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Review Article

DNA vaccines for cervical cancer

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Abstract: Human papillomavirus (HPV), particularly type 16, has been associated with more than 99% of cervical cancers. There are two HPV oncogenic proteins, E6 and E7, which play a major role in the induction and maintenance of cellular transformation. Thus, immunotherapy targeting these proteins may be employed for the control of HPV-associated cervical lesions. Although the commercially available preventive HPV vaccines are highly efficient in preventing new HPV infection, they do not have therapeutic effects against established HPV infection or HPV-associated lesions. Since T cell-mediated immunity is important for treating established HPV infections and HPV-associated lesions, therapeutic HPV vaccine should aim at generating potent E6 and E7-specific T cell-mediated immune responses. DNA vaccines have now developed into a promising approach for antigen-specific T cell-mediated immunotherapy to combat infection and cancer. Because dendritic cells are the most potent professional antigen-presenting cells, and are highly effective in priming antigen-specific T cells, several DNA vaccines have employed innovative strategies to modify the properties of dendritic cells (DCs) for the enhancement of the DNA vaccine potency. These studies have revealed impressive pre-clinical data that has led to several ongoing HPV DNA vaccine clinical trials.

Keywords: Human papillomavirus, cervical cancer, DNA vaccines

Introduction

Cervical cancer is a serious concern and remains a significant cause for cancer-related deaths in women worldwide, though mortality rates have reduced over the years due to early detection and screening programs [1]. It has been established that persistent infection with human papillomavirus (HPV) is the primary causative agent factor of cervical cancer and HPV DNA can been detected in 99.7% of cervical cancers [2]. There are more than 100 types of HPV [3] and among the multiple types, HPV types 16 as well as 18 are most frequently associated with cervical cancer [4]. The understanding of HPV as the etiological factor for cervical cancer has led to the notion of controlling cervical cancer through vaccination against HPV (for review, see [5]).

Understanding of HPV molecular biology will facilitate development of vaccines targeting HPV. The HPV genome is made up of circular, double-stranded DNA and contains about 8,000 base pairs encoding the early and late proteins. The early proteins E1 and E2 are involved in viral DNA replication and viral RNA transcription, E4 is involved in cytoskeleton reorganization and E5, E6 and E7 are responsible for cellular transformation. The late proteins L1 and L2 form the structural components of the viral capsid. In most cases of cervical cancer, the HPV genome integrates into the host chromosomal DNA and disrupts the viral E2 gene. E2 is a transcriptional regulator for the E6 and E7 genes. Thus, loss of the E2 leads to the uncontrolled expression of E6 and E7 proteins, which in turn leads to disruption of the normal cell cycle regulation by interacting with p53 and Rb respectively. This leads to uncontrolled cell cycle and suppression of apoptosis, progressing to HPV-associated cervical cancer (for review, see [6]).

Vaccination could be implemented for the prevention and treatment of HPV-associated diseases, either by generating neutralizing antibodies to block HPV viral infection (preventive vaccines) or by inducing HPV-specific T cell-mediated responses (therapeutic vaccines). The HPV viral capsid proteins, L1 and L2 have been employed as targets for the development of preventive vaccines and are used to generate neutralizing antibodies against HPV.

Currently, there are two commercially available preventive HPV vaccines; Gardasil (including HPV types 6, 11, 16 and 18) developed by Merck and Cervarix (including HPV types 16 and 18) developed by Glaxo Smith Kline. These vaccines are made up of HPV virus-like particles (VLP) derived from L1 major capsid protein. These VLPs can generate neutralizing antibodies against the HPV types that are included in the vaccine. In addition, Gardasil has been shown to effectively reduce the incidence of HPV-associated anogenital diseases in young women [7]. Cervarix has also been shown to protect against HPV-16/18-related persistent infections and CIN2 lesions [8].

