Cytolytic Activity of the Human Papillomavirus Type 16 E7_{11-20} Epitope-Specific Cytotoxic T Lymphocyte Is Enhanced by Heat Shock Protein 110 in HLA-A*0201 Transgenic Mice

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Heat shock proteins (HSPs) have been successfully applied to a broad range of vaccines as biological adjuvants to enhance the immune response. The recently defined HSP110, in particular, exhibits strong protein binding affinity and is capable of enhancing the immunogenicity of protein antigens remarkably more than other HSP family members. In our previous study, we verified that murine HSP110 (mHSP110) significantly enhanced the immune response of a C57BL/6 mouse model to the H-2d-restricted human papillomavirus (HPV) E7_{49-57} epitope (short peptide spanning the 49th to 57th amino acid residues in the E7 protein). To determine whether HSP110 similarly enhances the immunogenicity of human epitope peptides, we used the HLA-A2 transgenic mouse model to investigate the efficacy of the mHSP110 chaperone molecule as an immunoadjuvant of the human HLA-A2-restricted HPV16 E7_{11-20} epitope vaccine. Results showed that mHSP110 efficiently formed a noncovalently bound complex with the E7_{11-20} epitope. The mHSP110-E7_{11-20} complex induced epitope-specific splenocyte proliferation and E7_{11-20}-specific gamma interferon (IFN-γ) secretion. Importantly, cytotoxic T lymphocytes primed by the mHSP110-E7_{11-20} complex exerted strong cytolytic effects on target T2 cells pulsed with the E7_{11-20} peptide or TC-1 cells transfected with the HLA-A2 gene. In addition, the mHSP110-E7_{11-20} complex elicited stronger ex vivo and in vivo antitumor responses than either emulsified complete Freund’s adjuvant or HSP70-chaperoned E7_{11-20} peptide. These collective data suggest that HSP110 is a promising immunomodulator candidate for peptide-based human cancer vaccines, such as for the HLA-A2-restricted E7_{11-20} epitope.

Cervical cancer (CaCx) is the second leading cause of cancer deaths among women worldwide. CaCx is strongly associated with human papillomavirus (HPV) infection, particularly HPV types 16 and 18. Several clinical studies have demonstrated constitutive expression of the HPV16 oncoproteins E6 and E7 in the majority of cervical tumor cells (1–4). Since cervical tumors often recur after surgery and/or radiotherapy (5, 6), it is critical to develop prophylactic and therapeutic strategies that completely eliminate the tumor cells. HPV16 cytotoxic T lymphocyte (CTL) epitopes may be good candidates for the development of an effective peptide-based anticancer vaccine (7–9). In fact, studies in animal models have already shown that HPV16-specific CTLs can safely eradicate HPV16-transformed tumor cells in vivo (10).

To date, many therapeutic vaccine strategies have been developed targeting the HPV16 E6/E7 proteins (11–14). Among these, the synthetic peptide-based strategies have the particularly remarkable advantages of convenient and low-cost high-throughput preparation and rare occurrences of adverse reactions, including general toxicity, immunosuppression, and autoimmunity (15–17). More importantly, when generating specific CTLs it is possible to select the length of the amino acid sequence that is most suitable for the particular application. However, peptide-based vaccines are characterized by weak immunogenicity when used alone in vivo (18). To overcome this limitation, it is critical to identify adjuvant vehicles that can enhance the immunogenicity of peptides but are not specifically immunogenic themselves. A large number of immune adjuvants have been investigated for this purpose, including aluminum hydroxide, liposomes, cytokines (such as granulocyte-macrophage colony-stimulating factor [GM-CSF] and interleukin 2 [IL-2]), saponins, Toll-like receptor (TLR) agonists, and various heat shock proteins (HSPs) (19–21). Among these, the HSPs have shown especially promising results and are being held up as tools with which to develop effective and safer CaCx vaccines.

