antigen-presenting cells (APCs).[2,3] Internalization of the antigens by APCs, such as dendritic cells or macrophages, results in their presentation by major histocompatibility complex (MHC) class I molecules, eliciting antigen-specific CTL responses.[4–6] Much attention has been given to enhancing APC activation, manipulating processing of tumor-specific antigens, and ultimately improving the specific killing of tumor cells while sparing normal tissue, an aspect especially important to preservation of normal brain tissue in treatment of gliomas.[7–9] To exploit the ability of the immune system to generate antitumor responses, vaccinations against cancer that have been developed to date, which range from use of purified peptides and antigens to whole tumor lysates or cells. Active immunotherapy may also address some of the most challenging obstacles in cancer immunotherapy: the tumor's ability to escape immune detection or exertion of immunosuppressive mechanisms.[10] Endogenous heat shock proteins (HSPs) have been implicated in mediation of both adaptive and innate immunity, and there is a rising interest in the use of this safe and multifaceted HSP vaccine therapy as a promising treatment for human cancers, including GBM (**Fig. 1**).[11]

### *Discovery of HSP*

First discovered in flies, it was observed that environmental temperature increases led to the transient expression of specific proteins.[12] Other studies showed that these ''heat shock proteins (HSPs)'' were also stimulated by any environmental or pathologic insults or trauma that resulted in protein misfolding, including such conditions as hyperthermia, anoxia, glucose deprivation, oxidative damage, irradiation, infection, and inflammation.[13] Highly conserved, these abundant proteins are best known for their functions as molecular chaperones, playing important roles in the proper folding, assembly, transport of nascent peptides, and degradation of misfolded proteins. Under normal conditions, HSPs account for approximately 10% of proteins in the cell,[14] but exhibit as much as a threefold increase in expression levels in response to stressful cellular conditions to counteract abnormal protein folding and dysfunction.[14–16] There are five major classes of HSPs: Hsp60, Hsp70, Hsp90, Hsp100, and small HSPs.[17] In addition to these main HSPs that reside

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Neurosurg Clin N Am 21 (2010) 111–123
doi:10.1016/j.nec.2009.09.002
1042-3680/09/$ – see front matter 

