Potent Tumor-Specific Immunity Induced by an In vivo Heat Shock Protein-Suicide Gene–Based Tumor Vaccine

Wenhong Ren,1,2 Randy Strube,1,2 Xiaoping Zhang,5 Si-Yi Chen,1,2,3 and Xue F. Huang1,4

1Center for Cell and Gene Therapy, 2Departments of Molecular and Human Genetics, 3Immunology, and 4Pediatrics, Baylor College of Medicine, Houston, Texas; and 5United Gene Biotechnologies, Inc., Shanghai, China

ABSTRACT

Tumor cells harbor a repertoire of unique, mutated antigens and shared self-antigens but generally are incapable of provoking an effective immune response, likely because of inadequate antigen presentation by professional antigen-presenting cells. Heat shock proteins (HSPs) play important roles in eliciting innate and adaptive immunity by chaperoning peptides for antigen presentation and providing endogenous danger signaling. Although effective in inducing tumor-specific immune responses in mice and in some clinical trials, tumor-derived HSPs have many limitations like vaccines, such as the technical difficulty of ex vivo preparation of adequate quantities of HSPs from the resected tumors of individual patients. Here we have developed an in vivo HSP-suicide gene tumor vaccine by generating a recombinant replication-defective adenovirus (Ad-HT) that coexpresses HSP70 and a herpes simplex virus thymidine kinase suicide gene. The combination of HSP70 overexpression in situ and tumor killing by thymidine kinase/ganciclovir treatment, but neither strategy alone, provoked potent systemic antitumor activities after intratumor injection of Ad-HT. Tumor-specific CD8+ and CD4+ T-cell responses were induced by Ad-HT intratumor injection. CD11c+ dendritic cells (DCs) isolated from mice treated with Ad-HT were able to prime tumor-specific CTLs. Collectively, these results indicate that the combination of tumor killing by activation of a suicide gene to release tumor antigens and in situ HSP70 overexpression to enhance DC antigen presentation overcomes host immune tolerance to tumor antigens, leading to the induction of potent antitumor immunity. Our findings may have broad relevance to the use of the in vivo HSP/suicide gene tumor vaccine in therapy for human solid tumors.

INTRODUCTION

Increasing evidence indicates that heat shock proteins (HSPs) play important roles in eliciting innate and adaptive immunity. The ability of HSPs to chaperone peptides and to activate dendritic cells (DCs) seems to be the common feature associated with all the major HSPs, including HSP110, gp96, HSP90, and HSP70 (1). HSPs complex with antigenic peptides and gain access to the MHC class I and II antigen-presenting pathways in DCs via receptor-mediated uptake (2, 3), leading to CD8+ and CD4+ T-cell responses (4–14). Generating antitumor immune responses against tumor-associated self-antigens and mutated antigens requires endogenous nonmicrobial danger molecules that activate antigen-presenting cells (APCs). Besides their ability to chaperone antigenic peptides, HSPs may function as endogenous nonmicrobial danger molecules (1, 15, 16) that can up-regulate the expression of costimulatory and antigen-presenting molecules on DCs (2, 8, 17). They also stimulate the secretion of proinflammatory cytokines such as interleukin 12 (IL-12), leading to the activation of natural killer (NK) cells and other immune cells (2, 18–22).

Tumor-derived HSPs provide effective tumor vaccines in mouse models (1), and the ability of human melanoma-derived HSP70 to stimulate autologous melanoma-specific T cells also has been shown in vitro (23). Clinical trials of tumor-derived HSPs have been conducted in patients with a broad range of malignancies, including lymphoma, renal cell carcinoma, melanoma, colorectal cancer, gastric cancer, pancreatic cancer, and breast cancer (1). In a recent phase II clinical trial, vaccination of metastatic melanoma patients with autologous gp96 proteins induced clinical and tumor-specific T-cell responses in a substantial proportion of patients (24). There were no unacceptable side effects or autoimmune reactions associated with gp96 vaccination.

Because each tumor harbors a unique repertoire of mutated antigenic peptides, these HSP vaccines have been tailored to the properties of tumor cells in individual patients (1), but this approach has many limitations. For example, when HSP peptide vaccines represent a unique array of tumor antigens, it is difficult to establish a reproducible therapeutic standard. Moreover, the quantity of HSP used for tumor immunotherapy is strictly limited by the size of the surgically resected tumor mass, and some antigenic peptides are inevitably lost during HSP purification. Even if HSP vaccines could be produced, they could not be expected to destroy bulky tumors by themselves, thus limiting their utility.

