Virology 525 (2018) 205–215

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Epithelial boost enhances antigen expression by vaccinia virus for the generation of potent CD8+ T cell-mediated antitumor immunity following DNA priming vaccination

Jin Qiu[a,b], Shiwen Peng[b], Ying Ma[b,c], Andrew Yang[b], Emily Farmer[b], Max A. Cheng[b], Richard B.S. Roden[d], T.-C. Wu[e], Yung-Nien Chang[b,f,*], Chien-Fu Hung[g,**]

[a] Department of Obstetrics and Gynecology, Tongren Hospital, Shanghai Jiaotong University, School of Medicine, Shanghai, China
[b] Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, United States
[c] Department of Gynecology and Obstetrics, Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong province, China
[d] Department of Pathology, Department of Gynecology and Obstetrics, and Department of Oncology, Johns Hopkins Medical Institutions, Baltimore, MD, United States
[e] Departments of Pathology, Department of Obstetrics and Gynecology, Department of Molecular Microbiology and Immunology, and Department of Oncology. Johns Hopkins Medical Institutions, Baltimore, MD, United States
[f] Papivax Biotech Inc., Taipei, Taiwan, ROC
[g] Department of Pathology and Department of Oncology, Johns Hopkins Medical Institutions, Baltimore, MD, United States

ARTICLE INFO

Keywords:
Human papillomavirus
Therapeutic HPV vaccine
pNGVL4a-Sig/E7(detox)/HSP70
TA-HPV
Pre-clinical model

ABSTRACT

While both pNGVL4a-Sig/E7(detox)/HSP70 DNA vaccine and TA-HPV recombinant vaccinia viral vector-based vaccines have elicited HPV-specific CD8+ T cell responses in HPV16/E7-expressing tumor models, and been used as prime-boost regimen to enhance HPV-specific immune responses in humans (NCT00788164), the optimal route of administration for TA-HPV remains unclear. In a preclinical model, we examined the immunogenicity of priming with intramuscular pNGVL4a-Sig/E7(detox)/HSP70 followed by TA-HPV boost through different administration routes. We observed that priming twice with a pNGVL4a-Sig/E7(detox)/HSP70 followed by a single TA-HPV immunization boost through skin scarification generated the strongest antigen-specific CD8+ T cell response in C57BL/6 mice. These data translate to tumor control and prolonged survival of treated mice. Our results provide rationale for future clinical testing of intramuscular pNGVL4a-Sig/E7(detox)/HSP70 DNA vaccine prime, TA-HPV vaccine skin scarification boost immunization regimen for the control of HPV-associated diseases.

1. Introduction

The prevalence of human papillomavirus (HPV) infections and HPV-associated diseases has remained a global burden, even in an age where preventative HPV vaccines are proven effective (Gupta et al., 2017; Yang et al., 2016a). Not only is HPV a known biologic carcinogen for several cancers including penile, vaginal, anal, vulva, and oropharyngeal (Forman et al., 2012; Maxwell et al., 2016; Mehanna et al., 2013; Wakeham and Kavanagh, 2014), it is also recognized as an etiological factor for many other diseases (Forman et al., 2012; Wakeham and Kavanagh, 2014). Notably, HPV is responsible for causing nearly all cervical cancer cases worldwide, which remains the fourth deadliest female cancer (Wakeham and Kavanagh, 2014). Due to the adverse health effects associated with HPV infections, the need for treatment options for patients with established HPV infections and HPV-associated diseases is imperative.

HPV is a circular, double-stranded DNA virus belonging to the *Papillomaviridae* family (Lee et al., 2016). Uncleared HPV infection can progress into persistent infection, which may further develop into precancerous lesions or cancer, or regress at any stage of the transformation process (Ghittoni et al., 2015; Ostor, 1993). HPV oncoproteins E6 and E7 are believed to assist in the carcinogenesis of HPV-associated lesions by inhibiting the function of tumor suppressive proteins p53 and pRb (Doorbar, 2016; zur Hausen, 2002) and are therefore, required for