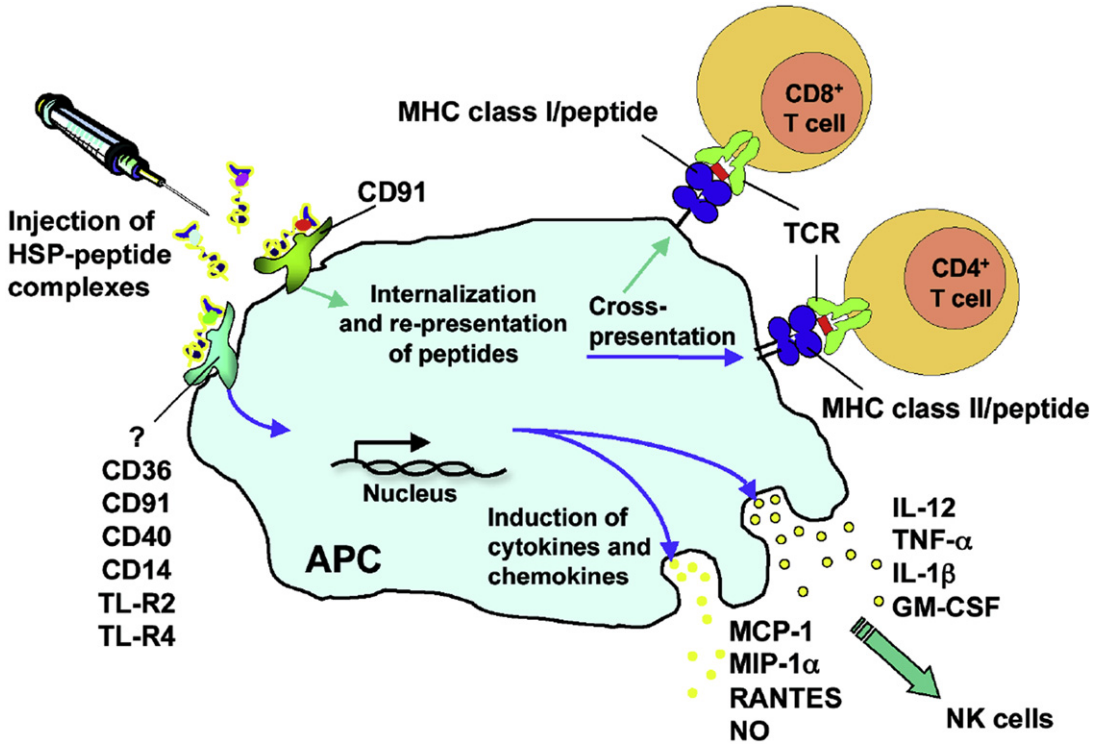


**Fig. 1.** Diagram for heat shock protein vaccine injection and postulated induced immune response and interactions with immune cells.

in the nucleus and cytosol, stress proteins include glucose[18–21] regulated proteins (Grp) that are found in the endoplasmic reticulum (ER).[22] This stress-inducible family of proteins (Grp78/BiP, grp94/96, and grp170) is induced by conditions effecting ER function, such as hyopoglycemia, hypoxia, heavy metals, and glycosylation and calcium homeostasis interference[23,24] and function to exert similar functions as HSPs. Although intracellular HSPs function to protect cells from death, extracellular and membrane-bound HSPs play important immunomodulatory functions.[17] Not all HSPs though have the ability to stimulate antigen-specific CTL immunity. Among stress proteins, Gp96, hsp90, hsp70, calreticulin, hsp110, and hsp170 appear to be among the most immunogenic stress proteins.[25,26]

### *Role of HSP in GBM*

The increasing interest in the use of immunogenic chaperone proteins in cancer has led to use of this vaccine for various cancers in immunotherapeutic clinical trials; however, the application of this therapy has not yet been widely explored for the treatment of brain tumors.

Some aspects of HSPs though have been characterized in human brain tumors.[11] Considering the stressful conditions of the glioma environment, such as hypoxia, high proliferation, increased levels of metabolism, and genetic instability, chaperone proteins have been found to be highly upregulated in brain tumor cells.[18–21] Tumor cell overexpression of HSPs may exploit the advantage of protection and homeostasis that is normally conferred by HSPs, and may permit cancer progression and therapeutic resistance of the cancerous tumor.[19] This abundance and overexpression of HSP and its functions in brain tumor cells may provide a possible therapeutic intervention to enhance targeted attacks against these tumor cells.

GBM is associated with a multitude of genetic mutations, the most frequent of which includes epidermal growth factor receptor (EGFR) amplification, PTEN deletion, inactivation of TP53, mdm2 overexpression, and loss of chromosomes 1p and 19q.[27–29] Many of these genes and proteins that play a role in glioma genesis have been found to interact with HSPs. HSPs are involved in the cellular proliferation, evasion of apopotosis, metastatic motility, invasion of normal issue, and angiogenesis associated with gliomas.[14,29] Although predominantly intracellular studies have begun to reveal that there are also cell surface expression and extracellular functions

of HSPs in cancerous tumors.[18,30–33] Under stressful conditions, glial cells have been shown to release chaperone proteins, such as Hsp70, Hsp110, and Grp78.[18,31,34–36]

### *Hsp90 interactions in GBM*

Expression of Hsp90 protein seems to play an important role in mediating mechanisms that promote tumor survival and growth.[14,37,38] It has been identified to be an important chaperone protein associating with key oncogenic proteins, growth factor receptors, and cell cycle regulators in known brain tumor signaling pathways. Hsp90 has been found to form complexes with many chaperone clients known to play important roles in glioma formation, such as EGFR (vIII), PDGFR, FAK, AKT, hTERT, p53, cdk4, MAPK, PI3K, EF-2 kinase, HIF-1$\alpha$, Akt, c-Src, Raf-1, Brc-Abl, and MMP 2.[14,29,31]

