Prediction and identification of human leukocyte antigen-A2-restricted cytotoxic T lymphocyte epitope peptides from the human papillomavirus 58 E7 protein

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Abstract. Persistent infection with high-risk human papilloma virus (HPV) is the primary cause of cervical intraepithelial neoplasia (CIN) and cervical carcinoma. HPV58 is the third most common HPV genotype in China after HPV16 and HPV18. HPV E6 and E7 are oncoproteins and are constitutively expressed in HPV-associated cancer cells, therefore they are considered to be ideal target antigens for immunotherapy, including HPV therapeutic vaccine. In the present study, human leukocyte antigen (HLA)-A2-restricted cytotoxic T lymphocyte (CTL) epitope peptides were predicted and screened from HPV58 E7 antigen and their immunogenicity was subsequently determined. A total of 6 HLA-A2-binding peptides derived from HPV58 E7 were predicted and selected using 3 different prediction programs. A negative control peptide and PBS were used as two negative controls. Peripheral blood mononuclear cells (PBMCs) with HLA-A2(+) allele were used to detect the specific cellular immune response among the 6 predicted peptides by enzyme-linked immunospot assay (ELISOPT). Following preliminary screening for the predicted peptides, the antigenicity of the peptide HPV58 $E7_{72-80}$ was further assessed by an immunoassay to a vaccine contained HPV58 E7 antigen. Specific humoral and cellular immunity were detected using the peptide HPV58 $E7_{72-80}$ as the specific antigen. A total of 6 peptides from HPV58 E7 protein were predicted and subsequently named P1 ($E7_{7-15}$: TLREYILDL), P2 ($E7_{14-22}$: DLHPEPTDL), P3 ($E7_{69-77}$: CINSTTTDV), and P4 ($E7_{72-80}$: STTTDVRTL), P5 ($E7_{79-87}$: TLQQLLMGT) and P6 ($E7_{83-91}$: LLMGTCTIV). In the ELISPOT assay on HLA-A2 (+) human PBMCs, interferon (IFN)-γ-production was evident in the P2 and P4 groups. The average numbers of IFN-γ associated spots in the P2 and P4 groups was 50.61±5.37 spot-forming cells (SFC)/1x10^5 and 266±34.42 SFC/1x10^5, respectively. The numbers of spots in the two peptides were significantly increased compared with the other 4 peptides and the control groups ($P<0.05$). In the further antigenicity verification of P4 (HPV58 $E7_{72-80}$), the peptide only stimulated the humoral immune response of the AD-HPV16/18/58 mE6E7 vaccine containing HPV58 E7 antigen. Compared with the 2 negative control groups (1:400), the antibody titers of the vaccine group (1:25,600) were significantly increased ($P<0.05$). In cellular immunoassays the average number of IFN-γ associated spots was 143.3±32.13 SFC/1x10^5 in the vaccine group, which was significantly enhanced compared with the PBS group (8±5.29 SFC/1x10^5; $P<0.01$) and the AD-NC group (28±5.13 SFC/1x10^5; $P<0.01$). The peptide HPV58 $E7_{72-80}$ (STTTDVRTL) displayed sufficient antigenicity to a vaccine contained HPV58 E7 antigen. Therefore, HPV58 $E7_{72-80}$ peptide may be considered as a candidate epitope peptide for the construction of HPV58 peptide vaccines.

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Introduction

Cervical cancer is the second most common female malignant tumor following breast cancer. High risk human papilloma virus (HPV) has been confirmed as a dominant factor responsible for several anogenital diseases, particularly cervical intraepithelial neoplasia (CIN) and cervical carcinoma (1). Of the more than two hundred HPV genotypes, 16, 18, 31, 45, 52 and 58 are six particularly important, as they are highly correlated with more than 90% of cervical carcinoma cases (2-4). Persistent infection with high risk HPV is the primary factor responsible for cervical cancer (3,4). Although strains 16 and 18 are the two most common high-risk HPV genotypes worldwide, genotype distribution studies in Chinese women have shown that HPV58 is the third most common genotype found in Chinese cervical carcinoma patients after HPV16 and -18 (5). Because HPV58 infection is not common in western countries, current domestic and foreign research on HPV58 is lacking. However, the high rate of HPV58 infection in China firmly indicates that studies investigating HPV58 therapeutic vaccines are necessary.

