Enhanced anti-tumor effects of HPV16E7\textsubscript{49−57}-based vaccine by combined immunization with poly(I:C) and oxygen-regulated protein 150

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Background: It is well known that both heat shock protein (HSP) and Toll-like receptor (TLR)3 agonist polyinosinic:polycytidylic acid (poly(I:C)) are capable of promoting the antigen-specific immune responses. In the current study, we assessed whether the anti-tumor effects of the HPV16E\textsubscript{749−57}-based vaccine can be elevated by combined applications of poly(I:C) and oxygen-regulated protein 150 (ORP150) in a mouse cervical cancer model. Methods: Recombinant mouse ORP150 and HPV E\textsubscript{749−57} peptide were combined to passively form the ORP150–E\textsubscript{749−57} complex under heat shock conditions. The effects of ORP150–E\textsubscript{749−57} complex plus poly(I:C) adjuvant on lymphocyte proliferation and functional cytotoxic T cells were investigated by methyl thiazolyl tetrazolium (MTT), ELISPOT, and non-radioactive cytotoxicity assays. Finally, the complex’s therapeutic anti-tumor effects with and without adjuvant therapy were observed in a tumor challenge experiment. Results: This combination vaccine approach significantly enhanced the proliferation of splenocytes and induced strong E\textsubscript{749−57}-specific CTL responses. More importantly, the ORP150–E\textsubscript{749−57} complex plus poly(I:C) vaccine format demonstrated more potent anti-tumor effects than ORP150–E\textsubscript{749−57} complex alone or E\textsubscript{749−57} plus poly(I:C) in TC-1 tumor-bearing mice. Conclusion: Both poly(I:C) and ORP150 chaperone can synergistically enhance the anti-tumor effects of the HPV16E\textsubscript{749−57}-based vaccine in vitro and in vivo. This strategy provides a platform for the design of a tumor therapeutic vaccine capable of inducing an effective anti-tumor immune response.

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1. Introduction

High-risk human papillomavirus (HPV) infections are associated with a variety of malignant neoplasms, such as cervical cancer [1,2]. Two currently available prophylactic vaccines effectively prevent cervical cancer, as evidenced by decreased incidence rates of new infections. However, these vaccines are not effective against pre-existing HPV infections [3]. It is requisite to develop a therapeutic vaccine that prevents and resolves HPV infection and related cervical cancer. Over the past decade, a number of alternative vaccine approaches have been developed and met with varying degrees of success in preclinical and clinical trials. These have included vaccines based on DNA, live vectors, cells, peptides or proteins, and combination approaches [4–6]. The peptide-based vaccines comprising minimal cytotoxic T lymphocyte (CTL) epitopes have shown particularly promising results in tests of efficacy and safety, and are easy to prepare [7].

While the immunogenicity of minimal CTL epitopes alone can elicit the CTL response that is essential for elimination of established tumors, the response itself is weak. In addition, the epitope peptide vaccines may induce specific T cell tolerance, possibly promoting tumor growth [8]. Immune-enhancing substances or adjuvants may overcome this limitation. Heat shock proteins (HSPs), such as Hsp70, Hsp90 and gp96, are capable of enhancing immunogenicity of antigen peptides, and have been demonstrated as effective molecular adjuvants [9–11]. Those HSPs are able to bind antigenic peptides in vitro to form stable complexes that interact with the professional antigen-presenting cells (APCs) in a receptor-dependent manner, thereby chaperoning the peptide to be expressed on major
histocompatibility complex class I (MHC I) molecules and inducing anti-tumor immunity and a specific CD8+ cytolytic T lymphocyte response [12].