The main functions of Hsp90 are the binding of unstable tertiary protein structures, prevention of protein degradation, and antiapoptosis properties.[37–40] Normally functioning to stabilize proteins and transcription factors for cell growth, Hsp90 acts in cancer as a buffer to tolerate the effects and altered signals of malignant genetic alterations.[14,41,42] Hsp90 has two isoforms: the minor Hsp90$\beta$ is expressed constitutively, whereas the expression of major Hsp90$\alpha$ is inducible.[38,43] Hsp90$\alpha$ protein and mRNA have been shown to be highly expressed in both glioma cells and in cancer tissue samples.[38]

### *HSP interaction with EGFR mutations*

The EGFR growth factor pathway is a strongly implicated molecular pathway in brain tumor pathogenesis; its mutations alter many factors that contribute to formation, maintenance, and progression of cancer. The most common genetic mutation found in GBM is the epidermal growth factor receptor (EGFRvIII), which results in the truncated form because of deletion mutations of exon 2-7.[44,45] The mutant form of EGFR is found in 40% to 50% of GBMs and has been associated with poorer prognosis of patients.[21,45–48] This mutation of EGFR receptor deletes the ligand binding region and subsequently results in constitutive expression of tyrosine kinase, leading to signaling alterations that promote malignant growth and survival.[49,50] Hsp90 associates with EGFRvIII by forming a complex along with proteins Cdc37 and p60/Hop to maintain elevated levels of EGFRvIII expression.[21] Hsp90 also directly interacts with c-Myc, a proto-oncogene highly expressed in high-grade gliomas, at its binding site on the Hsp90 promoter.[21,51] Like in other cancers, the Hsp90–c-Myc interaction is essential in mediating proliferation and promotion of malignant glioma cells survival, with a special role in the regulation of glioma stem cells.[51,52]

### *HSP interactions with AKT*

Another brain tumor relevant client protein of Hsp90$\alpha$ is Akt, a frequently hyperactivated downstream effector of phosphatidylinositol 3-kinase (PI3K) in the PTEN/PI3K/AKT pathway known to be aberrently upregulated in malignant gliomas.[29,53–55] In GBM, the common mutation of the AKT inhibitor, phosphatase, and tensin homolog deleted on chromosome 10 (PTEN) results in dysregulation of Akt and allows for downstream promotion of cell survival.[56–58] In addition to protection against apoptosis, activation of Akt is also known to be involved in immunoresistance of gliomas by upregulation of B7 homolog 1 (B7-H1) protein expression establishing Akt as a useful point for intervention.[59] Stabilization of the AKT protein kinase relies on interactions with Hsp90$\alpha$ to prevent protein degradation and to maintain its functions in tumor progression and cell survival.[37,53,60–63] Consistent with the essential stabilization of AKT by Hsp90$\alpha$, the suppression of Hsp90$\alpha$ using SiRNA results in decreased expression of phosphorylated AKT in glioma cells.[37] Inhibition of Hsp90$\alpha$ disrupts its complex with AKT and results in proteosome-dependent degradation of Akt protein.[62] Suppression of the PI3K-AKT-mammalian target of rapamycin pathway not only downregulates antiapoptotic FLIP, it also decreases expression of the immunosuppressive B7-H1 protein making tumor cells more amenable to immunotherapeutic treatments.[59,64]

Recent studies may explain the antiapoptotic effects of Hsp90$\alpha$ in glioma cells that are especially resistant to apoptosis. Tumor necrosis factor-$\alpha$ related apoptosis inducing ligand (TRAIL) is a key apoptotic factor in gliomas and is shown to interact with the $\alpha$ form of Hsp90. In a recent study, Hsp90$\alpha$ was found to bind FLIP and subsequently regulate the sensitivity of TRAIL-induced apoptosis in gliomas through recruitment and localization of antiapoptotic protein, FLIP, rather than through alteration of FLIP stability.[37] The recruitment and stabilization of FLIP and other antiapoptotic molecules to the death-inducing signaling complex allows Hsp90$\alpha$ to regulate TRAIL resistance through an ATP-dependent N-terminal domain interaction.[37] Using SiRNA targeting Hsp90$\alpha$, sensitization of previously resistant glioma cells rendered glioma cells vulnerable to apoptosis by TRAIL-dependent mechanisms.[37] The roles in cell cycle regulation and its ability to stabilize malignant characteristics suggest Hsp90