It has long been established that HSPs, particularly HSP70, can function as effective immunoadjuvants by virtue of their ability to bind tumor-specific peptides. However, recent research has indicated that a larger HSP, HSP110, is better suited for use in recombinant vaccines (22, 23). HSP110 is a major HSP of eukaryotic and mammalian cells in general. With a molecular mass of 110 kDa, HSP110 is composed of four functionally coupled domains, including the N-terminal domain that mediates ATP binding and the peptide-binding domain (amino acid residues 394 to 509) (24). Several studies have suggested that HSP110 can efficiently bind large proteins and peptides under heat shock conditions and that it produces excellent adjuvant effects (25–28).

The mouse H2-D b-restricted CTL epitope, E7_{49-57} (short...
peptide spanning the 49th to 57th amino acid residues in the E7 protein; RAHYNVYTF), was previously shown to form a noncovalent binding complex with HSP110 at a 1/50 molar ratio and to elicit antitumor immunity in C57BL/6 mice (29). Moreover, the epitope E7_{11-20} is not only a well-known HLA-A*0201-restricted human CTL epitope of the HPV16 E7 protein that shows high-affinity binding to HLA-A2 in vitro (8, 30, 31). In addition, its ability to induce peptide-specific CTLs has been verified in HLA-A2 transgenic mice (32). HLA-A2 is the most frequent HLA-A specificity in the human population, with an allele frequency of 10 to 40% in different ethnic groups (33). Specifically, about 50.7% of random individuals in the population in China carry HLA-A2, and HLA-A*0201 ranks first in frequency (allele frequency, 15.5%) (34). Thus, peptide vaccines based on this haplotype may have a particularly potent impact on the global HPV threat.

In the study presented herein, we investigated whether the CTL response induced by epitope E7_{11-20} can be enhanced by an HSP110 chaperone molecule. We found that murine HSP110 (mHSP110) can combine with E7_{11-20} under heat shock conditions. Application of this novel strategy in an HLA-A*0201 transgenic mouse model validated that the mHSP110-E7_{11-20} complex induces functional cytotoxic T cells in a peptide-specific manner and does so more efficiently than the complete Freund’s adjuvant (CFA)-emulsified peptide E7_{11-20} or HSP70 alone.

**MATERIALS AND METHODS**

**Mice, plasmids, and cell lines.** HLA-A*0201 transgenic mice (females, 4 to 8 weeks old) expressing HLA-A2.1 on a C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, ME) (35). All mice were housed in a pathogen-free environment and all procedures involving the animals were carried out with approval from the local ethics committee of the Third Military Medical University. TC-1 tumor cells, derived from primary pulmonary epithelial cells of C57BL/6 mice cotransformed with HPV16 E6 and E7 and c-Ha-ras oncogenes (36), were purchased from the Cell Center at the CAMS in Shanghai, China. The eukaryotic expression plasmid HLA-A2/Kk expressing the α1 and α2 domains of HLA-A*0201 and the α3 domain of H-2Kk (plasmid number 14906; Addgene, Cambridge, MA) was transfected into the TC-1 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and maintained in complete RPMI 1640 medium (Gibco, Invitrogen) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT), 2 mM l-glutamine (Gibco), 50 mM 2-mercaptoethanol (Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco) at 37°C in a humidified atmosphere of 5% CO₂ in air (37). To establish stable A2Kk-expressing TC-1/A2Kb cells, the cells were selected by addition of 500 μg/ml G418 (Sangon, Shanghai, China) to the complete RPMI 1640 medium for about 3 weeks until the control cells were dead. Tc cells, an HLA-A2-positive and antigen-processing-defective cell line, were obtained from the American Type Culture Collection (Manassas, VA) and maintained as previously described (35).

**Synthetic peptides.** The HPV E7_{11-20} peptide epitope (YMLDLQPETT) was synthesized with a free carboxy terminus by using solid-phase strategies on an automated multiple peptide synthesizer (Shenzhen Hanyu Pharmaceutical Corp., Shenzhen, China) with 9-fluorenylmethoxy carbonyl (Fmoc) chemistry. The purity of all synthesized peptides was >90% as detected by reverse-phase high-performance liquid chromatography (HPLC). Synthesized peptides were dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C. Fluorescein isothiocyanate (FITC)-labeled E7_{11-20} peptides (FITC conjugation at the N terminus; 90% pure) were synthesized (Shenzhen Hanyu Pharmaceutical Corp.) and dissolved in phosphate-buffered saline (PBS) with 1% DMSO and stored at −20°C.