We previously developed an HSP-mediated oncolytic tumor (“HOT”) vaccine by combining the versatile ability of HSPs to induce tumor-specific immune responses with the oncolytic activity of viruses (25), but enthusiasm for this strategy was tempered by concern regarding the safety of using replication-competent viruses in the clinic. Thus, we developed an in vivo HSP tumor vaccine by generating a recombinant replication-defective adenovirus (Ad-HT) that coexpresses HSP and a herpes simplex virus thymidine kinase suicide gene (HSV-TK). The combination of in situ HSP70 overexpression and tumor killing by thymidine kinase (TK)/ganciclovir (GCV) treatment after intratumor injection of Ad-HT, but not in situ HSP70 overexpression or tumor killing alone, provoked potent systemic antitumor immune responses. We also found that Ad-HT intratumor injection promoted tumor infiltration of DCs. The results described here suggest the broad therapeutic potential of this in vivo HSP/suicide vaccination approach against solid tumors.

MATERIALS AND METHODS

Cell Lines and Antibodies. The human tumor cell line Hep3B (hepatocellular carcinoma) was maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics. Murine mammary sarcoma tumor cells EMT6 were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 11.25 μg/mL l-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in a 5% CO2 atmosphere (26). Antibodies to human-inducible HSP70 were purchased from StressGen Biotechnologies, Inc. (Victoria, British Columbia, Canada). Dr. Sumers (Yale University, New Haven, CT) provided antibody to HSV-TK. Antimouse CD11c, CD8a, CD45RA (B220), and CD205 antibody conjugates and matched isotype controls were from BD PharMingen (San Diego, CA). Antimouse INF-γ antibodies were purchased from Mabtech, Inc. (Mariemont, OH).

Generation of Recombinant Replication-Defective Virus Ad-HT. An AdEasy system (E1 and E3 deletion; Quantum Biotechnologies Inc., Palo Alto, CA) was used to generate replication-defective adenovirus (Ad-HT) expressing HSP70. The HSP70 expression cassette was generated by PCR and subcloned into the HindIII and EcoRI sites of the helper-free replication-defective adenoviral shuttle vector pAd/ΔE1/ΔE3/H11545 using standard cloning methods. A construct expressing HSP70 under control of the human β-actin promoter and a herpes simplex virus thymidine kinase gene was also generated. The ΔE1/ΔE3 adenoviral genome was generated by linearization of the complete genome with HindIII and self-ligation, and the ΔE1/ΔE3 genome was packaged into adenovirus particles using the AdEasy system. Recombinant adenovirus was generated by infection of 293 cells with the ΔE1/ΔE3 adenovirus particles and packaging of the ΔE1/ΔE3 genome. The titre of Ad-HT is determined by cytopathic effect on Hep3B cells 2 days post infection.
CA) was used to construct and generate replication-defective adenoviruses. The HSV-TK gene was generated by PCR using the Ad-TK vector (27) as a template with a pair of primers (5'-ACTGCTAGCATGCGCTTCTGGTCGCTAC- 
CCTGC-3' and 5'-ACTGCTAGCATGCGCTTCTGGTCGCTAC-
CCTGC-3'). The resulting HSV-TK fragment contained an NheI restriction site at the 5'-end and XhoI at the 3'-end. The shuttle vector Ad-HT was constructed by inserting NheI/XhoI-digested HSV-TK into an NheI/XhoI-digested Ad-HE virus, replacing the E1A fragment. The recombinant replication-competent Ad-HE virus containing the human HSP70 and E1A linked with an encephalomyocarditis virus IRES sequence under control of the CMV promoter was constructed as described previously. PCR and DNA sequencing confirmed the insertion of HSP70 and HSV-TK. The recombinant adenovirus Ad-HT, which harbors the HSP70 and HSV-TK expression cassette under transcription control of the human CMVIE promoter in the E1 region, subsequently was generated according to the manufacturer’s instructions. The replication-defective virus, Ad-H, expressing human-inducible HSP70, was generated earlier (25). PCR and DNA sequencing confirmed the insertion of HSP70 and HSV-TK. The recombinant adenovirus Ad-HT, which harbors the HSP70 and HSV-TK expression cassette under transcription control of the human CMVIE promoter in the E1 region, subsequently was generated according to the manufacturer’s instructions. The replication-defective virus, Ad-H, expressing human-inducible HSP70, was generated earlier. Dr. Alan Davis (Baylor College of Medicine, Houston, TX) provided a replication-defective Ad-TK, HSV-TK expression vector. Recombinant adenoviruses were produced and titrated in 293 cells according to the manufacturer’s instructions (Quantum Biotechnologies Inc., Palo Alto, CA).