Oxynol-regulated protein 150 (ORP150, also known as GRP170) is an endoplasmic reticulum chaperone HSP and, unlike HSP70 and HSP110, is capable of binding peptides in its C-terminal region [13]. Tamura and co-workers showed that internalized ORP150-peptide complex was sorted to the Rab5*EEA1* static early endosome, then translocated to a recycling endosome and bound to MHC I molecules, and thus activate CD8+ T cells [14]. Vaccines based on GRP170 (ORP150) chaperone molecule have demonstrated strong anti-tumor effects. Wang et al. demonstrated that autologous ORP150 protein purified from the same tumor tissue or cell lines, or ORP150–antigen complexes by heat shock using recombinant HSPs led to a significant growth inhibition and regression of various kinds of tumors [15–20]. They further found that the secretosomal form of ORP150 promoted the immune-mediated inhibition of murine prostate and melanoma tumors [21,22], indicating the clinical relevance for ORP150-based candidate vaccines.

It is well known that dendritic cells (DCs) play an important role in anti-tumor immunity. ORP150, like many of the HSPs, is capable of inducing “danger signals” through interaction with DCs, which alert the immune system to mount a response against a tumor [23,24]. Tumors, however, have evolved counteractive regulatory mechanisms, such as expression of factors that decrease the proportion of DCs in the circulation and lymphoid organs of the tumor-bearing tissue [25]. Therefore, in order to achieve the best anti-tumor effects of ORP150-based vaccines, it is essential to activate DCs in tumor patients during vaccine application.

Polyinosinic:polycytidylic acid (poly(I:C)) has emerged as one of the most popular adjuvants of anti-virus and/or anti-tumor vaccines, according to its character as a ligand of the Toll-like receptor 3 (TLR3), which recognizes double-stranded (ds)RNA and triggers the innate immune response [26–28]. Poly(I:C) has been shown to reverse DC dysfunction that is induced by tumor-related factors and to be a superior adjuvant for CD4+ T cell responses to a peptide/protein vaccine [25,29]. Once activated by poly(I:C), TLR3 signals activate and maturate APCs, such as DCs, initiating processing and presentation of antigens [30].

In this study, we passively generated an ORP150 protein and HPV16E749–57 peptide complex and evaluated its anti-tumor effects in the presence of poly(I:C) adjuvant in vitro and in vivo. This combination strategy represents a promising therapy for HPV16-induced-tumors.

2. Materials and methods

2.1. Mice and cell lines

Six- to eight-week-old female C57BL/6 mice were purchased from the Animal Center at the Chinese Academy of Medical Science (CAMS) in Beijing, China. Animals were maintained under pathogen-free conditions. All procedures involving 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 co-transformed with HPV16 E6 and E7 and c-Ha-ras oncogenes [31], were purchased from the Cell Center at the CAMS in Shanghai, China. TC-1 cells were maintained in RPMI 1640 (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 2 mM L-glutamine (Gibco), 50 mM 2-mercaptoethanol (Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco) at 37℃ in a humidified atmosphere of 5% CO2 in air.

2.2. Peptide and reagents

The E749–57 CTL epitope (RAHYNIVTF) and FITC-labeled E749–57 peptides were synthesized (Shenzhen Hanyu Pharmaceutical Corporation, Shenzhen, China) with a free carboxy-terminus by solid-phase strategies on an automated multiple-peptide synthesizer ABI431A (PE Biosystems, Foster City, CA, USA) using the standard 9-fluorenyl methoxycarbonyl (FMoc) method. The purity of peptides was above 95%, as determined by reverse-phase high-performance liquid chromatography. Unlabeled peptides were dissolved in double-distilled H2O and FITC-labeled peptides in phosphate-buffered saline (PBS) containing 0.5% DMSO. All peptides were stored at –20℃ if not used immediately. Synthetic poly(I:C) was purchased from Sigma Corporation (Kanagawa, Japan) as a synthetic analog of dsRNA composed of a poly(I) strand (152–539 b) annealed to a poly(C) strand (319–1305 b).