**Expression, purification, and identification of recombinant proteins.** mHSP110 cDNA was cloned into the pQE-80L vector (Qiagen, Hilden, Germany) and transformed into recombinant Escherichia coli M15 and cultured in Luria broth. After induction by addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), the mHSP protein with 6×His tags was purified from the culture supernatant using an Ni-nitrilotriacetic acid (Ni-NTA) column, which was washed extensively with 1% Triton X-100/0.6 M guandine-HCl to remove endotoxins, as described previously (29). The lipopolysaccharide (LPS) in the final protein solution was undetectable by a Limulus test. The prepared mHSP110 and commercial source mHSP70 (LPS free; Cusabio, Wuhan, China) were stored at −70°C. Concentrations of the recombinant proteins were measured by Bradford assay. Protein purity was assessed with an SDS-PAGE assay with Coomasie blue staining. Western blot analysis was used to confirm the identity of the purified recombinant protein with rabbit anti-HSP110 or anti-mHSP70 polyclonal antibody (Stressgen, Ann Arbor, MI) as the primary antibody, and a chemiluminescence kit (Roche Diagnostics Limited, Shanghai, China) was used to detect the target protein band, followed by exposure to X-ray film.

**Reconstitution and potential of HSP110-HPV E7_{11-20} Peptide complex.** The noncovalent complex of mHSP110-E7_{11-20} was generated according to the previously published protocol for binding mHSP110 with peptide E7_{11-20} (29). Briefly, mHSP110 and E7_{11-20} or FITC-labeled E7_{11-20} peptide were mixed at a 1:50 molar ratio in PBS supplemented with 1 mM ATP and 1 mM MgCl₂. After 30 min of incubation at 50°C, the samples were cooled to room temperature for 1 h. Free peptides were cleared from the sample by a size exclusion filtration method using a Microcon 50 filter (Millipore, Billerica, MA) with a molecular mass cutoff of 50 kDa. The remaining bound complexes of E7_{11-20}/mHSP110-FITC were evaluated by native PAGE (NuPAGE Novex Tris-acetate minigels; Invitrogen), which was carried out in a darkroom. The gel was observed and photographed on a fluorescence imager and then stained with Coomassie brilliant blue R250. The PageRuler prestained protein ladder (Fermentas, Vilnius, Lithuania) was used to determine protein and complex sizes.

**Immunization, cell proliferation, and culturing.** Mice were divided into groups of five and administered one of the following immunogens via the intraperitoneal (i.p.) route: mHSP110-E7_{11-20} complex (100 μg mHSP110 plus 50 μg E7_{11-20}), mHSP70-E7_{11-20} complex (100 μg mHSP70 plus 50 μg E7_{11-20}), 100 μg of mHSP110 alone, 100 μg of mHSP70 alone, 50 μg of E7_{11-20} alone, or 100 μl PBS. All mice were immunized twice, with the initial immunization occurring on day 0 and the booster given 14 days later. Another group of five mice were immunized subcutaneously (s.c.) with 50 μg of E7_{11-20} together with CFA and then boosted with 50 μg of E7_{11-20} together with incomplete Freund’s adjuvant (Sigma-Aldrich, St. Louis, MO).

All immunizing agents were dissolved in PBS to achieve a final 100-μl volume. For all immunization groups, the mice were euthanized 2 weeks after the second vaccination. The spleens were aseptically removed and made into single-cell splenocyte suspensions. Red blood cells were removed by treating the suspension with red blood cell lysis buffer (Roche Diagnostics Limited). The remaining splenocytes were then washed in PBS containing 5% FBS and cultured at 3 × 10⁷/ml in 24-well culture plates with complete T-cell medium (RPMI 1640 containing 10% FBS, 2 mM l-glutamine, penicillin [100 U/ml], streptomycin [100 μg/ml], and 5 × 10⁻⁵ M 2-mercaptoethanol) at 37°C in 5% CO₂. The cultured cells were then subjected to various assays, including lymphocyte proliferation, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunosorbent spot (ELISPOT), and cytotoxicity.

