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Therapeutic potential of an AcHERV-HPV L1 DNA vaccine

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Cervical cancer is strongly associated with chronic human papillomavirus infections, among which HPV16 is the most common. Two commercial HPV vaccines, Gardasil and Cervarix are effective for preventing HPV infection, but cannot be used to treat existing HPV infections. Previously, we developed a human endogenous retrovirus (HERV)-enveloped recombinant baculovirus capable of delivering the L1 genes of HPV types 16, 18, and 58 (AcHERV-HP16/18/58L1, AcHERV-HPV). Intramuscular administration of AcHERV-HPV vaccines induced a strong cellular immune response as well as a humoral immune response. In this study, to examine the therapeutic effect of AcHERV-HPV in a mouse model, we established an HPV16 L1 expressing tumor cell line. Compared to Cervarix, immunization with AcHERV-HPV greatly enhanced HPV16 L1-specific cytotoxic T lymphocytes (CTL) in C57BL/6 mice. Although vaccination could not remove preexisting tumors, strong CTL activity retarded the growth of inoculated tumor cells. These results indicate that AcHERV-HPV could serve as a potential therapeutic DNA vaccine against concurrent infection with HPV 16, 18, and 58.

Keywords: human papillomavirus, vaccine, immunity, cytotoxic T-lymphocytes, anti-tumor

Introduction

Human papillomavirus (HPV) is the primary etiologic agent of cervical cancer. Two commercial HPV vaccines, Cervarix and Gardasil, effectively prevent persistent infections, as well as progressive diseases associated with HPV16 and HPV18 (Lu *et al.*, 2011; Romanowski, 2011; Kwang *et al.*, 2012; Kim *et al.*, 2013; Bissett *et al.*, 2014). However, these vaccines cannot be used to treat existing infections, or other HPV-associated diseases, including cervical cancer. Although vaccination has been successful at reducing new infections, treatment options for individuals already infected with HPV remain limited. The development of new therapeutic vaccines for the treatment of HPV-associated diseases is therefore urgently needed (Lehtinen and Dillner, 2013).

Numerous therapeutic vaccines targeting HPV16 or HPV18 have been developed over the past few decades, a small number of which having been investigated in clinical trials (Monie *et al.*, 2009; Bosch *et al.*, 2013). HPV early proteins E6 and E7 induce the malignant transformation of cells by binding to p53 and pRb, and disrupting normal cell cycle regulation. This central role in viral pathology has made these proteins the ideal target for therapeutic vaccines, with strategies ranging from chimeric virus-like particles (VLPs) incorporating E6 or/and E7, recombinant viruses, and peptides/protein fusions incorporating the E6 and E7 proteins (Trimble and Frazer, 2009). This approach has recently been validated clinically, with an E6/E7 peptide vaccine demonstrating modest efficacy for the treatment of cervical intraepithelial neoplasia (CIN), a pre-cancerous form of cervical cancer (Kenter *et al.*, 2009). This encouraging result brings hope that a papillomavirus VLP-based vaccine may also be effective in eradicating existing chronic papillomavirus infections if strong enough cellular immune responses can be achieved. However, despite promising results, no therapeutic VLP-based HPV vaccines have been shown to exhibit significant clinical efficacy against CIN (Kawana *et al.*, 2012).

Because HPV E6 and E7 antigens are selectively maintained and constitutively expressed only in infected cells and malignant tumors, most therapeutic HPV vaccines strategies have focused on eliciting T-cell immune responses against HPV E6 and E7 (De Bruijn *et al.*, 1998; Zheng *et al.*, 2008; Ma *et al.*, 2012). In contrast, cell-mediated immune responses against the HPV L1 antigen are believed to be of limited therapeutic value in established HPV-infected cervical lesions, and have therefore not been intensively investigated (Bellone *et al.*, 2009). Nevertheless, the L1 antigen has been shown to elicit neutralizing antibodies through the help of T cells, with potent antiviral activity in infected hosts (Rudolf *et al.*, 2001). Mucosal immunization with an HPV16 L1 VLP has been shown to inhibit growth of HPV16-expressing tumor cells in both prophylactic and therapeutic settings (De Bruijn *et al.*, 1998; Revaz *et al.*, 2001). Similarly, an intranasal HPV-16 L1 VLP vaccine induces vaginal IgA responses via the activation of vaginal Th1-like CD8+ T-cell-mediated cytotoxicity (Dupuy *et al.*, 1999). In addition, subcutaneous or nasal immunization of C57BL/6 mice with HPV16 L1 capsomere particles induces both L1-specific antibodies and cytotoxic T-lymphocytes (CTL) responses *in vitro*, resulting in the regression of L1-expressing tumor cells *in vivo* (Ohlschlager *et al.*, 2003).