2.3. Cloning, expression and purification of recombinant mouse ORP150

Total RNA was extracted from C57BL/6 mouse liver tissue with the TRIzol Reagent (Invitrogen), and used to amplify the mouse ORP150 (mORP150) DNA by reverse transcription–polymerase chain reaction (RT-PCR) with the following gene-specific primers: 5’-CGGATCCATGGCGGACCGCCACATCGAAG–3’ (forward) and 5’-CCGCGTCGACGTTTATAGTTCATCGCTTTGAAGGCC-3’ (reverse). The PCR product was digested with BamH1/Xho1 and cloned into the BamH1/Sall sites of the pQE80L expression vector (Invitrogen).

The resultant pQE80L-mORP150 recombinant plasmid was confirmed by DNA sequencing and transformed into E. coli Rosetta competent cells. The bacterial cultures were induced with 1 mM isopropyl-b-D-thiogalactopyranoside (IPTG) at 37℃ for 6 h. Cells were harvested by centrifugation and lysed by sonication. The lysate containing recombinant proteins were centrifuged at 12,000 × g for 20 min at 4℃, and the supernatant was harvested. The recombinant proteins were purified by running the supernatant through a nickel nitriloacetic acid-agarose column (Ni-NTA; GE Healthcare, Wauke-sha, WI, USA), according to manufacturer’s instructions. The column was washed extensively with 1% Triton-X 100/0.5 M guanidine-HCl to remove endotoxins, and then the target protein was eluted using an imidazole gradient (10–250 mM) and stored at –70℃ with protease inhibitors (Roche, Indianapolis, IN, USA). Protein purity was assessed by SDS-PAGE assay and the identity of the purified recombinant protein was verified by Western blot analysis with mouse anti-ORP150 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as the primary antibody and visualization by a chemiluminescence kit (Roche).

2.4. Preparation of the ORP150–E749–57 complex

ORP150 and FITC-labeled or unlabeled E749–57 were mixed in a 1:50 protein-to-peptide molar ratio and incubated in binding buffer (1 mM MgCl2 in PBS) at 50℃ for 30 min, followed by an additional incubation for 30 min at room temperature. The mixture was then ultra-filtrated by size exclusion (Microcon 50, molecular weight cut-off 50 kDa; Millipore, Billerica, MA, USA) to remove free peptides and any other small substances. To identify the complex, an aliquot (2–3 µg of protein) was subjected to nondenaturing gradient PAGE (NuPAGE® Novex® Tris-Acetate Mini Gels; Invitrogen) analysis, visualized and photographed on a fluorescence imager, and stained by Coomassie Brilliant Blue R250.

2.5. Mice vaccination

Eight groups of four C57BL/6 mice were immunized via the intraperitoneal (i.p.) route with different injection formulations:
ORP150–E749–57 complex (prepared with 95 μg of ORP150 and 50 μg of E749–57); ORP150–E749–57 admixed with poly(I:C) (50 μg); E749–57 (50 μg) admixed with poly(I:C) (50 μg); poly(I:C) (50 μg) alone; ORP150 (95 μg) alone; E749–57 (50 μg) alone; or PBS alone. For all mice, the vaccine volume was adjusted to 150 μL/mouse with PBS. All mice were immunized twice with the same dosage of the corresponding injection formulation with two-week intervals.

2.6. Splenocyte proliferation assay

Two weeks after the second immunization, single splenocyte suspensions were prepared from the spleen of each mouse. The splenocytes were seeded in triplicate in 96-well culture plates (2 × 10^4 cells/well in 100 μL) and incubated with E749–57 peptides (0 or 100 μg/mL) and mL-2 (10 IU/mL) at 37 °C in a humidified 5% CO₂ atmosphere for five days. The proliferation assay was performed according to the methyl thiazolyl tetrazolium (MTT) test kit protocol (Sigma–Aldrich, St. Louis, MO, USA). The proliferation index (ratio) was calculated as: (number of viable cells after E749–57 stimulation)/(number of viable cells after incubation without E749–57 stimulation).