Preventive vaccines, however, have not been shown to provide therapeutic effects against pre-existing HPV infections. Furthermore, because of the considerable burden of HPV infections worldwide, it is estimated that it will take decades for preventive vaccines to significantly reduce the prevalence of cervical cancer. Thus, for the current treatment of cervical cancer and their precursor lesions, it is important to focus on the development of therapeutic HPV vaccines that can generate cellular immunity against HPV-infected cells, thus potentially eliminating preexisting lesions and malignant tumors.

The choice of target antigen is an important factor that needs to be considered in the designing of therapeutic vaccines. While L1 and L2 are suitable targets for the development of preventive vaccines, they are not ideal targets for therapeutic HPV vaccine development. L1 and L2 are not expressed in the basal cells infected with HPV, unlike the HPV early proteins, E6 and E7. The early viral proteins E6 and E7 are expressed early in viral infection and help regulate the progression of the disease. Therefore, therapeutic vaccines should aim to generate T cell-mediated immune responses against the early proteins, E6 and E7. Furthermore, the E6 and E7 genes are co-expressed in HPV infected cells but not in normal cells and are essential for transformation [9]. Therefore, E6 and E7 represent ideal targets for the development of therapeutic HPV vaccines.

Several approaches employing therapeutic vaccines targeting the E6 and E7 antigens have been tested in preclinical and clinical trials, including peptide or protein-based vaccines, live vector vaccines, cell-based vaccines and DNA vaccines. Among the various forms of therapeutic HPV vaccines, DNA vaccines have emerged as an attractive approach for antigen-specific immunotherapy. Not only are naked DNA vaccines safe, stable and easy to produce, but they can also be used to sustain high levels of antigen expression in cells (for a review, see [10, 11]). In addition, DNA vaccines can be repeatedly administered since they do not elicit antibodies against DNA in the patient. However, DNA vaccines are poorly immunogenic since DNA lacks cell type specificity and the ability to amplify or spread to surrounding cells *in vivo*. Thus the strategies to enhance DNA vaccine potency have been an area of active investigation. Most of these strategies aim at targeting the gene of interest to antigen presenting cells (APCs) and modifying the properties of the APCs to enhance the immune responses.

It is now clear that dendritic cells (DCs) play a critical role in the generation of the antigen-specific antiviral and antitumor T cell immune responses. It has been established that cell-mediated immunity is important in the control of viral infections and malignant tumors. $CD8^+$ T cells are involved in the direct killing of viral-infected cells or tumors, while $CD4^+$ T helper cells lead to the expansion of $CD8^+$ immune responses. Immature DCs located in peripheral tissues express various surface receptors which enable them to respond to danger signals indicating the presence of an infection (for a review, see [12]). In response to a danger signal, the DCs undergo a maturation process, upregulating co-stimulatory molecules, thus generating efficient APCs and potent T cell activators. The DCs uptake and process antigens and load the peptides onto major histocompatibility (MHC) class I and class II molecules, which can then be presented on the cell surface. These DCs migrate to the lymphoid organs where they

activate antigen-specific T cells (for review, see [12-14]).

Several innovative strategies have been developed to modify the properties of DCs to enhance antigen-specific T cell immune responses, thus enhancing the DNA vaccine potency. These strategies aim to: 1) increase the number of antigen-expressing DCs; 2) improve antigen expression, processing, and presentation in DCs and; 3) enhance interaction between DCs and T cells. The current review discusses these strategies in detail and the related clinical trials. **Table 1** provides a sum-

Table 1. Strategies to enhance DNA vaccine potency.