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Previously, we demonstrated that human endogenous retrovirus (HERV) enveloped, replication-deficient baculovirus delivering HPV L1 genes (AcHERV-HPV) induced high levels of humoral and cellular immunity, conferring complete protection against HPV type-specific pseudovirions (Lee *et al.*, 2010, 2012; Cho *et al.*, 2014). Here, we examined whether the trivalent HPV L1 DNA vaccine exhibits antitumor efficacy in a murine model of HPV L1-associated cervical cancer.

Materials and Methods

Generation of AcHERV-HPV

AcHERV-HPV was produced using the Bac-to-Bac baculovirus expression system (Invitrogen) according to the manufacturer's instructions (Cho *et al.*, 2014). Briefly, a codon-optimized HERV envelope gene was embedded downstream from polyhedron promoter (GenBank accession number NM014590; GenScript Corp.) and three HPV genes, 16 L1, 18 L1, and 58 L1 (kindly supplied by Dr. Schiller, National Cancer Institute, National Institutes of Health) were constructed in recombinant baculoviruses under the control of the human elongation factor1α promoter, respectively. AcHERV-HPV was generated and amplified in *Spodoptera frugiperda* 9 (Sf9) cells.

Generation of stable B16F10-L1 cell lines

The HPV16 L1 gene was amplified from plasmid p16L1L2 DNA by PCR using primers 5′-AAAGCTAGCCCACCATGAGCCTGTGGC-3′ and 5′-AAATCTAGAAGCTTTCACAGCTTCCTC-3′, and then cloned into the *Not*I site of plasmid pcDNA3.1(+) (Invitrogen). The generated pcDNA3.1-1 was transfected into B16F10 mouse melanoma cells using Lipofectin (Invitrogen), and selection was performed with 800 μg/ml of G418 (Invitrogen). To confirm expression of the HPV16 L1 protein, B16F10-L1 cells were subjected to western blot analysis and immunofluorescence assays (IFA) using an anti-L1 antibody, Camvir-1 (Santa Cruz).

Mouse models

Six-week-old female C57BL/6 mice were purchased from Orient-Bio and housed in filter-top cages, with water and food provided *ad libitum*. The use of animals in these experiments was approved by the Institutional Animal Care and Use Committee of Konkuk University (Approval No. KU12078).

For each experiment, mice were divided into three groups: an AcHERV-HPV group (n = 14) which was immunized with 1×10^7 plaque-forming units (PFU) of AcHERV-HPV, a Cervarix group (n = 14) which received 5% of a normal human dose of Cervarix (GlaxoSmithKline), and a PBS control group (n = 14) which was treated with 50 μl of PBS. Each group was immunized intramuscularly on the left leg on days 0, 14, and 28. Serum and vaginal wash samples were then collected at 2, 4, and 6 weeks after the first immunization.

Anti-HPV16 L1 antibody assay

HPV16 pseudoviruses (PVs) were produced as described previously (Buck *et al.*, 2005; Xu *et al.*, 2006) using capsid-encoding plasmid p16L1/L2 and pYSEAP. ELISA was used to detect IgG and IgA antibodies to HPV16 L1 in the mice. Endpoint titers were defined as the highest serum dilutions at which the absorbance value was equivalent to that of non-immunized mice, and were expressed as group geometric means ± standard deviations (SDs). The PV neutralizing antibody (NAb) assay was performed as described previously (Cho *et al.*, 2014). Neutralization titers were defined as the reciprocal of the highest dilution factor that caused ≥ 50% reduction in SEAP activity.

Measurement of interferon (IFN)-γ and interleukin (IL)-4 production by enzyme-linked immunospot (ELISPOT) assay

To analyze T cell responses induced by immunization with AcHERV-HPV, spleens were collected 8 weeks after the first injection. HPV16 L1-specific CD8+ T cells were indirectly quantified by measuring production of IFN-γ and IL-4 following HPV challenge using murine IFN-γ and IL-4 ELISPOT kits (BD Biosciences) according to the manufacturer's instructions.