2.7. ELISPOT assay

The ELISPOT assay was performed using a commercial kit (BD PharMingen, Franklin Lakes, NJ, USA). Briefly, the red blood cell-free splenocytes of mice were prepared two weeks after the second immunization, as described above. Splenocytes (5 × 10^5/well) or CD8⁺ T cells (5 × 10^5/well) sorted from the splenocytes by fluorescence-activated cell sorter (FACS, MoFlo XDP Cell Sorter; Beckman Coulter, Inc., CA, USA), which were incubated with E749–57 (20 μg/mL) and mL-2 (10 IU/mL) at 37 °C in 5% CO₂ for 24 h. Positive control wells were generated by adding concanavalin A (ConA) (5 μg/mL) alone to cells, and background wells were generated by adding 10% PBS alone to cells. After incubation with 5 μg/mL biotinylated interferon-γ (IFN-γ) antibody (BD PharMingen) at 4 °C overnight, the IFN-γ immunoreactive spots were developed and counted using a dissecting microscope.

2.8. Specific cytolysis of CTL

Splenocytes were harvested two weeks after the second immunization and cocultured (1 × 10^7) with E749–57 (10 μg/mL) in RPMI-1640 supplemented with 10% fetal calf serum (FCS) and 10 U/mL of mouse interleukin-2 (mlL-2; R&D Systems, Minneapolis, MN, USA) at 37 °C in 5% CO₂. After five days of stimulation, the viable splenocytes were recovered for use as effector cells targeting the tumor cell line TC-1. The Non-Radioactive Cytotoxicity Assay Kit (Promega, Madison, WI, USA) was used to measure the cytolytic effect of these effector cells, according to the manufacturer’s protocol. Briefly, the stimulated splenocytes were cocultured with 5 × 10^4 target cells at different effector-to-target cell ratios (E/T) of 10:1, 50:1, and 100:1 in 96-well V-bottom culture plates at 37 °C in 5% CO₂. After 4 h of incubation, the supernatants (50 μL/well) were collected and transferred to wells on another ELISA plate. Substrate Mix (50 μL/well) was added and the plates were incubated in the dark for 30 min at room temperature. The reaction was terminated by adding 50 μL Stop Solution (1 M acetic acid), and sample absorbance at 490 nm wavelength was measured by an ELISA reader (Bio-Rad, Hercules, CA, USA). The spontaneous release of lactate dehydrogenase (LDH) by the target or effector cells was assessed by incubation of 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. Specific lysis (percent) was calculated as: [(experimental release – effector spontaneous release – target spontaneous release)/(target maximum release – target spontaneous release)] × 100. All the experiments were repeated three times.

2.9. Tumor challenge experiments

To evaluate the therapeutic anti-tumor effects of the various immunogens, tumors were established by subcutaneous (s.c.) injection of 5 × 10⁵ TC-1 cells in the right flank of C57BL/6 mice (day 0). On day 5, mice with obvious tumor development were assigned arbitrarily into eight groups (10/group). Mice were then i.p. immunized with different immunizing formulations (150 μL total volume with 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 diameter² × the longest diameter)/2. Survival of mice was recorded daily for each group.

2.10. Statistical analysis

Analysis of variance (ANOVA) and Student’s t-test were performed to assess statistical differences among the various treatment effects. Kaplan–Meier survival curves for tumor treatment experiments were applied. All graphical data is presented as mean ± SD. Differences were considered significant at P < 0.05.

3. Results

3.1. Characterization of the ORP150–E749–57 complex

The recombinant plasmid pQE80L-mORP150 was transformed into E. coli Rosetta competent cells that were further cultured in the presence of IPTG. ORP150 protein was then purified from the cell lysate supernatant using a Ni-NTA column. As shown in Fig. 1A, the purified ORP150 protein migrated through SDS-PAGE as one band at the expected molecular size of ~150 kDa. The purified ORP150 protein was recognized by ORP150 antibody in a Western blot assay (Fig. 1B). To ascertain and quantify the loading of peptides onto ORP150, FITC-labeled E749–57 was employed as a tracer.