**MTT Assay.** Tumor cells were infected in triplicate with adenovirus at multiplicity of infection (MOI) of 0, 1, 10, 100, and 1000 in a 96-well plate. Serum-free infection medium was replaced with complete culture medium containing 10% fetal bovine serum after 2 hours of infection. At 24 hours after infection, all of the wells were exposed to GCV at 30 μg/mL. Viability of the cell was quantitated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Roche Laboratories, Inc., Nutley, NJ), at day 5 after treatment with GCV.

**Animal Study.** Five- to 6-week-old female BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME), housed in a specific pathogen-free facility at Baylor College of Medicine, and treated according to institutional guidelines. EMT6 cells (5 × 10^5) were injected subcutaneously into the right flank of syngeneic BALB/c mice to establish a tumor model. Palpable tumors started to develop after 4 days, and their volumes were measured twice a week with a caliper until the experiment was completed. Tumor volume (mm^3) was calculated by the formula: length × width × 0.52. When tumor sizes reached 60 to 70 mm^3, all of the mice were randomly divided into groups of mice and injected with 50 μL of Ad-HT, Ad-HSP, or Ad-TK virus (endotoxin-free; Sigma, St. Louis, MO) or PBS control via intratumor injection. GCV (Roche) (50 mg/kg) dissolved in 0.5 mL PBS (endotoxin-free) was administered intraperitoneally twice a day for 5 consecutive days, beginning at 48 hours after injection of virus. All of the tumors were resected when tumor diameters in the PBS group reached 10 mm. In the tumor challenge experiment, animals were inoculated with 1 × 10^6 EMT6 cells on the contralateral flank 5 days after the viral treatment.

To examine the effect of intratumor injection on the growth of lung metastasis, we injected mice with 5 × 10^5 EMT6 cells via the tail vein 2 weeks after viral intratumor injection. Lungs were harvested 2 weeks later and fixed in Bouin’s solution. The resultant tissues were cut into 5-μm sections representing the front, middle, and back portions of the lung; each consecutive section was separated from the next by 500 μm. Sections were stained with hematoxylin and eosin, and lung metastases were quantified by counting the number of nodules in the lung sections.

**CTL and Natural Killer Assay.** At different times after intratumor injection of virus, mice in each treatment group were sacrificed. The splenocytes isolated from each group were assessed for the activity of NK cells or stimulated with EMT6 cell lysate (per 10^5 splenocytes stimulated with 10^5 cell lysate) in the presence of 20 units/mL of human IL-2 for 7 days to assess the activity of CTLs. Tumor cell lysate was prepared by five freeze-thaw cycles with 5 × 10^6 tumor cells resuspended in 2 mL of serum-free DM medium. The cells were sonicated for 10 minutes and then centrifuged at 15,000 × g for 30 minutes (4°C). Supernatant fluids were recovered, divided into aliquots, and stored at −80°C until further use (28). The cytotoxic activity of the effector cells was determined in a standard 4-hour 51Cr-release assay (25). To measure NK cell activity, splenocytes from treated mice were tested for cytotoxic activity against 51Cr-labeled YAC-1 cells in the presence of 20 units/mL of human IL-2.

**INF-γ Enzyme-Linked Immunospot Assay.** The enzyme-linked immunospot assay was carried out as described previously (25, 29). CD4^+ and CD8^+ T cells were isolated from splenocytes by using MACS CD4 (LJT4) or MACS CD8 (Ly-2) microbeads (Miltenyi Biotec, Auburn, CA).

**In vitro Priming of T Cells by Dendritic Cells.** CD11c^+ DCs from the splenocytes of tumor-bearing BALB/c mice treated with recombinant adenoviruses were purified with CD11c^+ microbeads (MiniMacs; Miltenyi Biotec) and used for subsequent experiments. Splenocytes (2.5 × 10^6 cells/mL) from naive BALB/c mice were cocultured with DCs (2.5 × 10^5 cells/mL) isolated from the spleens of treated mice in complete RPMI 1640 medium in six-well plates for 1 week with human IL-2 (20 units/mL) and then tested for CTL activity.

**Statistical Analysis.** Student’s t test was used to calculate the significance of statistical comparisons. The overall significance level was set at 5%. Results are presented as mean ± SE.