* Corresponding author at: Papivax Biotech Inc., 9F-1, No. 57, Section 3, Min-Shen East Road, Taipei 10478, Taiwan, ROC.
** Correspondence to: Departments of Pathology and Oncology, The Johns Hopkins University School of Medicine, CRB II Room 307, 1550 Orleans Street, Baltimore, MD 21231, USA.
E-mail addresses: speng@jhmi.edu (S. Peng), ayang35@jhmi.edu (A. Yang), femily1@jhmi.edu (E. Farmer), max.cheng@jhmi.edu (M.A. Cheng), roden@jhmi.edu (R.B.S. Roden), wutc@jhmi.edu (T.-C. Wu), chung2@jhmi.edu (C.-F. Hung).

https://doi.org/10.1016/j.virol.2018.09.019
Received 10 May 2018; Received in revised form 25 September 2018; Accepted 25 September 2018
0042-6822/ Published by Elsevier Inc.

the initiation and upkeep of HPV-associated malignancies (Doorbar, 2016). E6/E7 can circumvent immune tolerance against self-antigens because they are foreign proteins constitutively-expressed in transformed cells (Ma et al., 2012). For these reasons, E6/E7 have risen to the forefront of therapeutic HPV treatment strategies as ideal therapeutic HPV vaccine targets.

One promising treatment method for existing HPV infections and HPV-associated diseases are therapeutic HPV DNA vaccines (for review see Yang et al., 2016a). They have been widely studied in preclinical and clinical trials for the treatment of HPV-associated diseases (Bagarazzi et al., 2012; Kim et al., 2014; Maldonado et al., 2014). Therapeutic HPV DNA vaccines encode E6 and/or E7 antigens into the plasmid DNA, which are then introduced into the host cells upon vaccination. DNA vaccines do not lead to neutralizing antibodies against the DNA plasmids and can therefore be repeatedly administered (Ma et al., 2012); however, they have low intrinsic immunogenicity. To overcome this and enhance the therapeutic efficacy of DNA vaccines, strategies have been developed to strengthen the immune responses generated by DNA vaccination, including boosting the immune system with a heterologous therapeutic HPV vaccine (for review see Yang et al., 2016b).

Vaccinia virus is a viral vector commonly used to deliver E6/E7 antigens for therapeutic HPV vaccination (Hsieh et al., 2004) because it is extremely infectious and has a low probability of irregular DNA integration into the host's genome (Borysiewicz et al., 1996). TA-HPV is a live recombinant vaccinia virus expressing HPV16/18 E6/E7 proteins. TA-HPV was first used in a 2006 clinical trial in eight patients with late stage cervical cancer (Borysiewicz et al., 1996). TA-HPV has since been frequently used alone or in a prime boost regimen to enhance immune responses of several heterologous vaccines, including TA-CIN (Davidson et al., 2004; Smyth et al., 2004) and pNGVL4a-Sig/E7(detox)/HSP70 (Maldonado et al., 2014).

Both DNA vaccines and TA-HPV are often administered via intramuscular (IM) injection. Although this is a common administration route, it is still unclear what route of administration produces the most robust immune response. Previous data have shown that administration of vaccinia virus-based vaccines through skin scarification (SS) can induce potent immune responses (Liu et al., 2010; Rice et al., 2014). In this study, we examined the therapeutic efficacy of heterologous pNGVL4a-Sig/E7(detox)/HSP70 DNA prime, TA-HPV vaccinia virus boost vaccination regimen in preclinical TC-1 tumor model. We also explored the optimal route of vaccinia virus vaccination to elicit a desired potent antitumor immune response.

2. Materials and methods

2.1. Mice

5–8-week old female C57BL/6 mice were purchased from Charles River Laboratories (Frederick, MD). All mice were maintained at the Johns Hopkins University School of Medicine Oncology Animal Facility (Baltimore, MD) under specific-pathogen free conditions. All procedures were performed according to Johns Hopkins Institutional Animal Care and Use Committee and in accordance with recommendations for proper use and care of laboratory animals.