HPV E7 is an early oncoproteins in HPV and continuously present in cervical cancer cells. These proteins are responsible

for the carcinogenicity of HPV (1,6,7). Many studies have showed that sustained expression of oncoproteins E7 is necessary for the tumorigenicity and development of cervical cancer cells (8,9). Accordingly, HPV E7 could be applied as target antigens for a candidate HPV therapeutic vaccine due to their constitutive expression and the maintenance of cellular transformation in HPV-infected cells (3). Because of continuous E7 expression, it is impossible that cervical carcinoma cells can invoke immunologic escape through antigen loss (3). To date, E7 have been extensively confirmed as ideal target antigens for the development of HPV therapeutic vaccines against HPV-related CIN and cervical carcinoma (10).

HPV therapeutic vaccines designed using specific epitope peptides as target antigens can induce specific cellular immune responses, avoid the immunosuppression induced by natural proteins and enhance immune specificity and immune effects. At present, this type of vaccine has become the focus of research in the immunization field. Recently, researchers have found that the cytotoxic T lymphocyte (CTL) epitope of the HPV16 E6 and E7 oncoproteins match human leukocyte antigen (HLA)-A2, which is currently considered the most common human major histocompatibility complex (MHC)-I molecule; the immunogenicity of these epitope has been confirmed using transgenic mice (11). *In vitro*, lymphocytes from human peripheral blood previously immunized with the HPV16 polypeptide vaccine induced specific CTL immune responses and killed HPV16-positive tumor cells (12,13). However, the CTL epitope for HPV58 has not been found. In view of the high HPV58 infection rate in China, we attempted to find specific CTL epitope antigens in the HPV58 E7 proteins and to prepare corresponding polypeptide vaccines to treat cervical cancer.

To achieve our goal, we screened HLA-A2-restricted HPV58 E7 CTL epitope peptides and evaluated their immune responses. The resulting epitope peptides may serve as candidate epitope for the preparation of an HPV58 therapeutic epitope peptide vaccine.

Materials and methods

Peptide prediction and synthesis. The full-length amino acid sequences of the HPV58 E7 (accession no. AEJ33545.1) proteins were obtained from GenBank. The HPV58 E7 protein is composed of 98 amino acids, and its sequence is as follows: MRGNNPTLREYILDLHPEPTDLFCYEQLCDSSDEDEIGL DRPDGQAQPATANYYIVTCCYTCGTTVRLCINSTTT DVRTLQQLLMGTCTIVCPSCAQQ.

The predicted peptides used for the identification of CTL epitopes were selected on the basis of 3 different prediction programs from internet websites that include an HLA binding peptide prediction (http://www-bimas.cit.nih.gov/molbio/hla_bind/).

SYFPEITHI (http://www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm), and an MHC class I-binding prediction using an artificial neural network (http://tools.immuneepitope.org/analyze/html_mhci binding 20090901/mhc_binding. html).

The predicted peptides each containing nine amino acid were synthesized by solid phase peptide synthesis and purified by reversed-phase high performance liquid chromatography by Abgent (San Diego, CA, USA). The negative control peptide (irrelevant peptide) from the SARS coronavirus (CoV) spike protein (LYLTQDLFL) was synthesized and purified in the same way by GL Biochem Co., Ltd. (Shanghai, China). The mass of each peptide was 30 mg and the purity of exceeded 93%.

Isolation and culture of peripheral blood mononuclear cells (PBMC). A total of 100 ml peripheral blood from HLA-A2 (+) healthy women volunteers was drawn with a vacuum bag from the basilic vein. Our study using volunteers' samples was approved by the ethical committee of Guangxi Medical University Affiliated Tumor Hospital. Written informed consent was obtained from all volunteers. Human PBMC were separated from the peripheral blood using Ficoll density gradient separation method. The final concentration of PBMC was $1x10^5$, diluted with RPMI-1640 containing 10% FBS. The final volume added to the well was 100 ul. These cells were inoculated at 24-well culture plate and stimulated with 10 μg/ml of the six predicted peptides or negative control peptide, respectively. At the same time, the cells were added 100 U/ml interleukin 2 every other day. Three times of stimulation by the peptides and IL-2, the PBMC were collected for EILSPOT.