**RESULTS**

**Generation and Characterization of a Replication-Defective Adenovirus Coexpressing HSP70 and HSV-TK (Ad-HT).** An adenovirus AdEasy vector system was used to generate the recombinant replication-defective adenovirus (Ad-HT) that coexpresses human-inducible HSP70 and HSV-TK (Fig. 1A). Restriction enzyme digestion and DNA sequencing confirmed the insertion of HSP70 and HSV-TK genes in Ad-HT. The replication-defective virus Ad-HSP, which expresses human HSP70 only, and the replication-defective virus Ad-TK, which expresses HSV-TK only, were generated previously (27, 31). All of the recombinant viruses were produced in 293A cells, purified with CsCl gradients, and titrated on 293A cells with a commercial kit (BD Biosciences).

We first tested the ability of the recombinant adenovirus Ad-HT to...
enhance HSP70 expression because adenovirus infection alone also can increase HSP70 expression via responses to cellular stress. Mouse tumor cells (EMT6) that are susceptible to human adenovirus infection (data not shown) were seeded on 12-well plates (1 × 10^5 cells per well) in triplicate and were infected with Ad-HT, Ad-HSP, or Ad-TK at MOI 1 or mock infected for 2 hours and then replaced with fresh medium. At 48 hours after infection, the cell lysates were subjected to ELISA (HSP70 kits; StressGen Inc.). The results are presented as means of triplicate determinations from one of two experiments. High levels of HSP70 protein (mean, 106.2 or 99.2 ng/10^5 cells) were expressed in Ad-HT– or Ad-HSP–infected cells, whereas only low levels (mean, 14 or 8.16 ng/10^5 cells) were expressed in Ad-TK– or mock-infected cells, representing a 7.5-fold increase of HSP70 expression in Ad-HT– compared with Ad-TK–infected cells. We also tested whether Ad-HT retained TK-mediated cytotoxicity. Murine tumor cells were infected in triplicate with Ad-HT or control adenoviruses at a range of MOIs. Five days after virus infection in the presence of GCV (30 μg/mL), the percentage of viable cells in each well was determined by an MTT assay. At MOIs ranging from 10 to 1000, the cell death rates associated with Ad-HT and Ad-TK were
Intratumor Injection of Ad-HT Eradicates Primary Tumor and Prevents the Growth of Secondary Tumor. We next examined the ability of Ad-HT to inhibit local tumor growth in vivo. Four groups of BALB/c mice bearing syngeneic EMT6 murine mammary sarcoma tumors were intratumorally injected with Ad-HT, Ad-HSP, Ad-TK \(5 \times 10^8\) plaque-forming units (pfu/mouse), or PBS (26). After Ad injections on two consecutive days, all of the mice received GCV at 50 mg/kg twice a day for 5 days. As shown in Fig. 2A, the mice treated with Ad-HSP or Ad-TK had only a temporary delay in tumor growth, whereas Ad-HT uniformly eradicated tumors \((P < 0.01)\). Because Ad-HT and Ad-TK showed comparable cytotoxic activity in vitro, whereas Ad-HSP lacked apparent cytotoxicity, we attribute the enhanced ability of Ad-HT to eradicate primary tumors and the tumor inhibitory effect of Ad-HSP in the immunocompetent mice to HSP-mediated antitumor immune responses.

To test our hypothesis that intratumoral injection of Ad-HT coexpressing HSP70 and HSV-TK can induce a systemic antitumor response, we examined the susceptibility of mice to tumor rechallenge after treatment with Ad-HT or other adenoviruses. EMT6-bearing mice from different groups were intratumorally injected with Ad-HT, Ad-HSP, Ad-TK \(5 \times 10^8\) pfu/mouse, or PBS, followed by GCV treatment. All of the mice then were rechallenged with EMT6 cells on the contralateral side 5 days after the viral injection. The mice treated with Ad-HT were completely resistant to rechallenge with EMT6 cells, whereas those treated with Ad-HSP or Ad-TK were largely susceptible to such rechallenge (Fig. 2B). The resistance of the mice treated with Ad-HT was EMT6 specific because they were susceptible to rechallenge with a different murine mammary tumor (4T1) of the same genetic background (data not shown). Ad-HT-treated mice also remained resistant to EMT6 cells 2 months later, attesting to the durability of the antitumor response. We further used a lung metastasis model to test whether intratumor injection of Ad-HT could prevent the growth of metastatic tumors. Mice bearing EMT6 tumors were treated intratumorally with different Ad vectors twice and challenged with \(5 \times 10^4\) EMT6 cells via tail vein injection 2 weeks after the intratumor injection of adenoviruses. As shown in Fig. 3, none of the mice treated with Ad-HT developed lung metastases in contrast to large numbers of lung lesions in mice treated with Ad-HSP or Ad-TK. Collectively, these results indicate that intratumor injection of Ad-HT induces a more potent systemic antitumor activity than either Ad-HSP or Ad-TK treatment.