α may be a potential therapeutic target for brain tumors.[37,38]

### *HSP role in glioma angiogenesis*

Another tumor promoting function of Hsp90 is its involvement in cancer angiogenesis and malignant migration. Angiogenesis is important in cancer to sustain the growth of tumors because the highly proliferating tumors cells demand increased metabolism because of high proliferation states of tumor cells. Hsp90 binds and stabilizes hypoxia-inducible factor-1α (H1F1α), the major sensor of hypoxic conditions present in cancer cells.[14,39,65] In GBM, transcription factor H1F1α is highly overexpressed especially in the most invasive regions of the tumor, and also correlates with glioma tumor grades.[66–68] The hypoxic environment of GBM induces H1F1α to increase vascular endothelial growth factor stimulating nitric oxide synthase expression, resulting in proper signaling required for angiogenesis.[39,69] The treatment of glioma cells with an Hsp90 inhibitor in vitro results in the rapid proteolytic degradation of H1F1α and prevention of vascular endothelial growth factor induction.[69,70] The Hsp90 client protein, H1F1α, is also thought to contribute to malignant invasion and metastatic migration of gliomas. Interference of the Hsp90 and H1F1α interaction decreases the cellular migration of human glioma cells in culture by inhibition of focal adhesion kinase (FAK) phosphorylation.[67] These findings suggest the importance of Hsp90 in tumor angiogenesis and its therapeutic potential in mediating antiangiogenic mechanisms of GBM.

### *Hsp90 inhibition*

The instability of cancer signaling molecules resulting from disruption of HSP complexes using HSP inhibitors suggest that HSP-specific inhibitors could be used as therapeutic treatments or adjuvants.[31,62,71] Experiments using geldanamycin, an antibiotic inhibitor of Hsp90, demonstrated the widespread involvement of the HSP in many important tumor signaling pathways.[72] The anamycin antibiotics geldanamycin and 17-allyl-17-dimethoxygeldanamycin inhibit Hsp90 by binding to its ATP-binding domain so the treatment of cancer with Hsp90 inhibitors destabilize client proteins and causes their degradation.[55,73,74] In addition to decreasing stability of essential proteins, another mechanism of action of Hsp90 inhibitor may be the enhancement of complement-dependent cell lysis of cancer cells.[75]

17-Allyl-17-dimethoxygeldanamycin is the less hepatoxic geldanamycin analog that has recently been tested in phase I and II trials for metastatic melanoma, prostate, and breast cancer, renal cell carcinoma, leukemia, and other solid advanced cancers.[73,76–83] Combination treatments of 17-allyl-17-dimethoxygeldanamycin have been combined with chemotherapeutic agents, such as irinotecan, paclitaxel, and angiogenesis inhibitors, and have been shown to be promising in treating some cancers. Hsp90 inhibitors continue to be investigated as a potential therapeutic adjuvant.[14,79,83–85] Because Hsp90 interacts with client proteins that are highly dysregulated in the pathogenesis of GBM, such as EGFR, p53, AKT, HIF1α, and MMP2, Hsp90 inhibitors may provide a novel method for simultaneously targeting multiple aspects contributing to the rapid progression of GBM.