Cytotoxic T-lymphocyte (CTL) assay

Spleen cells were harvested from vaccinated mice two weeks after the final immunization. The CTL assay was performed by using the CytoTox 96 nonradioactive cytotoxicity assay (Promega) based on the detection of lactate dehydrogenase (LDH) release (Sharma *et al.*, 2012). The percent cytotoxicity was calculated as % Cytotoxicity = (Experimental - Effector Spontaneous - Target Spontaneous) / (Target Maximum - Target Spontaneous) × 100.

Tumor challenge

Two weeks after the final immunization, mice were injected subcutaneously in the left flank with 1×10^4 B16F10 or B16F10-L1 cells. Mice were monitored periodically, and tumor size was recorded for 30 days. Tumor growth was determined by measuring maximal and minimal diameters with a vernier caliper; tumor volumes were calculated according using the formula: volume = (length × width^2) × 0.52 (Li *et al.*, 2010).

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) and corrected for multiple hypothesis testing with the Student-Newman-Keuls post-hoc test using SigmaStat software (Systat Software). P values < 0.05 were considered significant.

Results

Humoral immune responses induced by AcHERV-HPV

The AcHERV-HPV- and Cervarix-immunized groups elicited strong anti-HPV16 L1 IgG immune responses relative

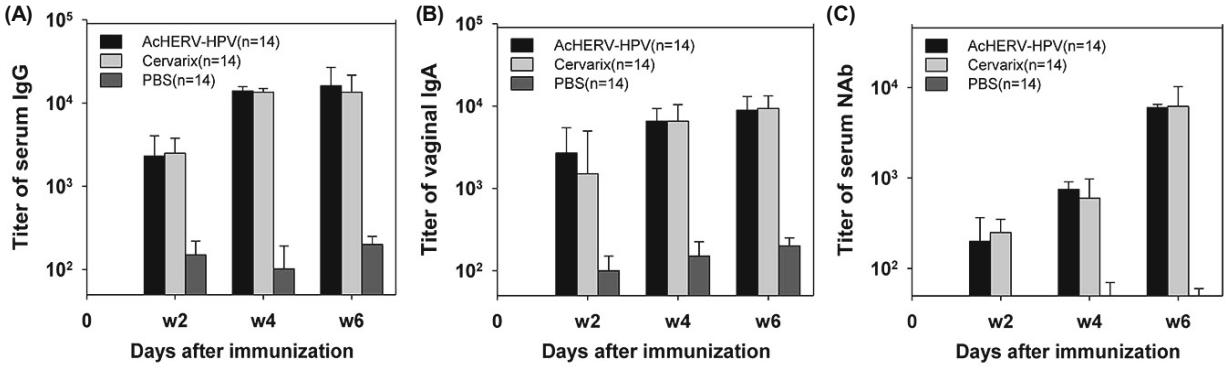

Fig. 1. Measurement of HPV16 L1-specific antibody titers after immunization. Mice were immunized with AcHERV-HPV, Cervarix, or PBS at two week intervals, three times a week. Blood collection and vaginal washes were performed at 2, 4, and 6 weeks after initial injection. HPV16 L1-specific serum IgG antibody titers (A), vaginal IgA antibody titers (B), and neutralizing antibody titers (C) were analyzed by ELISA. Representatives of two independent experiments performed in duplicate are shown. * $P < 0.05$ compared with other groups (ANOVA and Student-Newman-Keuls test).

to controls ($P < 0.002$). Serum IgG titers from mice immunized with AcHERV-HPV were similar to that of the Cervarix group (Fig. 1A).

Vaginal secretions of the AcHERV-HPV and Cervarix-immunized groups (Fig. 1B) exhibited significantly increased levels of anti-HPV16 L1 IgA relative to controls ($P < 0.005$).

Similar to serum IgG and vaginal IgA antibodies, high level of serum NAbs were induced in both the AcHERV-HPV and Cervarix groups (Fig. 1C). Taken together, these data clearly demonstrated that immunization with AcHERV-HPV is as effective as Cervarix® for inducing humoral responses against HPV L1.

HPV16 L1-specific Th1 and Th2 cell responses

Immunization with AcHERV-HPV induced two-fold higher production of IFN-γ compared to Cervarix (Fig. 2A), and triggered similar levels of IL-4 production compared to Cervarix plus HPV16PV stimulation (Fig. 2B). AcHERV-HPV immunization also yielded significantly higher levels of IFN-γ and IL-4 compared to PBS controls ($P < 0.001$). Together, these results suggest that AcHERV-HPV triggers the simultaneous inductions of Th1 and Th2 immune responses.

