Vaccine 28 (2010) 120–131

Contents lists available at ScienceDirect

Vaccine

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

# *In silico* DNA vaccine designing against human papillomavirus (HPV) causing cervical cancer

Shishir Kumar Gupta[a,*], Archana Singh[a], Mugdha Srivastava[a], Shailendra K. Gupta[b], Bashir Akhlaq Akhoon[c]

[a] *Society for Biological Research & Rural Development, Lucknow 226010, UP, India*
[b] *Indian Institute of Toxicology Research, Lucknow, UP, CSIR, India*
[c] *Department of Bioinformatics, Shri Mata Vaishno Devi University, Katra, Jammu, India*

ARTICLE INFO

*Article history:*
Received 5 June 2009
Received in revised form
17 September 2009
Accepted 22 September 2009
Available online 30 September 2009

*Keywords:*
HPV
Cervical cancer
Vaccine designing
DNA vaccine
Clinical informatics

ABSTRACT

HPV vaccines available in the market are not effective against different strains of papillomavirus, therefore, there is a need to develop a new prophylactic DNA vaccine which can work against different strains of HPVs and may lead to protection of cervical cancer against new pandemic viruses. We designed a potential prophylactic DNA vaccine by using all the consensus epitopic sequences of HPVs L2 capsid protein and performed *in silico* cloning of multiepitopic antigenic DNA sequence in pVAX-1 vector. Immunogenicity of vaccine has been enhanced by techniques like codon optimization, engineering CpG motifs, introducing promoters and co-injection with plasmids expressing immune-stimulatory molecules.


## 1. Introduction

Cervical cancer is 5th most common cancer in women worldwide. An estimated 371,000 new cases of invasive cervical cancer are diagnosed worldwide each year, representing nearly 10% of all cancers in women [1]. Approximately 20 million Americans are currently infected with HPV, and another 6.2 million people become newly infected each year. The American Cancer Society estimates that in 2008, a total of 11,070 women will be diagnosed with cervical cancer in the U.S. [2]. Cervical cancer is a slow growing malignant cancer present in the tissue of the cervix or cervical area in women. The human papillomavirus (HPV) is the etiological agent in cervical cancer and has been identified as being a necessary, but not the only cause of cervical cancer [3]. More than 100 different types of human papillomaviruses (HPV) have been identified [4] and on the basis of epidemiologic and phylogenetic relationship 15 HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); 3 were classified as probable high-risk types (26, 53, and 66); and 12 were classified as low-risk types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) [5,6]. The HPV strains 16 and 18 are together responsible for approximately 90% of all cervical cancers [7]. Numerous studies of the epidemiology of cervical cancer have shown strong associations with religious, marital and sexual patterns [8]. The studies conducted during the past 30 years have consistently indicated that cervical cancer risk is strongly influenced by measures of sexual activity: number of sexual partners, age at first sexual intercourse and sexual behavior of the woman's male partners [9,10]. Although it is well established that women with multiple partners and early ages at first intercourse are at high risk, less is known about how these factors interact or how risk is affected by specific sexual characteristics [8]. Tobacco smoking has been a well-known risk factor for cervical cancer [11]. There is an excess risk of cervical cancer associated with long-term use (12 years or more) of oral contraceptives [12]. HPVs have two specific capsid proteins, L1 and L2 that is common in almost all HPV types [13]. Both innate and acquired immune responses are activated against HPV [14]. Acquired immunity is mediated by the activity of B and T cells. T cells become activated upon recognizing viral proteins and produce additional cytokines that induce growth and maturation of B cells. B cells reside in the lymphoid tissue in the genital tract and are activated by interaction with T cells and binding of HPV antigens to their surface antibodies. An antibody response to HPV is initiated by the capacity of neutralizing antibodies to specifically recognize or react with L1 or L2 [15] major and minor HPV capsid proteins, respectively. The introduction of the HPV vaccine presents several unique challenges, for example,

* Corresponding author. Tel.: +91 9451157798.
*E-mail address:* shishir.bioinfo@gmail.com (S.K. Gupta).