**Splenocyte proliferation assay.** Splenocytes from the various groups of immunized mice were cultured (2 × 10⁴ cells/well, 100 μl) in triplicate in 96-well culture plates in the presence of the E7_{11-20} peptide for 5 days at 37°C in 5% CO₂. The number of viable cells was then determined using a 3-(4,5-dimethyl-2-thiazoly)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test kit (Sigma-Aldrich).

**Cytotoxicity assay.** Splenocytes (1 × 10⁷ in RPMI 1640 containing 10% FBS) from the various groups of immunized mice were stimulated in

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vivo with E7\textsubscript{11-20} (10 \mu g/ml) and murine interleukin 2 (mIL-2) (10 U/ml) (R&D Systems, Minneapolis, MN). At day 5 of culture, the viable splenocytes were used as effector cells. Meanwhile, T\textsubscript{2} cells were preincubated with peptide E7\textsubscript{11-20} (10 \mu g/ml) for 4 h, washed in RPMI 1640 containing 5% FBS, and then used as the target cells of the stimulated splenocytes. T\textsubscript{2} cells alone were used as the target cell control. Furthermore, to test whether the T cells from mice that were immunized with immunogens recognize the E7\textsubscript{11-20} epitope naturally processed from E7 protein, the TC-1/A2Kb and TC-1 cells were applied as the target cells.

The CTL activity of the effector cells was measured using a nonradioactive cytotoxicity kit (Promega, Madison, WI) as previously described \cite{28}. Briefly, the stimulated splenocytes were cocultured with 5 \times 10^4 target cells at different effector/target cell (E/T) ratios of 50:1, 25:1, and 12.5:1 in 96-well V-bottom culture plates at 37°C in 5% CO\textsubscript{2}. After 4 h of incubation, the supernatants (50 \mu l/well) were removed and the substrate mix (50 \mu l/well) was added. The plates were incubated in the dark for 30 min at room temperature, after which the reaction was terminated by adding 50 \mu l of stop solution (1 M acetic acid). Sample absorbance at a 490-nm wavelength was measured by an ELISA plate reader (Bio-Rad, Hercules, CA).

Spontaneous release of lactate dehydrogenase (LDH) by the target or effector cells was assessed by incubating the target cells in the absence of effector cells and vice versa. The maximum release of LDH was determined by incubating target cells in 1% Triton X-100 in assay medium. The specific lysis percentage was calculated as ([experimental release – effector spontaneous release]/target spontaneous release) \times 100. All experiments were repeated three times.

ELISA. Levels of gamma interferon (IFN-\gamma) in cell culture supernatants were determined by using a mouse IFN-\gamma precoated ELISA kit (R&D Systems) according to the manufacturer’s instructions. Briefly, the splenocyte supernatants (100 \mu l/well) were harvested on day 7 of culture and were added to the precoated wells. After incubation at room temperature for 2 h and washing 4 to 6 times, biotinylated antibody (100 \mu l/well) was added and the plates were incubated for 1 h at 37°C. After an additional 15-min incubation with enzyme conjugate at 37°C, the reaction was stopped by adding 100 \mu l of stop solution and the optical density was read at 450 nm. Three independent experiments were carried out for each sample.

ELISPOT assay. Antigen-specific IFN-\gamma-secreting T cells were detected by a mouse IFN-\gamma precoated ELISPOT kit (Dakewe, Shenzhen, China) according to the manufacturer’s protocol. Briefly, the precoated plate was activated by adding Lympho-Spot serum-free medium. Splenocytes (5 \times 10^5 cells/well, 100 \mu l) were added in triplicate wells and incubated in the presence of either E7\textsubscript{11-20} (10 \mu g/ml) or HSP110 (10 \mu g/ml) at 37°C in 5% CO\textsubscript{2} for 36 h. Positive control wells (1 \times 10^5 cells/well) were established with phytohemagglutinin alone (20 \mu g/ml). Wells containing only Lympho-Spot serum-free medium were established to measure the background signal. Then, ddH\textsubscript{2}O (100 \mu l/ml) was added to the wells and the plates were incubated for 10 min at 4°C. After extensive washing (5 to 7 times), biotinylated IFN-\gamma antibody (Ab) (100 \mu l/well) was added and the plates were incubated for 1 h at 37°C. After five more washes, streptavidin horseradish peroxidase (HRP) (100 \mu l/well) was added and the plates were incubated for 1 h at room temperature. After a final five washes, the immunoreactive spots were developed by incubating with aminothiol carbazole (AEC) chromogenic solution (100 \mu l/well) for 25 min at room temperature. The spots were counted using a dissecting microscope.