2.2. Peptides, antibodies and reagents

HPV16/E7aa49–57 peptide, RAHYNIVTF and HPV18/E6aa67–75 peptide, KCIDFYSRI, were synthesized by GenScript (Piscataway, NJ) at a purity of ≥ 80%. FITC and PE-conjugated anti-mouse CD8a (clone 53.6.7), and FITC-conjugated anti-mouse IFN-γ (clone XMG1.2) antibodies, purified anti-mouse CD16/32 (Fc Block™), and 7-Amino-Actinomycin D (7-AAD) were purchased from BD Pharmingen (BD Pharmingen, San Diego, CA). Purified anti-HPV16/E7 monoclonal antibody (clone 8C9) was purchased from Thermo Scientific (Rockford, IL). PE-conjugated, HPV16/E7aa49–57 peptide loaded H2-D^b tetramers were obtained from the National Institute of Allergy and Infectious Diseases Tetramer Facility (Atlanta, GA). G 418 disulfate salt was purchased from Sigma-Aldrich (St. Louis, MO). Purified HPV16 E7 protein was purchased from Protein X Lab (San Diego, CA). Bifurcated needles were purchased from Precision Medical Products, INC (Denver, PA).

2.3. Cell line

TC-1/luc cells expressing the HPV16-E6/E7 proteins and firefly luciferase were developed in our laboratory and have been described previously (Huang et al., 2007). The cells were maintained in RPMI medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin, 400 μg/mL of G418, and 10% fetal bovine serum (FBS).

2.4. Vaccine and vaccination

The generation of pNGVL4a-Sig/E7(detox)/HSP70 (Trimble et al., 2003), pcDNA3-CRT, and pcDNA3-CRT/HPV16/E7 (Cheng et al., 2001a) DNA vaccines have been described previously. To generate pcDNA3-CRT/HPV18/E6 DNA vaccine, HPV18/E6 DNA was synthesized by GenScript and cloned into *Eco*RI/*Hin*dIII of pcDNA3-CRT. A recombinant vaccinia virus expressing HPV16/18-E6/E7, TA-HPV, has been described previously (Borysiewicz et al., 1996). The generation of vaccinia virus expressing luciferase (Lister strain, rVV4) (Chang et al., 2009) and wild-type vaccinia virus (WR strain) (Wu et al., 1995) has also been described previously. For DNA vaccination, 25 μg of designated DNA was prepared in 50 μL using endotoxin-free kit (Qiagen) and injected intramuscularly into biceps femoris muscle. For TA-HPV intramuscular vaccination, mice were injected with TA-HPV virus (50 μL) intramuscularly (biceps femoris muscle). For virus skin scarification, mice were anesthetized and 5 μL of virus at designated dose was applied to tail skin 1 cm from the base of the tail or on the ear. The skin area was then gently scratched 15 times with a bifurcated needle.

2.5. Tetramer staining

For tetramer staining, mice PBMCs were stained with purified anti-mouse CD16/32 first, and then stained with anti-mouse CD8-FITC, and PE-conjugated H-$2D^b$ tetramer loaded with HPV16/E7aa49–57 peptide at 4 °C. After washing, the cells were stained with 7-AAD before flow cytometry analysis to exclude dead cells. The cells were acquired with FACSCalibur flow cytometer and analyzed with CellQuest Pro software.

2.6. Intracellular cytokine staining and flow cytometry analysis

To detect HPV16/E7- and HPV18/E6-specific CD8+ T cell responses by IFN-γ intracellular staining, splenocytes were stimulated with either HPV16/E7aa49–57 or HPV18/E6aa67–75 peptide (1 μg/mL) in the presence of GolgiPlug (BD Pharmingen, San Diego, CA) at 37 °C overnight. The stimulated splenocytes were then washed once with PBS containing 0.5% BSA and stained with PE-conjugated anti-mouse CD8 antibody. Cells were permeabilized and fixed with Cytofix/Cytoperm kit according to the manufacturer's instruction (BD Pharmingen, San Diego, CA). Intracellular IFN-γ was stained with FITC-conjugated rat antimouse IFN-γ. Flow cytometry analysis was performed using FACSCalibur flow cytometer with CellQuest Pro software (BD biosciences, Mountain View, CA).

2.7. ELISA

HPV16 E7-specific antibody response was detected through enzyme-linked immunoabsorbent assays (ELISA) as described previously (Cheng et al., 2001b). Optical density (OD) value was read with xMark