### *Hsp70 interactions in GBM*

Hsp70 is an important target for anticancer therapies because of its expression on the tumor cell surface, acting as a target for natural killer (NK) cells.[86] The CD94 lectin receptor on NK cell recognizes a specific 14 amino acid peptide of Hsp70 that is presented on the plasma membrane of tumor cells and initiates cell lysis.[87–89] Detection of the peptide causes NK cells to release cytotoxic lymphocyte product, granzyme B, which is subsequently taken up by tumor cells and rendering these Hsp70-positive tumor cells more vulnerable to cytolytic killing.[90] In addition to soluble chaperone Hsp70 that are known to be released by glial cells,[34] some tumor cells are also known to release detergent-soluble exosomes containing the membrane-bound Hsp70, which can stimulate the cytotoxic activities of NK cells.[17,90,91] These exosomes derived from Hsp70-positive tumor cells can also induce the specific migration of CD94-positive NK cells to target tumor cells.[90] The ability of these secreted exosomes to stimulate immune reactions of macrophages and dendritic cells is functionally effective in causing tumor reduction of autologous and allogeneic animal models bearing cancerous tumors.[92]

P53 is a crucial tumor suppressor protein normally functioning to regulate genomic damage and defects, abnormal oncogene activation, and hypoxic and metabolic stresses.[29,93,94] TP53 is a frequent genetic mutation in gliomas and is deleted or absent in approximately 40% of GBM.[95,96] Inactivated in almost all cancers, p53 deletion leads to the dysregulation of normal cellular growth cycle, angiogenesis, apoptosis, and oncogenic regulation, all of which are important processes that contribute to tumorigenesis.[94,97]

Interestingly, a major regulator of HSP is p53 and the loss of HSP promoter repression by p53 in cancer may contribute to the increased rates of HSP transcription in cancers.[39,98,99] Human

Hsp70 is normally transcriptionally repressed by p53 binding of transcription factors including NF-Y and (CCAATT binding factor) CBF.[39,98,100] Absence of p53 caused by TP53 mutations results in the lack of normal defenses against tumorigenesis, and studies have demonstrated that Hsp70 binds mutant p53 and accumulates abnormally in tumor cells of various cancers.[39,101–103]

One of the mechanisms by which Hsp70 may be exerting its antiapoptotic protection of cancer cells is stabilization of lysosomes. In human cancers, Hsp70 localizes to the plasma membrane of lysosomes and prevents permealization ultimately halting tumor necrosis factor–induced apoptosis.[104] Indeed, depletion of Hsp70 using antisense Hsp70 cDNA decreases survival of glioblastoma cells in vitro and induces caspase independent cell death. In vivo, its depletion results in tumor reduction and promotes survival of glioblastoma xenograft mice compared with control mice.[105] The variety of immunostimulatory and antiapoptotic capabilities of Hsp70 makes this chaperone protein a potent activator of the immune system and a potentially effective therapeutic target.

### *Hsp27*

A prognostic role has been suggested for antiapoptotic chaperone protein Hsp27, both in murine models and in human cancers.[106,107] Hsp27 is characterized as an apoptotic HSP that is associated with improved prognosis in esophageal squamous cell carcinoma and malignant fibrous histiocytomas, but decreased survival in prostate and gastric cancer for patients with high expression of Hsp27.[108–110] A recent study revealed that constitutional expression of Hsp27 in tumor cells did not correlate significantly with clinical outcome of patients with medulloblastoma.[107] In a large study of 198 human brain tumors, all glioblastomas were found to be intensely immunopositive for Hsp27.[20] Although increased expression levels of Hsp27 has been associated with higher-grade gliomas compared with their lower-grade counterpart, it is not shown to be prognostic and the Hsp27 expression level has not been found to be significantly increased in GBM patients undergoing radiation treatment.[19,20,111] The lack of prognostic correlation may be in part attributed to the high baseline overexpression of Hsp27 in GBM, in which further elevation may not contribute to the already present chemotherapy- and radiation-resistant phenotype of GBM.[30,101] In vitro experiments using GBM cell culture also did not reveal any protective effects of induced Hsp27.[19]

## HSP CANCER VACCINES

### *Immunogenic Properties of HSP*

Realization that cancer cells extracted from tumor provided immune protection in host mice on subsequent challenge prompted studies that revealed the importance of HSPs in the immune response. That mice were immunized against the specific cancers used for rechallenge, but not against other types of cancers they harbored, established the unique and individual-tumor specificity of cancer immunity.[112–114] To isolate the immunogenic component of tumors, cancer homogenates were fractionated by chromatographic techniques and represented to host animals testing for cancer rejection potential.[115] Molecules capable of immunizing animals were then purified by repeated fractionation until homogenous, and further identified to be proteins belonging to the heat shock family, such as Hsp90, Hsp70, Hsp110, Hsp170, calreticulin, and Gp96.[25,114–116] This surprising discovery revealed the immunogenic properties of these HSPs; however, the HSPs isolated from adjacent normal tissue or other tumors were unable to elicit the immune protection seen by its tumor-derived counterpart.[114]