Tumor challenge experiments. To evaluate the therapeutic antitumor effects of the various immunogens, tumors were established by s.c. injection of E7\textsubscript{11-20} into the left flank of mice (day 0). On day 5, mice with obvious tumor development were assigned arbitrarily into 8 groups of 10. Mice were then i.p. injected with different immunization formulations (150 \mu l total volume in PBS) on days 5, 9, and 14. Tumor volume was recorded twice a week by measuring perpendicular tumor diameters with a caliper and calculating (the shortest diameter \times \sqrt{2} \times \text{the longest diameter})/2. Survival of mice was recorded daily for each group.

Results

Noncovalent binding of mHSPs to E7\textsubscript{11-20} under heat shock conditions. Recombinant mHSP110 was prepared as described in our previous study \cite{29}. The recombinant mHSP110 protein preparation yielded high purity, as shown in the SDS-PAGE assay (Fig. 1, lanes 1 to 3). The purified mHSP110 and the commercial source mHSP70 were further confirmed by Western blotting (Fig. 1, lanes 4 and 6). Prior studies in our laboratory indicated that mHSP110 efficiently binds the HPV16 epitope E7\textsubscript{49-57} (RAHYNIVTF) at heat shock temperature \cite{29}. Herein, to determine whether the mHSP110 and mHSP70 can bind the E7\textsubscript{11-20} peptide, the FITC-labeled E7\textsubscript{11-20} peptides were incubated with mHSP110 or mHSP70 in the binding buffer under heat shock conditions, and the complex was then subjected to 10% nondenaturing PAGE analysis and imaged under a fluorescence imager. As shown in lanes 5 and 7 of Fig. 1, an FITC signal was detected at the position corresponding to the mHSP110 or mHSP70 protein in the gel, indicating that mHSP110 and mHSP70 could complex with the FITC-E7\textsubscript{11-20} peptides. However, when the complex was run on an SDS-PAGE gel, no fluorescent signal was observed at the position corresponding to the mHSP110 band (data not shown), which indicated noncovalent binding of mHSP110 with the peptide E7\textsubscript{11-20}.

mHSPs-E7\textsubscript{11-20} complexes promoted antigen-specific splenocyte proliferation. After HLA-A*0201 transgenic mice were immunized with the various immunogens, their splenocytes were harvested and restimulated in vitro with the E7\textsubscript{11-20} peptides for 5 days. MTT assay showed that the mice immunized with the mHSP110-E7\textsubscript{11-20} complex exhibited the strongest lymphocyte
proliferation compared with all other groups (Fig. 2). The mHSP70-E711-20 complex and the CFA-emulsified E711-20 peptide primed similar lymphocyte proliferation levels, both of which were markedly higher than those observed in the E711-20, mHSP110, and mHSP70 groups. In contrast, the spleen cells from mice immunized with mHSP110, mHSP70, and E711-20 did not stimulate significant cell proliferation after in vitro restimulation, as evidenced by the indexes not being significantly different from that detected in the PBS negative-control group.

mHSP110-E711-20 chaperone complex stimulates enhanced IFN-γ secretion in immunized mice. To determine whether the mHSP110-E711-20 complex stimulated the cellular immune response, IFN-γ production by splenocytes of the immunized mice was measured by using the ELISA method. HLA-A*0201 transgenic mice were immunized twice with the indicated immunogens, with a 2-week interval between immunizations. Two weeks after the second immunization, splenocytes harvested from the immunized mice were stimulated with E711-20 in vitro, as described above. As shown in Fig. 3A, significantly increased IFN-γ production was observed in the supernatants of cells from mice vaccinated with the mHSP110-E711-20 chaperone complex compared to the supernatants of cells from mice vaccinated with all other immunogens. However, the mHSP70-E711-20 complex and the CFA-emulsified E711-20 peptide stimulated much higher IFN-γ production than the immunogens E711-20, mHSP110, and mHSP70 or the PBS control.