Immunization with AcHERV-HPV induces L1 specific CTLs

Splenocytes harvested from mice immunized with AcHERV-HPV or Cervarix exhibited L1-specific cytotoxic responses, which were completely absent from immunized with PBS alone (Fig. 3A–C). The AcHERV-HPV group exhibited higher cytotoxicity in response to HPV16 L1 compared to Cervarix group. The extent of L1-specific lysis directly correlated with the ratio of effector cells to target cells. These data show that immunization with AcHERV-HPV leads to efficient re-stimulation of L1-specific CTLs, with greater efficacy than that of Cervarix.

Expression of HPV L1 protein in cells

Expression of the HPV16 L1 protein in B16F10-L1 cells was confirmed by western blot analysis using Camvir-1 as the primary antibody (~52 kDa; Fig. 3D). HPV16 L1 expression was also confirmed via immunofluorescent staining of B16F10-L1 cells using the Camvir-1 antibody (Fig. 3E).

Antitumor activity of the AcHERV-HPV vaccine

To evaluate the therapeutic potential of AcHERV-HPV, its antitumor activity was evaluated in a B16F10-L1 cell-transplanted tumor model. Tumor growth was measured and the mean volumes are reported in Fig. 4A. Variable tumor take or growth rate was observed between individual mice. In Fig. 4B, the mean tumor volume of mice immunized with AcHERV-HPV was significantly reduced compared

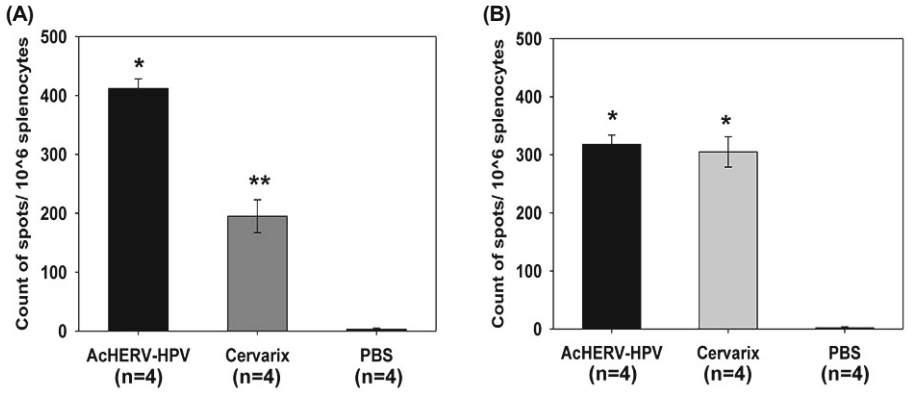

Fig. 2. Analysis of Th1 and Th2 cytokines present in the splenocytes of immunized mice. Splenocytes were harvested 7 weeks after initial immunization, and analyzed by ELISPOT assay to determine the number of (A) IFN-γ-producing HPV16-specific $CD8^+$ T cells, and (B) IL-4-producing HPV-16-specific $CD4^+$ T cells. Values represent the number of spots per 10^6 splenocytes following stimulation with HPV16 PVs. Representatives of two independent experiments performed in triplicate are shown. * $P <$ 0.001 vs PBS control; ** $P < 0.05$ vs PBS control.