![Fig. 1. Preparation and identification of ORP150–E749–57 complex. Panel A.](image-url)
ORP150 was found to bind the labeled peptides efficiently, as shown in a non-denaturing gradient PAGE assay followed by imaging under fluorescence microscope and staining with Coomassie brilliant blue (Fig. 1C and D).

3.2. ORP150–E749–57 complex plus poly(I:C) elicited strong E749–57-specific lymphocyte proliferation and IFNγ secretion

Mice were immunized twice with the indicated amount of immunogens at two-week intervals: ORP150–E749–57 plus poly(I:C), E749–57 plus poly(I:C), ORP150 plus poly(I:C), ORP150–E749–57, ORP150, poly(I:C), E749–57, or PBS. Two weeks after the second immunization, the splenocytes from the immunized mice were harvested and re-stimulated in vitro with E749–57 in the presence of mIL-2 for the lymphocyte proliferation assay and IFNγ ELISPOT assay, respectively. As shown in Fig. 2A, the splenocytes from mice inoculated with ORP150–E749–57 plus poly(I:C) showed significantly higher cell proliferation than any of the other groups. ORP150–E749–57 complex and E749–57 plus poly(I:C) also had the ability to induce cell proliferation, as indicated by the proliferation index >2.0. In contrast, neither E749–57 peptide, ORP150 alone, poly(I:C) alone, nor PBS alone could induce remarkable cell proliferation, as indicated by the respective indexes all being <1.5.

ELISPOT assay demonstrated that the in vitro re-stimulation with E749–57 led to significantly enhanced frequency of IFNγ-secreting cells in the splenocytes isolated from mice immunized with ORP150–E749–57 plus poly(I:C), ORP150–E749–57 complex, and poly(I:C)–E749–57 (Fig. 2B). In accordance with the proliferation assay, splenocytes from mice inoculated with ORP150–E749–57 plus poly(I:C) showed evidently more IFNγ spots than any of the other groups (Fig. 2B). As expected, very few IFNγ immunoreactive spots were detected from animals immunized with poly(I:C) plus ORP150, poly(I:C) alone, ORP150 alone, E749–57, or PBS alone.

It has been reported that poly(I:C) is a competent direct activator and modulator of natural killer (NK) cell functions [32], and the adjuvant effects of poly (I:C) on antigen-specific T cell responses are partially dependent on NK cells with the induction of beneficial cytokines including IFNγ [33]. Thus, to further dissect the contribution of poly(I:C) to the IFNγ production, the ELISPOT assays were performed on CD8+ T cells that were sorted from splenocytes by FACS. Results showed that ORP150–E749–57 plus poly(I:C), and ORP150–E749–57 and poly(I:C) plus E749–57 groups had many more IFNγ spots than other groups (Fig. 2C). However, CD8+ T cells from poly(I:C) plus E749–57 group showed less IFNγ-producing cells than ORP150–E749–57 group (Fig. 2C), though the splenocytes from both groups showed similar amounts of IFNγ spots in the ELISPOT assays (Fig. 2B and C).

3.3. ORP150–E749–57 complex plus poly(I:C) stimulated the enhanced E749–57-specific CTL response

Non-radioactive cytotoxicity assay was performed to estimate the specific CTL cytolysis induced by various immunogens, in which the re-stimulated splenocytes were used as the effector cells against TC-1 target cells. As shown in Fig. 3, compared with other immunogens, ORP150–E749–57 complex plus poly(I:C), ORP150–E749–57 complex, and E749–57 plus poly(I:C) immunizations showed significantly more cytolytic effects on target cells at E/T ratios of 100:1, 50:1, and even 10:1. The effector cells from mice immunized with poly(I:C) plus ORP150, poly(I:C) alone, ORP150 alone, E749–57 alone, or PBS alone did not elicit any detectable cytotoxicity against the TC-1 cells. Similar to the results from ELISPOT assay (Fig. 2B), ORP150–E749–57 complex plus poly(I:C) immunization showed the highest cytotoxic capacity of all the groups examined (Fig. 3).
Fig. 3. Strong CTL effects induced by ORP150–E7<sub>49–57</sub> plus poly(I:C) immunization. Mice were immunized twice with the indicated immunogens with a two-week interval. Two weeks after the second immunization, splenocytes were harvested from each mouse and were cocultured with E7<sub>49–57</sub> (10 μg/ml) in the presence of 10 U/ml of mlt-2 for five days. The LDH release rate from the target cell TC-1 was assessed as the E7-specific cytolytic activity of effector T cells. Each experiment was repeated three times and data shown are mean ± SD. **P < 0.01 vs. ORP150 + poly(I:C), E7<sub>49–57</sub>, ORP150, poly(I:C), and PBS groups; *P < 0.01 vs. all other groups.