Compared with the ELISA method, the ELISPOT assay is a sensitive functional method for measuring IFN-γ production at the single-cell level. Therefore, ELISPOT was employed to further investigate whether the HSP110-E711-20 complex elicited antigen-specific IFN-γ production. In accordance with the ELISA results, the HSP110-E711-20-immunized mice primed the most IFN-γ-producing cells, compared with all other groups (Fig. 3B). Again, the mHSP70-E711-20 complex and the CFA-emulsified E711-20 peptide primed more IFN-γ-producing cells than mHSP110, mHSP70, and E711-20 alone or the PBS control (Fig. 3B).

mHSP110-E711-20 complex primed potent and specific cytotoxicity. We next used an LDH release assay to investigate whether the mHSP110-E711-20 complex elicited the antigen-specific CTLs from HLA-A*0201 transgenic mice immunized with the mHSP110-E711-20 complex and several control agents. T2 cells pulsed with the E711-20 peptide were used as the target cells, because T2 cells express HLA-A2 molecules but do not process intracellular antigens. As shown in Fig. 4A, in the mice vaccinated with the mHSP110-E711-20 complex, the cytolytic activity of effector T cells on the T2 cells pulsed with the E711-20 peptide was significantly higher than in other groups when the E/T ratio was 50:1. At this ratio, however, both mHSP70-E711-20 and CFA-E711-20 stimulated higher cytotoxicity than E711-20 alone, mHSP110 alone, mHSP70 alone, and the PBS control. When the untreated T2 cells were used as target cells to investigate whether natural killer (NK) cells contribute to the nonspecific cytotoxicity in this assay, no apparent difference of cytotoxicity was observed between any of the groups (Fig. 4B).

We further investigated whether the CTLs from immunized HLA-A*0201 transgenic mice recognized the E711-20 epitope naturally processed from the E7 protein by using TC-1/A2Kb as the target cells and TC-1 cells as the control target cells. The results were similar to those obtained when the E711-20-loaded T2 cells were used as the target cells (Fig. 4C). Likewise, none of the cytotoxic T cells from any of the groups killed the TC-1 cells (Fig. 4D).

FIG 3 Enhanced IFN-γ production elicited by the indicated immunogens in HLA-A*0201 transgenic mice. (A) IFN-γ in the splenocyte culture supernatants measured by ELISA. (B) IFN-γ-producing splenocytes detected by ELISPOT assay. #, P < 0.01 compared to all other groups; *, P < 0.05 compared to the mHSP110, mHSP70, E711-20, or PBS groups.
Potent in vivo antitumor effect of immunization with mHSP110-E711-20 complex. Therapeutic efficacy against established tumors in vivo is the most important indication for the potential application of vaccines. Thus, to determine whether the enhanced ex vivo E7-specific T cell response generated by the mHSP110-E711-20 complex elicited therapeutic antitumor effects, we performed in vivo tumor treatment experiments using the HLA-A2Kb/TC-1 tumor model by s.c. inoculation of HLA-A2Kb-expressing TC-1 cells into HLA-A*0201 transgenic mice. As shown in Fig. 5A, although the mHSP70-E711-20 and CFA-emulsified E711-20 immunizations inhibited tumor growth, the tumor-bearing mice treated with the mHSP110-E711-20 complex exhibited a stronger decrease in tumor growth than the tumor-bearing mice treated with the other immunogens (P < 0.01). Likewise, Kaplan-Meier survival analysis further showed that tumor-challenged mice treated with the mHSP110-E711-20 complex exhibited the most markedly prolonged survival; however, the mHSP70-E711-20 and CFA-emulsified E711-20 immunizations also produced significant antitumor effects compared to the mHSP110 alone, mHSP70 alone, and E749-57 alone and the PBS group (Fig. 5B).