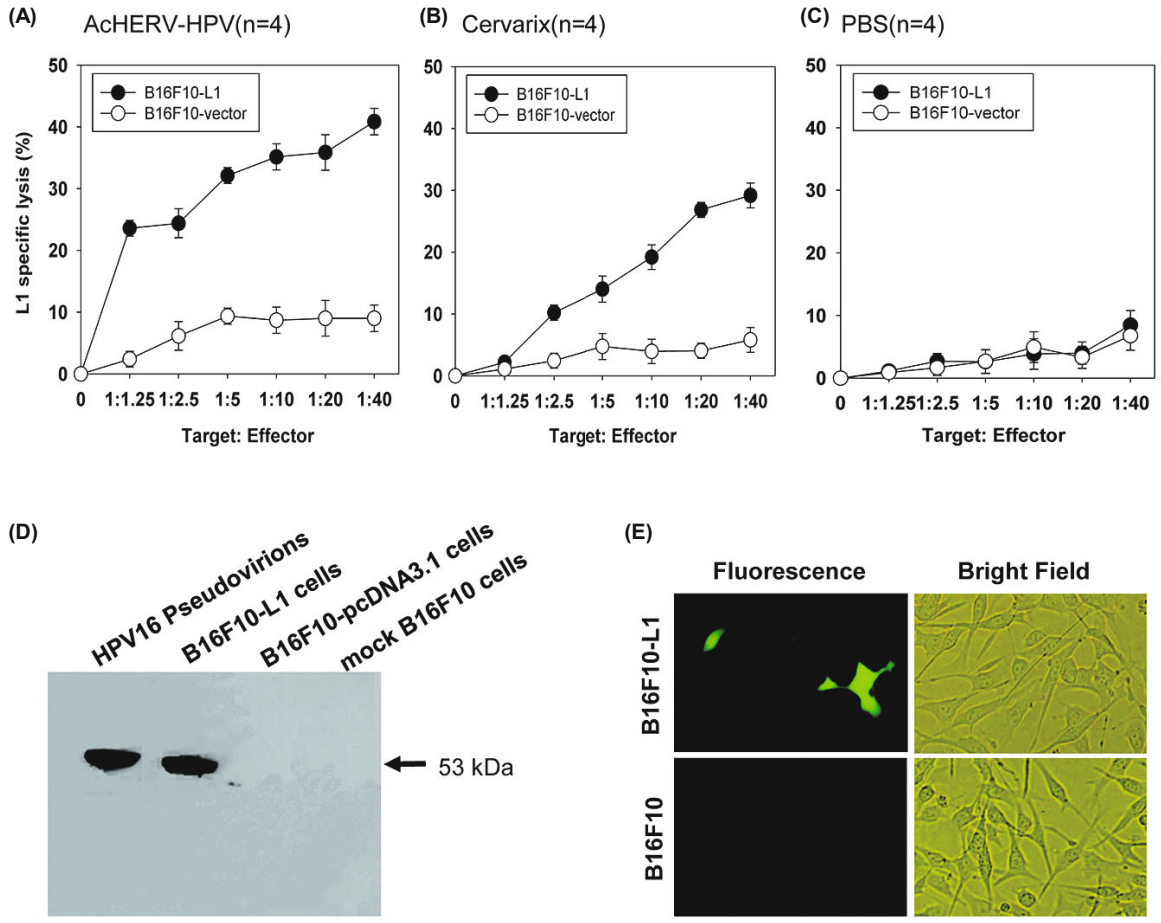

Fig. 3. Analysis of CTL activity and detection of HPV16 L1 protein. CTL responses were measured in C57BL/6 immunized mice with AcHERV-HPV (A), Cervarix (B), or PBS (C) intramuscularly by indirect detection of LDH released from dead target cells. The percentage of L1-specific lysis was denoted as black circles in B16F10-L1 cells and open circles in B16F10-vector cells. Representatives of two independent experiments performed in triplicate are shown. Expression of HPV16 L1 protein was determined by western blot (D) and IFA (E).

with that of the negative control group at day 30 after tumor challenge and 2 of 10 mice remaining tumor free ($P < 0.01$). Although tumor growth was also retarded in the mice immunized with Cervarix ($P < 0.02$), AcHERV-HPV immunization retarded the tumor growth more effectively than Cervarix. Taken together, the antitumor effects of AcHERV-HPV suggest that it has potential as a therapeutic vaccine candidate.