0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.09.095

to obtain maximum effectiveness, the vaccine needs to be administered prior to the onset of sexual activity, which means vaccinating young adolescents [16]. Potential opposition to the vaccine ascribed to fears that vaccination will lead to increased sexual disinhibition have been explored [17,18]. Yet, studies to date have suggested that the vaccine's protective effect and ability to prevent cervical cancer override concerns of increased sexual promiscuity [19]. Two HPV vaccines GARDASIL and CERVARIX are present but they are not completely effective against all the strains of this virus. Therefore the development of new prophylactic DNA vaccine is desirable that can work against all the different strains of HPV virus. Both the L1 and L2 capsid proteins have been well investigated as potential vaccine candidates [20]. *In vitro* neutralization studies with L2 antisera demonstrate high cross-reactivity and suggest common epitopes in HPV L2 proteins [21,22]. Viral protein degrades into small peptides in the cells by a sequence of event called antigen processing pathway. In predicted peptides, identifying epitopes that can elicit helper T cells immune responses is the crucial step [23]. We used *in silico* techniques to design a computational prophylactic DNA vaccine by using all the consensus epitopic sequences of HPV-56 L2 capsid protein. Further searching of MHC class-I and -II epitopes can be done by using computational algorithm. These algorithms predict motifs for both MHC class-I and -II restricted epitopes and identify clusters of these motifs as most likely candidate for epitopes prediction [24]. In the present work an efficient DNA vaccine is designed using choice of a suitable expression vector, ensuring optimal expression by codon optimization, engineering CpG motifs for enhancing immune responses and providing additional sequence signals for efficient translation. DNA vaccines have been one of the latest developments in vaccine technology. DNA vaccines are essentially plasmids capable of expressing an antigenic peptide in the host [25]. These expressed proteins are recognized as foreign in the cells of the body. They are processed by the host cells and displayed on their surface to alert the immune system and trigger body's immune responses. DNA vaccines have become an attractive alternative to conventional methods due to the fact that it can elicit sustained cell-mediated as well as humoral immune responses, which is very much important in combating pathogenic organisms, especially intracellular pathogens. Vaccine efficacy can be assessed by correlating the vaccine's immunogenicity such as its ability to induce CD8+ or CD4+ T cells to the HPV oncoproteins with its ability to protect vaccinated animals against formation of tumors or to cause clearance of already established tumors [26]. Recently several techniques like optimizing codons, CpG optimization and promoter and resistance gene insertion have been tried to enhance the immunogenicity of DNA vaccines [25].

## 2. Materials and methods

### 2.1. Sequence retrieval

Sequence of putative minor capsid protein L2 from HPV-56 (accession no. ABO76822.1) was retrieved from Entrez protein database available at NCBI (http://www.ncbi.nlm.nih.gov).

### 2.2. Phylogenetics of HPV L2 capsid

79 sequences of different strains of HPV L2 capsid proteins were collected from the Entrez protein database available at NCBI and UniProtKB database available at UniProt (http://www.uniprot.org). The reference sequence and the above collected sequences were subjected to multiple sequence alignment (MSA) using ClustalX 2.0.11. Alignment file was used to estimate evolutionary distance of HPV-56 from other strains using distmat program of mEMBOSS 6.0.1. Uncorrected distances algorithm was employed to observe number of substitution per 100 amino acids. Considering

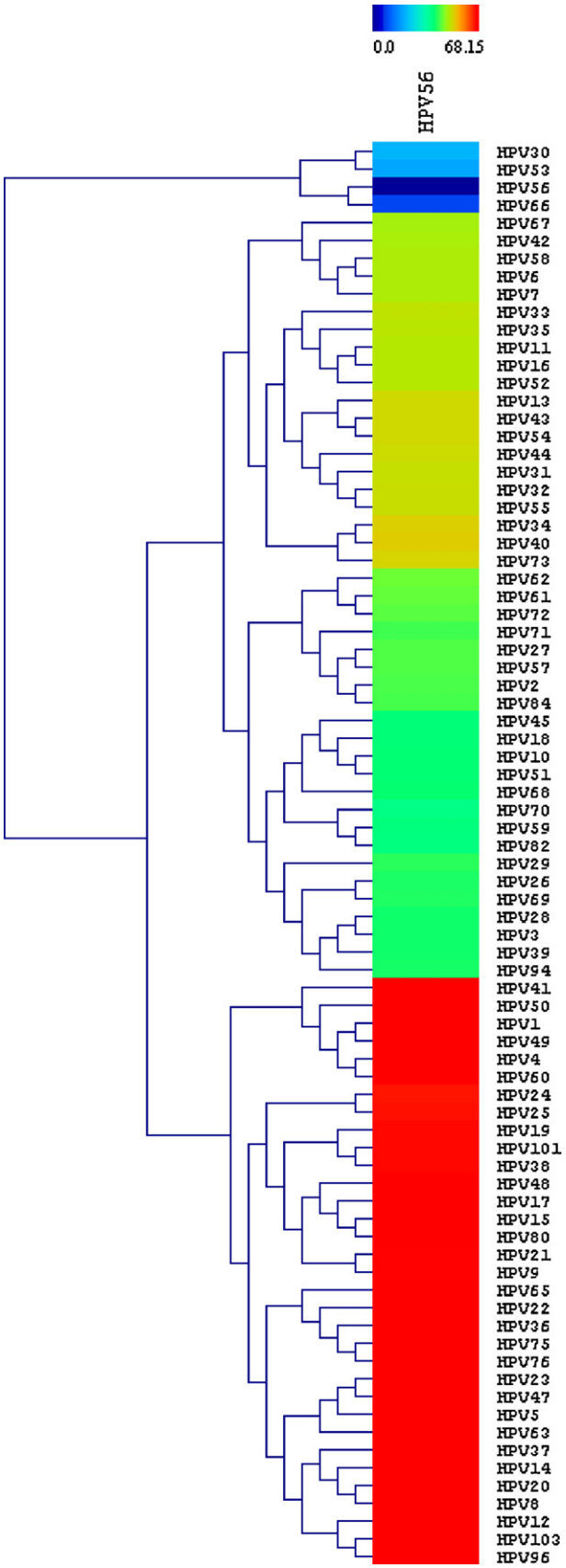


**Fig. 1.** Dendogram relating L2 protein of HPV strains.