### *Origin of Immunogenicity*

Attempts to explain the tumor specificity of cancer-derived HSP were not revealed by differences in DNA sequencing, structural variation, or somatic polymorphisms when compared with nontumor HSPs.[22] It was a series of experiments showing large groups of peptides to be associated with homogenous Gp96-HSP preparations that suggested peptide groups to be the immunogenic origins. The ability of HSP-protein complex to induce cellular immunity was later confirmed by loss of immune protection under peptide deprivation conditions.[114] Furthermore, the replenishment of peptide to HSP proteins reconstituted effective antitumor protection in host animals.[117] Interestingly, these peptides carried by other types of proteins, such as serum albumin, did not elicit CTL induction and failed to provide immunogenicity.[117] These studies confirmed that neither peptide nor HSP were immunogenic individually, but antigenic peptides chaperoned by HSPs could induce antigen-specific CD8+ T cell[117,118] in immunized animals.[119–121]

### *HSP vaccine*

Based on the properties of chaperone proteins to generate the desired specialized targeting of malignant cells, the idea of harnessing HSP immunogenicity led to the development of HSP-peptide

complex cancer vaccines. Although HSP vaccines have been successfully developed for clinical trials of melanoma, sarcoma, colorectal, renal, and pancreatic cancer, and non-Hodgkin's lymphoma, there is currently a single clinical trial studying the use of HSP in malignant brain tumors.[122–125] This promising vaccine therapy uses heat shock peptide-complex to generate a combination of tumor-specific adaptive immunity but also induces the activation of innate immune mechanisms, maximizing antitumor activity. Oncophage (HSP-peptide complex-96; vitespen) vaccines are composed of Hsp96 (Gp96) complexed with the autologous tumor antigenic peptides.[126] The specificity of the vaccine against the tumor of origin is caused by the ability of chaperone proteins to form strong noncovalent bonds with the unique antigens of the individual tumor. An ongoing clinical trial is currently investigating the HSP-peptide complex-96 in the treatment of patients with recurrent or progressive high-grade gliomas, such as GBM, gliosarcoma, anaplastic gliomas, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic infiltrating glioma, and mixed malignant glioma (http://clinicaltrials.gov/ct2/show/NCT00293423). The vaccine is derived from autologous tumor cells obtained from individual patients during standard intracranial surgical resection, generating immune responses only against the specific tumor from which it was derived.

### *HSP polyvalent antigen interaction*

By representing the broad range of tumor-associated antigen peptides characterizing individual cancers, the HSP vaccine provides a polyvalent method to improve targeting of tumors. The ability for the HSP-peptide complex vaccine to represent the wide antigenic fingerprint of gliomas makes this vaccine an individualized cancer therapy, conveniently circumventing the need to characterize specific antigens for a certain cancer type, especially in such cancers as GBM, in which these antigenic epitopes have not yet been sufficiently identified.[25,117,127,128] In addition, HSP peptide complexes encompass the entire antigenic repertoire, which can also overcome the common issue of immunoresistance and immune escape mechanisms by cancerous tumors.[14,127]

### *Presentation on APCs*

The Gp96-peptide complexes isolated and purified from patients' glioma samples are complexed with the specific variety of tumor-specific peptides making up the antigenic profile of the tumor. The ability of HSPs to chaperone these antigenic peptides confers the ability to elicit specific immunity against the origin of the peptides, within tumors and other malignancies. The specific mechanism by which HSP-peptide complexes accomplish immune induction is beginning to be understood through investigation of its interaction with APCs. To generate a successful immune response against tumor tissue, antigens must be processed and displayed by APCs on MHC class I molecules for induction and expansion of antigen-specific CD8+ T cells. The ability of tumor-derived HSP vaccination to elicit specific immunity against the origin of the antigenic peptides indicates that HSP-peptide complexes are involved in the antigen processing pathway. Depletion of both macrophages and APCs from host animals confirmed the dependence of HSP-peptide complex to induce immune protection on phagocytic mechanisms.[114,128] These studies also suggested the essential role of macrophages in transfer of antigenic material from chaperone proteins to APCs for MHC class I presentation, rather than a direct exchange of peptides between chaperone and APCs, to exert immune induction.[112]