ELISPOT Assay on human PBMC. The human PBMC, which were HLA-A2 (+) and activated by the 6 predicted peptides or negative control peptide, were detected special immune spots by an ELISPOT kit (DKW22-1000-096S; Dakewe Biotech Co., Ltd., Shenzhen, China). Briefly, a 96-well nitrocellulose plate precoated with anti-human interferon (IFN)-γ antibody was placed at 4˚C overnight and then blocked the following day with RPMI-1640 medium containing 10% FBS. PBMC were added to the wells at a concentration of $1x10^5$ cells/well in a volume of 100 μl. These cells were stimulated with 10 μg/ml of the six predicted peptides, negative control peptide, phorbol-12-myristate-13-acetate (PHA) (5 ng/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and PBS, respectively. PHA served as the positive control, and the negative control peptide and PBS served as the negative controls. The plate was incubated at 37˚C in 5% carbon dioxide for 24 h. The next day, the plate was incubated with a biotinylated anti-human IFN-γ antibody (primary antibody) for 2 h at room temperature. Unbound primary antibody was removed by washing with scrubbing solution, and a streptavidin-HRP antibody (secondary antibody) was added. Following 1 h of incubation at room temperature, the unbound secondary antibody was removed by washing with scrubbing solution, and staining with AEC (3-amino-9-ethylcarbazole) substrate solution was carried out for 20 min. The plate was washed and air dried for 24 h. The immune spots, which represented individual IFN-γ-producing cells as spot-forming cells (SFC) on the membrane, were enumerated by the KS-ELISPOT automatic system (Dakewe Biotech Co., Ltd.). The responses were considered positive when the IFN-γ associated spot numbers produced by predicted peptides stimulation were above 50 SFC/well compared with the responses obtained with all the negative control peptide, P-values ≤ 0.05 were considered significant.

Antigenicity verification of HPV58 $E7_{72-80}$ peptide. Before further immune evaluation to the peptide HPV58 $E7_{72-80}$ (STTTDVRTL), a mice cervical cancer cell line U14/LV-HPV58E6E7 and an

adenovirus vector vaccine AD-HPV16/18/58 mE6E7 containing HPV58 E7 antigen were constructed by the authors. Our animal experiments were approved by the ethical committee of GuangXi medical university affiliated tumor hospital. Thirty female C57BL/6 mice, 6-8 weeks old, weighed approximately 20 to 25 g, were randomly divided into three groups as follows: PBS group, AD-NC (empty adenovirus vector) group, and AD-HPV16/18/58 mE6E7 vaccine group. It is n=10 in each group. C57BL/6 mice were subcutaneously injected on the back of the mice with $1x10^6$/100 ul the mice cervical cancer U14/LV-HPV58E6E7 cells which can express HPV58 E6E7 fusion protein. The PBS, AD-NC or AD-HPV16/18/58 mE6E7 vaccine was individually administered by tattooing on day 7, 14, and 21 after the cancer cells challenge. Two weeks after the last immunization, serum and splenocytes were isolated individually from blood and spleen of the immunized mice. The peptide HPV58 $E7_{72-80}$ was used as antigen to activate the immunized mice serum and splenocytes before ELISA and ELISPOT assay. HPV58 related serum specific antibody was detected by enzyme-linked immunosorbent assay (ELISA) using an ELISA kit following the manufacturer's instructions (DKW-F1; Dakewe Biotech Co., Ltd.). IFN-γ-spots of the splenocytes were detected using an ELISPOT kit (DKW22-2000-096S; Dakewe Biotech Co., Ltd.). Briefly, a 96-well nitrocellulose plate precoated with anti-mice IFN-γ antibody was placed at 4˚C overnight and then blocked the following day with RPMI-1640 medium containing 10% FBS. Immunized mice splenocytes were added to the wells at a concentration of $1x10^5$ cells/well in a volume of 100 μl. These cells were stimulated with 10 μg/ml of the peptide HPV58 $E7_{72-80}$. The plate was incubated at 37˚C in 5% carbon dioxide for 24 h. The next day, the plate was incubated with a biotinylated anti-mice IFN-γ antibody (primary antibody) for 2 h at room temperature. Unbound primary antibody was removed by washing with scrubbing solution, and a streptavidin-HRP antibody (secondary antibody) was added. Following 1 h of incubation at room temperature, the unbound secondary antibody was removed by washing with scrubbing solution, and staining with AEC (3-amino-9-ethylcarbazole) substrate solution was carried out for 20 min. The plate was washed and air dried for 24 h. The immune spots, which represented individual IFN-γ-producing cells as SFC on the membrane, were enumerated by the KS-ELISPOT automatic system (Dakewe Biotech Co., Ltd.). The production of IFN-γ associated spots indicated that the peptide HPV58 $E7_{72-80}$ can activate the mice splenocytes which were immumed by the AD-HPV16/18/58 mE6E7 vaccine containing HPV58E7 gene. The responses were considered positive when the spot numbers produced in AD-HPV16/18/58 mE6E7 vaccine group were above 50 SFC/well compared with the responses obtained with PBS group and AD-NC group, P-values ≤0.05 were considered significant.