Both In situ HSP Overexpression and Cytotoxicity Are Required to Induce Potent Tumor-Specific T-Cell Responses. To determine whether a systemic antitumor immune response is induced by intratumor injection of Ad-HT, we assessed the T-cell responses in mice treated with different Ad vectors using enzyme-linked immunospot assays. As shown in Fig. 4A, the splenocytes from mice treated with Ad-HT responded vigorously to EMT6 lysate-pulsed DCs, whereas the responses from mice treated with Ad-HSP or Ad-TK were significantly weaker \((P < 0.01)\). To analyze the contribution of CD4\(^+\) and CD8\(^+\) T lymphocytes to the observed responses, we isolated CD4\(^+\) T cells and CD8\(^+\) T cells from the spleens of immu-

![Fig. 4. Enzyme-linked immunospot assays of tumor-specific CD4\(^+\) and CD8\(^+\) T cells induced by Ad-HT. Splenocytes from BALB/c mice bearing EMT6 tumors treated with Ad-HT, Ad-TK, Ad-HSP, or PBS once a day for two consecutive days were harvested and pooled 10 days after the second intratumor injection. The number of IFN-γ-producing T-cell precursors in the splenocyte population \((A)\), isolated CD4\(^+\) T cells \((B)\), or CD8\(^+\) T cells \((C)\) was determined with the enzyme-linked immunospot assay (200,000 cells/well). Results are the mean numbers of spots (±SE) observed after stimulation with EMT6 lysate-pulsed BALB/c mouse bone marrow-derived DCs \((P < 0.01, \text{Ad-HT versus Ad-HSP, Ad-TK, or PBS group})\). The numbers of IFN-γ CD4\(^+\) or CD8\(^+\) T cells from Ad-HT–treated mice stimulated with DCs pulsed with EMT6 lysate or irrelevant tumor lysate (CT26) also are shown \((P < 0.01)\) \((D)\). Data are representative of three independent experiments.](https://cancerres.aacrjournals.org/content/66/15/6648)
nized mice using the anti-CD4 or -CD8 microbeads (Miltenyi Biotec). When stimulated with EMT6 lysate-pulsed DCs, significantly more CD4+ and CD8+ T cells from the Ad-HT treatment group secreted IFN-γ compared with results for the remaining groups (Fig. 4B and C; P < 0.01 for all of the comparisons between Ad-HT and other groups). The CD4+ and CD8+ T-cell responses were tumor specific because the CD4+ and CD8+ T cells from the Ad-HT–treated mice responded to EMT6 lysate-pulsed DCs but not to DCs pulsed with the cell lysate from an irrelevant murine tumor (CT26; Fig. 4D).

We further examined the CTL activity in mice treated with Ad-HT. Splenocytes from Ad-HT mice showed significantly higher CTL activity against the target EMT6 tumor cells than did splenocytes from the Ad-HSP−, Ad-TK−, or PBS-treated mice (Fig. 5A). Splenocytes from the Ad-HT mice lacked any apparent CTL activity against irrelevant murine tumor cells, including JC and CT26 (Fig. 5B), indicating that the CTL activity was tumor specific. Antibody blocking assays showed that T lymphocytes were the major effector cells responsible for tumor cell killing (Fig. 5C). We also tested the NK cell status of treated mice by chromium release assay. The data showed that NK cells from Ad-HT–treated mice performed a significantly higher YAC-1 cell killing efficiency (Fig. 5D). Collectively, these results indicate that the combination of HSP70 overexpression in situ and tumor killing by TK/GCV treatment postinjection of Ad-HT, but not either mechanism alone, can elicit potent tumor-specific CD8+ CTL, CD4+ T-helper, and NK cell responses.