## MORE EFFICIENT ANTIGEN PRESENTATION ON MHC

The high sensitivity of APCs to HSP-peptide complexes led to early suspicion of HSP-specific receptors on APCs that account for the efficient uptake of small quantities of HSP complexes.[129] Further evidence supporting indirect antigen is the finding that HSP-chaperoned peptides are significantly more efficient at loading antigens onto MHC class I molecules than that of free peptides and inducing recognition by CTLs.[112,130,131] There is now evidence that HSP-peptide complexes undergo internalization by macrophages through clathrin-coated receptor-mediated endocytic mechanisms, and some HSP-specific receptors have been identified on the surface of APCs.[132]

On injection of Oncophage into the host, the HSP-peptide complex is internalized by HSP-specific receptors, such as CD91 on APCs. The exogenous peptides complexed with chaperone proteins then undergo antigen processing through intracellular compartments of MHC class I and II molecules to be presented or re-expressed on the surface of APCs.[120,129,133] Activated APCs carrying antigenic peptides, including dendritic cells, macrophages, or Langerhans cells, exert their immunostimulatory effects by circulating systemically to be recognized by naive T cells in the lymph node of the host.[112,113,129] Naive T cells stimulated by recognition of these tumor-specific

peptides expand into CD8+ and CD4+ T cells that exert specific immunity against the range of antigens present in the tumor. Both glioma relevant HSPs (Hsp70 and Hsp90) have been shown to play essential roles in the activation of CD8+ T cells by this process of representing tumor-specific antigens on surfaces of MHC class I molecules.[40]

### *HSP Activation of Innate Immunity*

In addition to eliciting adaptive immunity, this HSP vaccine also has the advantage of generating activation of innate immunity. Apart from its involvement in the antigen presentation pathway, the internalization of chaperone-peptide complexes results in the maturation and subsequent functional activation of APCs, defined by the expression of costimulatory molecules and cytokine activity.[134,135] Exposure to various HSPs causes differential expression of MHC class II and costimulatory molecules, such as CD80 (B7-1), CD86 (B7-2), and CD40, to be upregulated on the surface of APCs.[39,131] Interaction with Gp96 induces the surface expression MHC class II and B7-2, but not B7-1 on dendritic cells, whereas another HSP, Hsp70, induces upregulation of B7-1, but not MHC II and B7-2.[23,136,137]

The other consequence of the interaction between isolated HSP and APCs is the natural stimulation of cytokine release by macrophages and dendritic cells.[136] Important cytokines released by APCs on exposure to gp96, Hsp70, and Hsp 90 include interleukin-1b, -12, and -6; tumor necrosis factor-$\alpha$; granulocyte-macrophage colony–stimulating factor; chemokines, such as MCP-1, MIP-1, and RANTES; and nitric oxide.[23,25,138] In particular, it is thought that the cytokine interleukin-12 released from exposure to HSP may be responsible for expansion of NK cells[26] in vaccine-treated cancer patients. The presence of HSP itself, regardless of bound peptides, can stimulate release of these potent proinflammatory agents known to be important in antigen-specific immunity, serving as a convenient adjuvant for enhancing protective immunity.[23]

Specific interaction between HSP-peptide complex and APCs leads to induction of dendritic cell maturation, release of cytokines by both macrophages and DCs, and the stimulation of NK cells to enhance antitumor activity of T cells. NK cells are essential for innate immunity and the activation of these cells has been observed following immunization by tumor derived HSP-peptide complexes.[26,113] The multiple effects on APCs may be caused by the observation that exposure to Gp96 and Hsp70 causes translocation of NFkB into the nucleus of APC, a well conserved pathway for immunologic signal transduction.[26,39,135] Activation of NFkB through HSP influence mediates important downstream proteins that regulate the growth, progression, and antiapoptotic capabilities of cancer cells to enhance malignancy.[119,136] Acting in conjunction with the activation of antigen-specific CD8 T cell and CD4 T cells, important players in innate immunity are stimulated by the HSP-peptide complex vaccine.