Statistical analysis. The SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. IFN-ELISPOT assay and ELISA were evaluated using an analysis of variance (ANOVA). Results are expressed as means ± standard deviation (SD). To identify significant differences between groups, Student's t-test was used. For all comparisons, P<0.05 was considered to indicate a statistically significant difference. All findings were confirmed in at least one additional independent experiment.

Results

Peptide prediction and analysis results. Several peptide prediction programs were used to assist in the identification of candidate CTL epitope. According to the prediction results of the three programs, the top-ranked six peptides in HPV58 E7 were selected are shown in Table I.

Evaluation of HPV58 E7 peptide-specific T cell immunity in HLA-A2 (+) human PBMC. IFN-γ ELISPOT assay was performed on the HLA-A2 (+) human PBMC using a final concentration of 10 μg/ml of the 6 candidate peptides of HPV58 E7. IFN-γ-positive spots were detected in the PHA (the positive control), P2 and P4 groups, whereas other experimental groups and the two negative control groups (negative control peptide group and PBS group) exhibited no or few spots formation (Fig. 1). The average numbers of IFN-γ-positive spots in the PHA, the negative control peptide, PBS, P1, P2, P3, P4, P5 and P6 groups were 737±17.54 SFC/$1x10^5$, 0, 0,0, 50.61±5.37 SFC/$1x10^5$, 5.62±4.78 SFC/$1x10^5$, 266±34.42 SFC/$1x10^5$, 13.33±1.53 SFC/$1x10^5$, 21.61±5.37 SFC/$1x10^5$, respectively. The average numbers of IFN-γ-positive spots in the PHA, P2 and P4 groups were significantly higher than other experiment groups and the two negative control groups (P<0.05; Fig. 1).

Antigenicity verification of the peptide HPV58 $E7_{72-80}$

The humoral immunogenicity of the peptide HPV58 $E7_{72-80}$. On day 14 after the last immunization, blood was collected from the orbital veins of the mice, and sera were isolated. The peptide HPV58 $E7_{72-80}$ was added to 96-well plate as stimulation antigen. Then the serum of mice immunized with PBS, AD-NC, and AD-HPV16/18/58 mE6E7 vaccine was added to 96-well plate. Specific humoral immune response associated with HPV58E7 was detected by ELISA. After ELISA was completed, the absorbance of each hole of 96 holes was detected. Absorbance value more than 0.1 can be judged as positive. The maximum dilution of the positive results was regarded as the antibody titer of the predicted peptides. As shown in Fig. 2, on the 14th day of the last immunization, with the prediction peptide HPV58 $E7_{72-80}$ as antigen, the AD-HPV16/18/58 mE6E7 vaccine containing HPV58 E7 protein can produce specific antibody in the immunized mice serum. The antibody titers of vaccine group were 1:25,600, but the antibody titers of PBS group and AD-NC group were 1:400. Compared with the two control groups, the antibody titers of AD-HPV16/18/58 mE6E7 vaccine were significantly increased (P<0.05). HPV58 $E7_{72-80}$ peptide can activate humoral immunogenicity of the mice immunized by the vaccine containing HPV58E7 antigen.