DCs Isolated from Ad-HT–Treated Mice Prime Tumor-Specific CTLs. It generally is believed that mature antigen-presenting DCs migrate into lymphoid organs where they prime the responses of T cells and their precursors. Although DCs were present in the tumors of all of the groups, we reasoned that tumor-infiltrating DCs from different Ad-treated mice might differ in their ability to take up tumor antigens, migrate into lymphoid organs, and prime a tumor-specific CTL response. Thus, we isolated CD11c+ DCs from the spleens of mice treated with different Ad vectors and cocultured them with splenocytes from naive mice in vitro for 1 week to prime T-cell responses (30). After in vitro priming, the splenocytes were used to test CTL activity against target tumor cells. As shown in Fig. 6, the T cells primed by DCs isolated from Ad-HT–treated EMT6-bearing mice were active in killing EMT6 target cells, whereas those primed by DCs isolated from mice treated with other Ad vectors were significantly less active. The EMT6 tumor specificity of the CTL activity primed by DCs from Ad-HT–treated mice was shown by the inability of the primed T cells to kill irrelevant 4T1 tumor cells (data not shown). Thus, the combination of HSP overexpression in situ and TK/GCV-mediated cytotoxicity enhances DC antigen presentation and/or homing to lymphoid organs.

DISCUSSION

Tumor cell populations represent clonal expansions of cells bearing largely random genetic mutations (~11,000 mutations per tumor cell)
and shared antigens (32). Antigenic peptide repertoires derived from mutated antigens and shared antigens (33, 34) could potentially induce specific immunity against tumor cells. However, the host immune system largely ignores or tolerates these tumor antigens, most likely because of inadequate antigen presentation by professional APCs such as DCs (35). In this study, we show that the in situ HSP-suicide gene tumor vaccine can effectively induce systemic tumor-specific immune responses that control the growth of primary and metastatic tumors in immunocompetent mice, suggesting that such a vaccine may have broad therapeutic potential for the management of human solid tumors.

Transfection of tumor cells with HSP expression vectors to overexpress HSPs has been used before to induce tumor-specific immune responses (36, 37). Intratumor injection of HSP expression vectors or enhancement of endogenous HSP expression has been reported to induce antigen-specific immune responses (38–40). Suicide gene therapy as exemplified by HSV-TK/GCV treatment also has been investigated in cancer patients. The results of clinical trials indicate that this therapy is safe and produces local tumor killing via direct and bystander effects (41–44). However, suicide gene therapy has not resulted in either eradication of local tumor or control of metastasis. In this study we found that in situ overexpression of HSP70 or TK/GCV-mediated tumor killing alone was insufficient to provoke potent antigen immune responses. Our data indicate that the combination of TK/ GVC-mediated cytotoxicity that facilitates tumor antigen releases and in situ HSP overexpression that promotes DC antigen presentation are required to break the body’s tolerance of self or mutated tumor antigens, leading to the induction of potent tumor-specific immune responses.

Injection of tumors with recombinant viruses expressing various cytokines, including IL-12, granulocyte macrophage colony-stimulating factor, or other immune-enhancing molecules, such as B-7, has been exploited to induce tumor-specific immune responses (45–47). The HSP/suicide gene tumor vaccine we describe is aimed at enhancing DC antigen presentation and maturation, a key step in provoking antigen immune responses, by combining the cytolytic activity of suicide molecules and the ability of overexpressed HSPs to promote DC maturation and antigenic presentation. Because our strategy uses HSP-mediated antigen presentation to induce tumor-specific immune responses, we predict that it would readily complement other previously described approaches and may even produce synergistic effect overall (45–47). In summary, the results of this study indicate that the combination of tumor killing by activation of a suicide gene to release tumor antigens and in situ HSP70 overexpression to enhance DC antigen presentation overcomes host immune tolerance to tumor antigens, leading to the induction of potent antitumor immunity. Our findings may have broad relevance to the use of the in vivo HSP/suicide gene tumor vaccine in therapy for human solid tumors.

ACKNOWLEDGMENTS

We thank M. Brenner and colleagues at Baylor’s Center for Cell and Gene Therapy for helpful suggestions. We also thank Lisa Rollins, Xiao-Tong Song, and Natasha Lapteva for technical assistance and helpful suggestions.

REFERENCES


6650
Potent Tumor-Specific Immunity Induced by an *In vivo* Heat Shock Protein-Suicide Gene–Based Tumor Vaccine

Wenhong Ren, Randy Strube, Xiaoqing Zhang, et al.

*Cancer Res* 2004;64:6645-6651.

**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/18/6645

**Cited articles**
This article cites 46 articles, 28 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/18/6645.full#ref-list-1

**Citing articles**
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/64/18/6645.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.