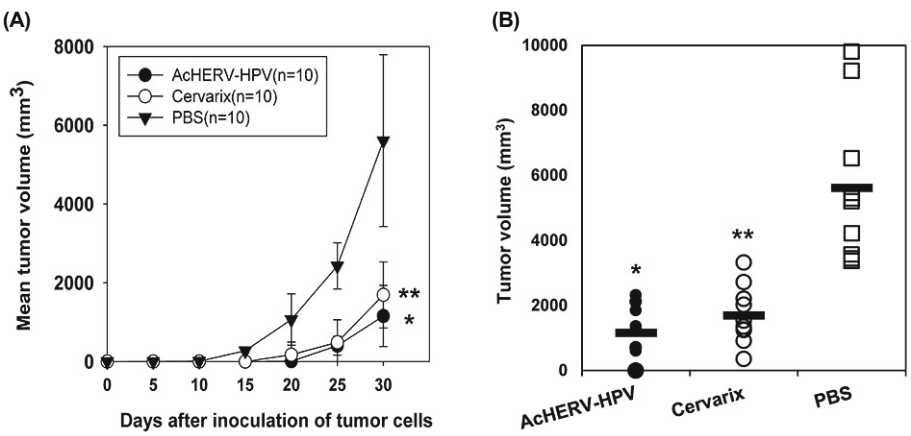

Fig. 4. Measurement of tumor growth in immunized and tumor challenged mice. Tumor size and volume were monitored for 30 days after tumor challenge, in mice immunized with AcHERV-HPV, Cervarix, or PBS. (A) Mean tumor volumes are shown in each immunization group at the indicated time points. Error bars indicate standard errors of the mean. (B) The tumor volumes of individual mice are shown for each immunization group at day 30 after B16F10-L1 cell challenge. Mean tumor volumes are shown using thick horizontal bars. Statistical comparisons of the means were carried out using Student's *t* test. * $P < 0.01$ vs PBS control; ** $P < 0.02$ vs PBS control.

Discussion

HPV vaccines are highly effective at preventing infection by blocking viral entry; however, these vaccines are unable to clear existing infections in patients or HPV-associated malignancies. A variety of therapeutic vaccines have been examined in both preclinical and clinical trials, including proteins, peptides, DNA vaccines, recombinant viruses (Einstein *et al.*, 2007; Gan *et al.*, 2014). Among the many strategies employed, most therapeutic vaccines are focused on E7 protein and suppressing the growth of E7 tumor cells in C57BL/6 mice (Schafer *et al.*, 1999). However, L1 epitopes are prominent on HPV-infected cervical squamous epithelium during the chronic stage. Thus, an HPV L1-targeting DNA vaccine should be more efficient than an E7-targeting vaccine for eradicating HPV during the pre-cancer stage.

The goal of this study was to explore the therapeutic potential of an AcHERV-HPV L1 vaccine. Previously, we described a trivalent HERV-enveloped, baculovirus-based DNA vaccine against HPV16 L1, -18L1, and -58L1 (AcHERV-HPV) capable of inducing high levels of both humoral and cellular immunity (Cho *et al.*, 2014). In this study, we found that IFN-γ and IL-4 levels in splenocytes isolated from mice immunized with AcHERV-HPV were significantly higher than PBS controls. Especially, IFN-γ levels were higher than those seen in response to Cervarix (Fig. 2). We observed that immunization with AcHERV-HPV induced both Th1 and Th2 immune responses in mice. It might be explained by Th2-stimulating adjuvant activity of baculoviral vector (AcHERV). In other study, baculovirus was reported to induce both Th1 and Th2 immune responses, by inducing baculovirus-specific INF-γ and IL4-expressing splenocytes in mice (Luo *et al.*, 2013). Therefore, it is considered that AcHERV baculoviral vector could stimulate the activation of both Th1 and Th2 cell subsets for expressed HPV antigens.

This dual cytokine response was particularly important, as the inhibition of tumor growth and subsequent regression of tumor volume are strongly associated with T cells production both IFN-γ and IL-4. The Th1 cytokine likely induces effector cells, such as CD8+ T cells and NK-T cells, that function to eliminate tumor cells and promote tumor regression (Garcia Paz *et al.*, 2014). In addition to T cell-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity (ADCC) may also be important in tumor therapy (Gan *et al.*, 2014). Anti-HPV antibodies identify E6 or E7 peptides presented on the surfaces of tumor cells, resulting in ADCC, and clearance of tumor cells. Therapeutic vaccines capable of strengthening the immune response against targets such as HPV L1 and E7 may therefore be the key for controlling the progress of HPV-associated malignancies.

Here, we used the B16F10 tumor cell line expressing HPV-16 L1 to establish a murine model of HPV tumorigenesis. Immunization with AcHERV-HPV greatly enhanced HPV16 L1-specific anti-tumor effects in mice. Although such immunization could not remove preexisting tumors, strong CTL activity and Th2 response retarded the growth of the inoculated tumor cells.

In conclusion, the strong induction of humoral and cell-mediated immunity by AcHERV-HPV vaccination suggests that this vaccine provides both therapeutic and prophylactic treatment of HPV-associated malignancies.

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References

Bellone, S., El-Sahwi, K., Cocco, E., Casagrande, F., Cargnelutti, M., Palmieri, M., Bignotti, E., Romani, C., Silasi, D.A., Azodi, M., *et al.* 2009. Human papillomavirus type 16 (HPV-16) virus-like particle L1-specific CD8+ cytotoxic T lymphocytes (CTLs) are equally effective as E7-specific CD8+ CTLs in killing autologous HPV-16-positive tumor cells in cervical cancer patients: implications for L1 dendritic cell-based therapeutic vaccines. *J. Virol.* **83**, 6779–6789.