Cellular immunogenicity of the peptide HPV58 $E7_{72-80}$. To test cellular immunogenicity of the peptide HPV58 $E7_{72-80}$, PBMC from the immunized mice were isolated and added to a 24-well plate at a concentration of $2x10^5$ cells/well and in a volume of 200 μl. Then the cells were stimulated with 10 μg/ml of the peptide HPV58 $E7_{72-80}$. IL-2 100 u/ml was added to the cells every other day. The splenocytes were co-cultured with the peptide HPV58 $E7_{72-80}$ for a week. Seven days later, the splenocytes from the immunized mice were tested directly ex vivo in IFN-γ Elispot assays against the peptide HPV58 $E7_{72-80}$. AD-HPV16/18/58 mE6E7 vaccine can induce specific cellular

Table I. Prediction results for HPV58 E7 epitope peptides.

Peptide number	The initial position of the amino acid	The terminal position of the amino acid	Amino acid sequence
P1	7	15	TLREYILDL
P2	14	22	DLHPEPTDL
P3	69	77	CINSTTTDV
P4	72	80	STTTDVRTL
P5	79	87	TLQQLLMGT
P6	83	91	LLMGTCTIV

HPV, human papilloma virus.

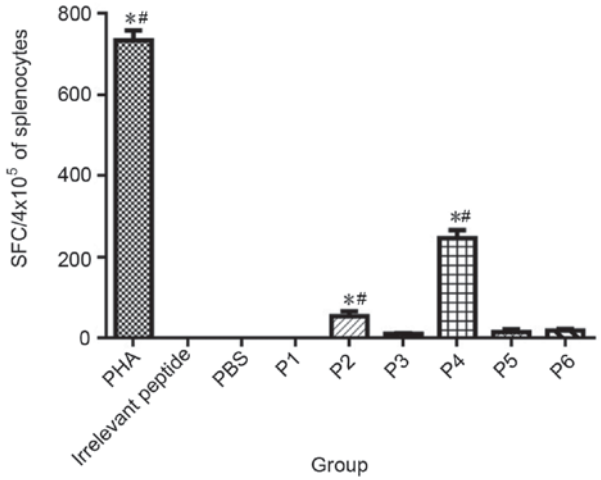

Figure 1. HPV58 E7-specific T-cell response to the candidate peptides in human leukocyte antigen-A2 (+) human PBMC as measured by IFN-γ enzyme-linked immunospot assay. IFN-γ-positive spots were detected in the PHA, P2 and P4 groups, whereas other experimental groups and the two negative control groups exhibited no or few spots formation. The average numbers of IFN-γ-positive spots in the PHA, P2 and P4 groups were 737±17.54 SFC/1x10^5, 50.61±5.37 SFC/1x10^5, 266±34.42 SFC/1x10^5, respectively. The spot numbers in these three groups were significantly higher than other experiment groups and the two negative control groups. *P<0.05 vs. the PBS group. $^\#$P<0.05 vs. the irrelevant peptide group. IFN, interferon; PBS, phosphate-buffered saline; SFC, spot-forming cells; HPV58 E7, human papillomavirus; PBMC, peripheral blood mononuclear cells; PHA, positive control.

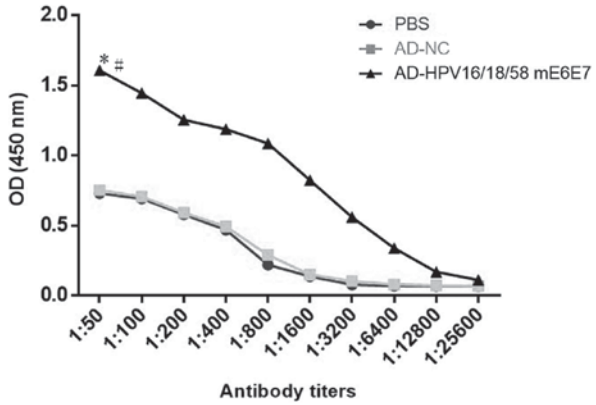

Figure 2. The 14th day antibody titers in three groups following the final immunization. The antibody titers of AD-HPV16/18/58 mE6E7 vaccine group were 1:25,600. The antibody titers of the PBS and AD-NC group were 1:400. Compared with the two control groups, the antibody titers of the AD-HPV16/18/58 mE6E7 vaccine group were significantly increased *P<0.05 vs. the PBS group and $^\#$P<0.05 vs. the AD-NC group. PBS, phosphate-buffered saline; HPV, human papillomavirus.

immune responses against the HPV58 E7$_{72\text{-}80}$ peptide in the C57BL/6 mice after three immunizations, the average number of IFN-γ-positive spots was 143±32.13 SFC/1x10^5. The positive spots were significantly increased compared with the negative groups as PBS (8±5.29 SFC/1x10^5, P<0.01) and AD-NC empty vector (8±5.13 SFC/1x10^5, P<0.01) (Fig. 3). HPV58 E7$_{72\text{-}80}$ peptide can also activate cellular immunogenicity of the mice immunized by the vaccine containing HPV58 E7 antigen.