3.4. Potent in vivo anti-tumor effect of immunization with ORP150–E7<sub>49–57</sub> complex plus poly(I:C)

Treatment of the established tumors in vivo by certain vaccines is the most important indication for the potential application of the vaccines. Thus, to determine whether the enhanced ex vivo E7-specific T cell response generated by ORP150–E7<sub>49–57</sub> complex plus poly(I:C) contributed to therapeutic anti-tumor effects, we performed in vivo tumor treatment experiments using the TC-1 tumor model. As shown in Fig. 4A, although ORP150–E7<sub>49–57</sub> complex and E7<sub>49–57</sub> plus poly(I:C) immunizations showed inhibition of tumor growth, the tumor-bearing mice treated with ORP150–E7<sub>49–57</sub> complex plus poly(I:C) exhibited the most significant decrease of tumor growth, as compared with the tumor-bearing mice treated with the other agents (P < 0.01). Likewise, Kaplan–Meier survival analysis further showed that tumor-challenged mice treated with ORP150–E7<sub>49–57</sub> complex plus poly(I:C) exhibited the most markedly prolonged survival, although ORP150–E7<sub>49–57</sub> complex and E7<sub>49–57</sub> plus poly(I:C) immunizations also showed significant anti-tumor effects, as compared with the other treatment groups (Fig. 4B).

4. Discussion

Although the causal relation between human papillomavirus and cervical cancer has been established for nearly 30 years, an effective treatment for HPV infection remains elusive. Traditional methods of management for cervical cancer are focused on resolving the tumor and not the underlying viral infection, providing no protection against recurrence after successful treatment.

While peptide-based immunotherapy is promising for resolving both cancer and viral infection, the approach has yet to realize its full potential and inherent limitations have yet to be overcome [34]. One of the major obstacles is the infection-induced perturbations of immune homeostasis and the related microenvironment in patients requiring treatment. As our understanding of the mechanisms of immunocompromise in tumor-bearing bodies increases, novel strategies are developed to overcome negative regulation of anti-tumor immune response [35]. In the current study, we showed that E7<sub>49–57</sub> peptide can elicit significant anti-tumor specific T cell response with the help of a combination of poly(I:C) and ORP150 adjuvants because we found that immunization with ORP150–E7<sub>49–57</sub> complex and E7<sub>49–57</sub> plus poly(I:C) elicited strong anti-tumor immune responses in vitro and in vivo, as evidenced by significant enhanced lymphocyte proliferation, IFNγ production, cytotoxicity and in vivo anti-tumor effects, compared with control groups.

To our knowledge, there are three strategies for ORP150-based vaccine types to date: (1) autologous ORP150 protein purified from the same tumor tissue or cell lines [15–17]; (2) ORP150–antigen complexes by heat shock using recombinant HSPs [17–20]; (3) secretable form of ORP150 by engineering the tumor cells [21,22]. Though each type of ORP150-based vaccines has its own advantages and all have been verified to have potent antitumor effects in animal tumor models [15–22], we used the
in vitro-prepared ORP150–peptide antigen complex plus poly(I:C) strategy to investigate its antitumor effects in a cervical cancer model in this study. Such an ORP150-based vaccine has several advantages over autologous ORP150 vaccines, including no requirement for a surgical tumor specimen, unlimited quantities of off-the-shelf vaccines with uniformity, broad applicability and easy immunomonitoring using well-defined antigens [20]. Therefore, the strategy used in this study provides additional convenient choice for the potential treatment of cancer patients in the future.