Bissett, S.L., Draper, E., Myers, R.E., Godi, A., and Beddows, S. 2014. Cross-neutralizing antibodies elicited by the Cervarix(R) human papillomavirus vaccine display a range of Alpha-9 intertype specificities. *Vaccine* **32**, 1139–1146.

Bosch, F.X., Broker, T.R., Forman, D., Moscicki, A.B., Gillison, M.L., Doorbar, J., Stern, P.L., Stanley, M., Arbyn, M., Poljak, M., *et al.* 2013. Comprehensive control of human papillomavirus infections and related diseases. *Vaccine* **31 Suppl 7**, H1–31.

Buck, C.B., Thompson, C.D., Pang, Y.Y., Lowy, D.R., and Schiller, J.T. 2005. Maturation of papillomavirus capsids. *J. Virol.* **79**, 2839–2846.

Cho, H., Lee, H.J., Heo, Y.K., Cho, Y., Gwon, Y.D., Kim, M.G., Park, K.H., Oh, Y.K., and Kim, Y.B. 2014. Immunogenicity of a trivalent human papillomavirus L1 DNA-encapsidated, non-replicable baculovirus nanovaccine. *PLoS One* **9**, e95961.

De Bruijn, M.L., Greenstone, H.L., Vermeulen, H., Melief, C.J., Lowy, D.R., Schiller, J.T., and Kast, W.M. 1998. L1-specific protection from tumor challenge elicited by HPV16 virus-like particles. *Virology* **250**, 371–376.

Dupuy, C., Buzoni-Gatel, D., Touze, A., Bout, D., and Coursaget, P. 1999. Nasal immunization of mice with human papillomavirus type 16 (HPV-16) virus-like particles or with the HPV-16 L1 gene elicits specific cytotoxic T lymphocytes in vaginal draining lymph nodes. *J. Virol.* **73**, 9063–9071.

Einstein, M.H., Kadish, A.S., Burk, R.D., Kim, M.Y., Wadler, S., Streicher, H., Goldberg, G.L., and Runowicz, C.D. 2007. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. *Gynecol. Oncol.* **106**, 453–460.

Gan, L., Jia, R., Zhou, L., Guo, J., and Fan, M. 2014. Fusion of CTLA-4 with HPV16 E7 and E6 enhanced the potency of therapeutic HPV DNA vaccine. *PLoS One* **9**, e108892.

Garcia Paz, F., Madrid Marina, V., Morales Ortega, A., Santander Gonzalez, A., Peralta Zaragoza, O., Burguete Garcia, A., Torres Poveda, K., Moreno, J., Alcocer Gonzalez, J., Hernandez Marquez, E., *et al.* 2014. The relationship between the antitumor effect of the IL-12 gene therapy and the expression of Th1 cytokines in an HPV16-positive murine tumor model. *Med. Inflamm.* **2014**, 510846.

Kawana, K., Adachi, K., Kojima, S., Kozuma, S., and Fujii, T. 2012.

Therapeutic human papillomavirus (HPV) Vaccines: A novel approach. *Open Virol. J.* **6**, 264–269.

Kenter, G.G., Welters, M.J., Valentijn, A.R., Lowik, M.J., Berends-van der Meer, D.M., Vloon, A.P., Essahsah, F., Fathers, L.M., Offringa, R., Drijfhout, J.W., *et al.* 2009. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl. J. Med.* **361**, 1838–1847.

Kim, Y.J., Kwon, M.J., Woo, H.Y., and Paik, S.Y. 2013. Prevalence of human papillomavirus infection and genotype distribution determined by the cyclic-catcher melting temperature analysis in Korean medical checkup population. *J. Microbiol.* **51**, 665–670.

Kwag, H.L., Kim, H.J., and Chang, D.Y. 2012. The production and immunogenicity of human papillomavirus type 58 virus-like particles produced in *Saccharomyces cerevisiae*. *J. Microbiol.* **50**, 813–820.

Lee, H.J., Hur, Y.K., Cho, Y.D., Kim, M.G., Lee, H.T., Oh, Y.K., and Kim, Y.B. 2012. Immunogenicity of bivalent human papillomavirus DNA vaccine using human endogenous retrovirus envelope-coated baculoviral vectors in mice and pigs. *PLoS One* **7**, e50296.