Discussion

Peptide vaccines have attracted increasing attention because they possess the ability to induce a single specific immune response. At present, studies evaluating HPV therapeutic polypeptide vaccines have focused on the HPV E6 and E7 proteins and their CTL epitope (14). The HPV E6 and E7 proteins contain multiple CTL epitope that have high affinity for HLA class I molecules. Therefore, we can identify epitope peptides with both strong immunogenicity and high specificity (15). This process is key for the preparation of HPV therapeutic polypeptide vaccines. Despite the high HPV58 infection rate in south China, where HPV58 is third after HPV16 and 18, the HPV58 infection rate is very low in western countries; moreover, HPV58 infection distribution differs by region. Research and development of HPV58 peptide vaccines has not received much attention because no accepted CTL epitope has been identified to date. Thus, the study and development of related therapeutic vaccines are difficult.

Specific cellular immunity mediated by CTL plays a key role in cancer immunity. Effector T cells mainly recognize CTL epitopes that are bound to MHC-I molecules to induce cytotoxic cytotoxicity (16). HLA-I molecules are key to antigen presentation and activation of T cells. It is very important for the antigen presentation of virus antigen, tumor antigen, and so on. HLA-A2 is the most specific HLA-I molecule. Its distribution has significant ethnic, ethnic and geographical differences. Studies have shown that the frequency of allele distribution varies from 10 to 40% in different ethnic groups. HLA-A2 (+) accounts for approximately 53% of Chinese population (17).

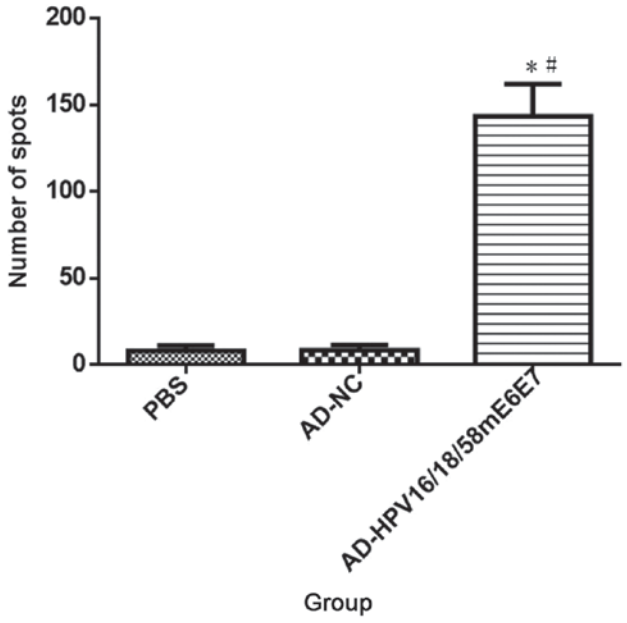

Figure 3. Specific T-cell responses were tested directly *ex vivo* in IFN-γ enzyme-linked immunospot assay assays against the HPV58 $E7_{72-80}$ peptide. The average number of IFN-γ-positive spots was 143.3±32.13 SFC/1x10^5 in the AD-HPV16/18/58 mE6E7 vaccine group. The positive spots were significantly enhanced compared with those of control mice immunized with PBS group (8±5.29 SFC/1x10^5), or AD-NC empty vector group (28±5.13 SFC/1x10^5). *P<0.01 vs. the PBS group and #P<0.01 vs. the AD-NC group. IFN, interferon; PBS, phosphate-buffered saline; SFC, spot-forming cells; HPV58 E7, human papillomavirus; AD, adenovirus; NC, negative control.