Wang et al. found that GRP170 (ORP150) purified from both tumor tissue and culture cells was more efficient than HSPI10 when administered on an equal-mass basis, by comparing the results with HSPI10 and GRP170 as immunogens in Colon 26 and Meth A tumor models and in the DC study [15]. However, in this study, we find that the in vitro prepared ORP150–E749–57 complex shows similar anti-tumor effects with our previously reported data for HSPI10–E749–57 as the immunogen in the same TC-1 cervical tumor model [36]. This observation might reflect the different biological features of HSPI10 or ORP150 purified from the tumor tissue, which naturally binds peptides in vivo and probably binds several kinds of peptides, with the in vitro prepared HSPI10/E749–57 or ORP150/E749–57 that only contains peptide E749–57. Therefore, the fact that in our TC-1 tumor model, the in vitro-prepared HSPI10/E749–57 and ORP150/E749–57 showed synergistic anti-tumor effects, and ORP150/E749–57 plus poly(I:C) showed highest antitumor effects than all other groups indicates the synergistic adjuvant effects between ORP150 and poly(I:C).

DCs bridge the innate and adaptive arms of immunity, and play a pivotal role in anti-tumor immunity [37,38]. T cells can be activated via their TCR stimulation by epitope in context of MHC molecules on DCs. As ORP150 can help antigen presentation of DCs, it thus promotes T cell activation and the following expansion with the help of the costimulatory signals including B7/CD28 and IL-2. Nonetheless, it is known that a single maturation stimulus is likely not sufficient to fully stimulate DCs [39]. This feature may explain why we did not observe the most advantageous anti-tumor effects of ORP150–E749–57 complex in this study. Many kinds of molecules have been defined as activators of DCs and work through a myriad of signaling pathways [40–42]. Among these, we chose poly(I:C) as the adjuvant with the expectation of achieving more abundant effects on DC activation. As a dsRNA, poly(I:C) is able to trigger DC activation through the TLR3 signaling pathway and to activate IFN production [27,29]. Recently, poly(I:C) has been reported to promote antigen-specific T cell immune response by modulating NK cells to produce cytokines including IFNγ, which are beneficial for T cell activation [32,33]. In this study, the ELISPOT assays for the splenocytes and CD8+ T cells from the immunized mice indicate that non-CD8+ T cells that probably include NK cells contribute to IFNγ production (Fig. 2B and C). Thus, poly(I:C) has antitumor effects at least partially through the cytokine production from DCs and NK cells, which are further involved in tumor antigen-specific T cell activation. Results in the present study showed that E749–57 plus poly(I:C) induced stronger anti-tumor immune responses than the E749–57 peptide alone. Most importantly, combined application of ORP150 and poly(I:C) elicited significant anti-tumor effects of peptide E749–57 in vitro and in vivo, indicating that both ORP150 and poly(I:C) play crucial roles in the therapeutic efficacy of ORP150–E749–57 complex plus poly(I:C) vaccine.

In conclusion, our findings indicate that both poly(I:C) and ORP150 chaperone can synergistically enhance the anti-tumor effects of the HPV16 E7 49–57–based vaccine in vitro and in vivo. This strategy provides a platform for the design of a tumor therapeutic vaccine that will induce an effective anti-tumor immune response.

Conflict of interest statement

There is no conflict of interest.

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References


[33] Salem ML, El-Naggar SA, Kadima A, Gillanders WE, Cole DJ. The adjuvant effects of the toll-like receptor 3 ligand polyinosinic-cytidylic acid poly(I:C) on antigen-specific CD8+ T cell responses are partially dependent on NK cells with the induction of a beneficial cytokine milieu. Vaccine 2006;24:5119–32.