Lee, H.J., Park, N., Cho, H.J., Yoon, J.K., Van, N.D., Oh, Y.K., and Kim, Y.B. 2010. Development of a novel viral DNA vaccine against human papillomavirus: AcHERV-HP16L1. *Vaccine* **28**, 1613–1619.

Lehtinen, M. and Dillner, J. 2013. Clinical trials of human papillomavirus vaccines and beyond. *Nat. Rev. Clin. Oncol.* **10**, 400–410.

Li, Y.L., Qiu, X.H., Shen, C., Liu, J.N., and Zhang, J. 2010. Vaccination of full-length HPV16 E6 or E7 protein inhibits the growth of HPV16 associated tumors. *Oncol. Reports* **24**, 1323–1329.

Lu, B., Kumar, A., Castellsague, X., and Giuliano, A.R. 2011. Efficacy and safety of prophylactic vaccines against cervical HPV infection and diseases among women: a systematic review & meta-analysis. *BMC Infect. Dis.* **11**, 13.

Luo, W.Y., Lin, S.Y., Lo, K.W., Lu, C.H., Hung, C.L., Chen, C.Y., Chang, C.C., and Hu, Y.C. 2013. Adaptive immune responses elicited by baculovirus and impacts on subsequent transgene expression *in vivo*. *J. Virol.* **87**, 4965–4973.

Ma, B., Maraj, B., Tran, N.P., Knoff, J., Chen, A., Alvarez, R.D., Hung, C.F., and Wu, T.C. 2012. Emerging human papillomavirus vaccines. *Expert Opin. Emerg. Drugs* **17**, 469–492.

Monie, A., Tsen, S.W., Hung, C.F., and Wu, T.C. 2009. Therapeutic HPV DNA vaccines. *Expert Rev. Vaccines* **8**, 1221–1235.

Ohlschlager, P., Osen, W., Dell, K., Faath, S., Garcea, R.L., Jochmus, I., Muller, M., Pawlita, M., Schafer, K., Sehr, P., *et al.* 2003. Human papillomavirus type 16 L1 capsomeres induce L1-specific cytotoxic T lymphocytes and tumor regression in C57BL/6 mice. *J. Virol.* **77**, 4635–4645.

Revaz, V., Benyacoub, J., Kast, W.M., Schiller, J.T., De Grandi, P., and Nardelli-Haefliger, D. 2001. Mucosal vaccination with a recombinant Salmonella typhimurium expressing human papillomavirus type 16 (HPV16) L1 virus-like particles (VLPs) or HPV16 VLPs purified from insect cells inhibits the growth of HPV16-expressing tumor cells in mice. *Virology* **279**, 354–360.

Romanowski, B. 2011. Long term protection against cervical infection with the human papillomavirus: review of currently available vaccines. *Human Vaccines* **7**, 161–169.

Rudolf, M.P., Fausch, S.C., Da Silva, D.M., and Kast, W.M. 2001. Human dendritic cells are activated by chimeric human papillomavirus type-16 virus-like particles and induce epitope-specific human T cell responses *in vitro*. *J. Immunol.* **166**, 5917–5924.

Schafer, K., Muller, M., Faath, S., Henn, A., Osen, W., Zentgraf, H., Benner, A., Gissmann, L., and Jochmus, I. 1999. Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumor protection. *Int. J. Cancer* **81**, 881–888.

Sharma, C., Dey, B., Wahiduzzaman, M., and Singh, N. 2012. Human papillomavirus 16 L1-E7 chimeric virus like particles show prophylactic and therapeutic efficacy in murine model of cervical cancer. *Vaccine* **30**, 5417–5424.

Trimble, C.L. and Frazer, I.H. 2009. Development of therapeutic HPV vaccines. *Lancet Oncol.* **10**, 975–980.

Xu, Y.F., Zhang, Y.Q., Xu, X.M., and Song, G.X. 2006. Papillomavirus virus-like particles as vehicles for the delivery of epitopes or genes. *Arch. Virol.* **151**, 2133–2148.

Zheng, Y., Zhang, Y., Ma, Y., Wan, J., Shi, C., and Huang, L. 2008. Enhancement of immunotherapeutic effects of HPV16E7 on cervical cancer by fusion with CTLA4 extracellular region. *J. Microbiol.* **46**, 728–736.