Therefore, it is important to study the HPV58 E6 and E7 HLA-A2-restricted CTL epitope peptide in Chinese population. At present, some HLA-A2 (+) CTL epitopes in HPV16 E7 protein, such as $E7_{11-19}$ and $E7_{49-57,}$ have been identified and accepted (18-20). At present, many studies have confirmed the effectiveness of these epitopes as antigen targets (21-24). However, there are few studies about HPV58. Chan identified three HLA-A11-restricted HPV58 E7 peptides (amino acid positions 78 to 86, 74 to 82, and 88 to 96) showed a positive response, but without HLA-A2-restricted E7 peptides (25). Sabah confirmed the peptide region for the E7 protein ranged from27 to 33amino acids and a 9-m epitopes, SSDEDEIGL, in HPV58 E7 were the most potential T cell epitope. And the peptide sequences could interact with as many as seven MHC-1 alleles and showed population coverage up to 90.31% (26).

The CTL epitope for the HPV E6 and E7 antigens were screened by acid elution or the use of synthetic overlapping peptides. However, these two methods are costly and time consuming; additionally, the methods can result in a high miss rate because four to eight amino acid residues are separated by two overlapping peptide segments. Time and money can be saved by using internationally recognized epitope prediction sites. Therefore, in our study, HPV58 E7 HLA-A2-restricted CTL epitope peptides were predicte d using several related websites: the HLA binding peptide prediction website HLA Peptide Binding Predictions, http://www-bimas.cit.nih.gov/molbio/hla_bind/; SYFPEITH, http://www.syfpeithi.de/; and epitope prediction and analysis tools, http://tools.immune epitope.org/main/ (27). After a comprehensive analysis, we preliminarily identified 6 CTL antigen peptides in HPV58 E7 that were subsequently named P1 ($E7_{7-15}$: TLREYILDL), P2 ($E7_{14-22}$: DLHPEPTDL), P3 ($E7_{69-77}$: CINSTTTDV), and P4 ($E7_{72-80}$: STTTDVRTL), P5 ($E7_{79-87}$: TLQQLLMGT), P6 ($E7_{83-91}$: LLMGTCTIV).

In the next study, HLA-A2 (+) PBMC were collected and co-cultured with the six predicted peptides, respectively. In the evaluation of HPV58 E7 peptide-specific T cell immunity in HLA-A2 (+) human PBMC, IFN-γ-production was evident by ELISPOT assay in the P2 and P4 groups. The average numbers of IFN-γ-positive spots in groups P2 and P4 were significantly increased compared to other four experiment groups and the two negative control groups. And P4 showed the strongest response. Therefore, we chose P4 ($E7_{72-80}$: STTTDVRTL) to continue our study. To be viewed as good peptide epitope, a particular sequence must have some key properties. Firstly, the epitope has to be fairly well conserved among HPV's proteome. Secondly, the epitope has capacity that ensures processivity. In addition, the processed peptide has to be able to interact with MHC alleles, and finally the interacting MHC allele should have good population coverage. $E7_{72-80}$ was chose from HPV58 E7 conserved sequence and fulfilled all the parameters mentioned here.

In the further antigenicity verification of the peptide HPV58 $E7_{72-80}$, this peptide can activate not only humoral but also cellular immune reaction of AD-HPV16/18/58 mE6E7 vaccine containing HPV58 E7 antigen, but not the negative control groups as PBS and AD-NC. the antibody titers of the vaccine group were significantly increased. In cellular immunoassays, the average number of IFN-γ-positive spots in the AD-HPV16/18/58 mE6E7 vaccine group wa significantly enhanced compared with PBS group and AD-NC group. This result preliminarily confirmed that the peptide HPV58 $E7_{72-80}$ can be used an HLA-A2-restricted CTL epitope peptide. It can be used as a candidate peptide for peptide vaccine development.

Overall, peptide vaccines are considered to be safe, easy to produce and stable (28). However, their low immunogenicity must first be overcome. Another limitation of peptide-based vaccines is the need to match the patient's HLA that makes it difficult to develop a peptide-based vaccine applicable to the whole population. HPV58 $E7_{72-80}$ is a HLA-A2-restricted CTL epitope peptide and has good antigenicity. HLA-A2 (+) accounts for more than 50% of Chinese population. Therefore, this result provided a potential target epitope for HPV58 E7 protein and lay the foundation for the construction and identification of HPV58 peptide vaccines.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HW performed the experiments and wrote the manuscript. LC predicted and selected the epitopes. WM performed the animals experiment about immunity. YZ divided the lymphocyte from peripheral blood. LQ carried out the immunity test. MC analyzed the experiments data. LL designed and conducted the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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