DISCUSSION

The utility of HSPs as peptide adjuvants has been widely researched. The HSP family members HSP70, HSP90, GP96, and HSP110 have been successfully applied in the clinical setting as vaccine adjuvants targeting cancers and infections (39–41). Peptides can also be complexed with either tissue-derived or recombinant HSPs in vitro to generate HSP-peptide complexes for use as peptide-specific vaccines (22, 42, 43). However, clinical application of tissue-derived HSPs is limited by the requirement of a surgical specimen of sufficient quantity to purify the HSP. We had previously verified that mHSP110 significantly enhances the immunity of the H-2d-restricted HPV E7 49-57 epitope in a mouse model (29), but it remained unknown whether HSP110 enhances the immunogenicity of different major histocompatibility complex (MHC)-restricted epitope peptides. In the current study, we investigated the efficacy of the mHSP110 molecular chaperone as an immunoadjuvant to enhance the immunogenicity of the HLA-A2-restricted HPV16 E711-20 peptide vaccine. The results further proved the broad applicability of a peptide vaccine strategy based on the HSP110 chaperone.

We selected the HPV16 E711-20 epitope peptide as the target molecule because it had been previously verified as an HLA-A*0201-restricted CTL epitope of HPV16 E7. Furthermore, this epitope had been detected in patients with squamous cell carcinoma of the oropharynx (44), and an HPV16 E711-20-specific CTL cell line had been established (45). Several strategies based on the E711-20 peptide, including DNA vaccine format and single amino acid substitution, have shown improved immunogenicity in in
The mHSP110-E7_{11-20} complex showed higher antitumor effects than CFA-emulsified E7_{11-20} Peptide. This observation is in accordance with previous findings by Wang et al., which showed that the HSP110 chaperone gp100 generated a higher antitumor immune response than CFA-gp100 (28). In addition, we also found that the mHSP110-E7_{11-20} complex is stronger than the mHSP70-E7_{11-20} complex in priming the host antitumor response. This observation may reflect the fact that HSP110 has a much higher protein- and peptide-binding affinity than the HSP70 family (28, 47–49).

In conclusion, our data encourage further investigations of HSP110 as a promising candidate immunomodulator for peptide-based human cancer vaccines, such as the HLA-A2-restricted E7_{11-20} epitope.

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FIG 5  Antitumor effects of the indicated immunogens on established tumors. (A) Data represent the mean tumor volumes of all 10 mice per group. *, P < 0.01 compared to the mHSP110, mHSP70, E7_{11-20}, or PBS groups; #, P < 0.01 compared to all other groups. (B) Time to death over a 70-day period is plotted as a Kaplan-Meier survival curve. Deaths include those of mice that were euthanized upon becoming moribund due to tumor burden.

vivo systems (38, 46). However, the human E7_{11-20} epitope has been less extensively studied than the mouse H2-D{\textsuperscript{b}}-restricted CTL epitope E7_{49-57}. Thus, further investigations on the E7_{11-20} epitope are necessary, especially for improving the immunity of this epitope in transgenic animal models, which will have direct implications for applications in human HPV infection cases.

The CTL response is a key component of the immune response against tumor cells. Therefore, the focus of this study was to observe whether mHSP110 enhanced the efficient activation and expansion of functional CTLs specific to the HPV16 E7_{11-20} Peptide. Indeed, the results presented herein demonstrate that the mHSP110-E7_{11-20} complex elicits epitope-specific splenocyte proliferation and E7_{11-20}-specific IFN-γ secretion. Importantly, the mHSP110-E7_{11-20} complex immunization produced very strong cytolytic effects on target T\textsubscript{c} cells pulsed with the E7_{11-20} peptide, and even the HLA-A2 transgenic TC-1 cells that process and present the E7_{11-20} epitope from the E7 protein in TC-1 cells. Besides the strong ex vivo antitumor immune response, the mHSP110-E7_{11-20} complex can also reduce the tumor volume and enhance the survival of tumor-bearing mice.

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23. HSP110 Improves Antitumor Effects of Epitope E711–20