### *Limitations of an HSP Vaccine for GBM*

The HSP-peptide complex vaccine requires the isolation of significant amounts of HSP-peptide complexes from the patient's tumor, which can be limited by the size of tumors extracted during standard surgical resection, even though the vaccine itself is relatively easy to produce.[23] Although this may not be an issue for cancers characterized by larger tumors, the significantly smaller tumor able to be resected from GBM patients sometimes limits the availability of HSP vaccine treatment as an option.[23,121,139] In addition to the quantity of HSPs and tumor sample, another level of challenge for patients to receive the potent immune vaccines is the purity criteria during processing necessary to generate the vaccine for patients to proceed with the treatment. Although the sample is processed as soon as possible after surgery, the time required to generate the vaccine may sometimes create difficulty especially in aggressive tumors that can progress rapidly and prevent use of this treatment modality.[139,140]

The polyvalent chaperoning capabilities of the HSP-peptide vaccine are advantageous and effective in treatment of cancers not yet sufficiently characterized antigenically, as in the case of GBM. The lack of characterization also results in less efficient immunomonitoring, however, which may assist in the further understanding and development of other targeted cancer strategies.[121] Concern for adverse autoimmune responses is countered by excellent safety profiles have been observed for all autologous HSP clinical trials. Patients report good quality of life and only occasional patients experience transient side effects, such as low-grade fever and mild local responses.[139,140] The ongoing clinical trial of the vaccine for patients with recurrent high-grade gliomas is rigorously assessing the safety, dosage, and efficacy of this vaccine for the treatment of these fatal brain tumors (http://clinicaltrials.gov/ct2/show/NCT00293423).

### *Advantages of an HSP vaccine for GBM*

The multifactorial effects of HSP vaccines seem to be a promising immunotherapeutic approach for treating malignant gliomas, which tend to have very high rates of recurrence even after standard modern treatment. The potential to efficiently chaperone potent antigenic peptides, activate APCs, and exert proinflammatory stimulation of NK cells are important properties of HSPs that allow the use of tumor-derived HSP-peptide complexes to maximize specific immunity against cancer cells. The unique ability to chaperone the wide range of a tumor's antigenic fingerprint is not only capable of stimulating potent specific immunity against this tumor but also activates innate immunity to aid targeting of the malignancy.

Although tumor-associated antigens are now being investigated and characterized in human cancers, most current immunotherapeutic treatments require identification of characteristic tumor antigens for respective cancers. Although tumor-associated antigens are continuing to be investigated in human gliomas, most specific glioma antigens remain to be sufficiently characterized. By representing the antigenic repertoire of glioma antigens from the particular tumor extracted from the patient, the HSP vaccine provides a strictly individualized treatment vaccine and also bypasses the usual difficulty of identifying a set of immunogenic antigens that characterize a certain cancer, such as GBM. In addition, the chaperoning of the entire antigenic fingerprint precludes the immune evasion and escape capabilities from single antigen therapies, issues faced especially by aggressive cancers like GBM. Because the variety of antigens chaperoned by the isolated HSPs include some self-antigens expected to be chaperoned in addition to the desired tumor antigens, the risk of autoimmunity from administration of the vaccine might be expected.[23] The occurrence of autoimmune reactions or serious side effects has not been observed, however, in any HSP-peptide vaccine patients to date.[139,141,142] The ability of these potent immunogenic properties of the HSP vaccine to efficiently elicit protective immunity against cancers, combined with the safety and success of clinical trials against other cancers, has established a promising and novel avenue of investigation for neuro-oncologists against malignant gliomas.

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