Strategies	Sub-strategies	References
Increasing the number of antigen-expressing DCs	*Route of administration:*	
	Gene gun	[18]
	Laser treatment	[24]
	Electroporation	[22]
	Intercellular antigen spreading as a strategy to increase the number of antigen-expressing DCs	[25-28]
	Linkage of antigen to molecules capable of binding to DCs as a method to target antigen to DCs	[30, 33, 34]
	Employment of chemotherapy-induced apoptotic cell death to increase the number of antigen-loaded DCs	[35-39]
Improving antigen expression, processing, and presentation in DCs	*Codon optimization as a strategy to enhance antigen expression in DCs*	[40]
	Employment of demethylating agents to increase antigen expression	[42]
	Employment of intracellular targeting strategies:	
	- to enhance MHC class I antigen presentation in DCs	[33, 43-45, 49]
	- to enhance MHC class II antigen presentation in DCs	[51, 53, 66]
	Enhancing the expression of MHC class I/II molecules	[53]
	Bypassing antigen processing as a method for generating stable antigen presentation in DCs	[54, 55]
Enhancing DC and T cell interaction	*Employing cytokines and costimulatory molecules to enhance T-cell activation*	[56-58]
	Prolonging DC survival to enhance T cell interaction	[59-63]
	Induction of $CD4^+$ T cell help as a strategy for augmenting $CD8^+$ T cell responses	[61, 62] [66, 67]
	Eliminating immunosuppressive regulatory T cells	[68]

mary of each of the strategies employed with specific citations.

Strategies to increase the number of antigen expressing DCs

Several strategies have been developed to increase the number of antigen-expressing DCs including i) optimizing the route of delivery of DNA vaccines, ii) employment of intercellular antigenic spreading, iii) linkage of antigen to molecules capable of binding to DCs as a method to target antigen to DCs and iv) employment of chemotherapy-induced apoptotic tumor cell death to increase the number of antigen-loaded DCs.

The route of delivery plays an important role on the antigen-specific immune response elicited by DNA vaccines. Conventionally, intramuscular or intradermal administration has been used for DNA vaccine delivery (for a review, see [11]). In general, it is desirable to employ efficient routes for *in vivo* delivery of DNA vaccines directly into DCs. Gene gun has emerged as a novel tool for delivery of DNA vaccines directly into DCs compared to previously explored routes (for reviews, see [11, 15]). The gene gun is used to deliver DNA which is coated on gold particles, *in vivo* into intradermal Langerhans cells which then mature and migrate to the lymphoid organs for T cell priming [16, 17] (see **Figure 1**). Recently, it has been shown that cluster (short-interval) intradermal DNA vaccination using gene gun can rapidly induce E7-specific CD8+ T-cell immune responses leading to therapeutic antitumor effects compared to long-interval vaccination [18]. A new model of gene gun, called low-pressured gene gun has been used to deliver naked DNA in solution. The low-pressured gene gun has been shown to generate comparable immunologic responses and antitumor effects compared to the conventional gene gun that delivers DNA loaded on gold particles. Such an approach has also been shown to have less burning effects of the skin compared to conventional gene gun [19]. Electroporation has also emerged as an efficient method for improving the transfection efficiency of HPV DNA vaccines to increase the number of antigen-expressing DCs [20-22]. For example, Best et al. have shown that in a head-to-head comparison study, electroporation-mediated intramuscular delivery could generate the highest $CD8^+$ T cell immune responses compared to gene gun and syringe intramuscular injection [22].

Several innovative delivery mechanisms have been explored in other antigenic systems. For example, a novel route of delivery for DNA vaccines is the liposome-DNA complex patch; this novel topical liposomal route has proven to be an effective DNA vaccination pathway against Japanese encephalitis virus infection [23]. Another recently developed method to improve the transfection efficiency of DNA vaccines in vivo involves the employment of low-energy laser technology. It has been shown that treatment with femtosecond laser immediately after delivery of DNA vaccines can enhance the transfection efficiency of the DNA vaccine [24]. These strategies may potentially be used for the delivery of therapeutic HPV DNA vaccines.

Intercellular antigen spreading has also emerged as a novel approach to increase the number of DCs expressing antigen. It has been shown that linkage of antigen to proteins involved in intercellular transport can improve the spread of encoded antigen. The herpes simplex virus type 1 (HSV-1) tegument protein VP22 has previously been used for DNA vaccine development. VP22 has been linked with several antigens in the context of a DNA vaccine to enhance antigen-specific $CD8^+$ T cell immune responses [25-28]. Although some concerns have been raised whether the intercellular trafficking ability of VP22 is a true phenomenon or attributed to fixation artifacts [26], intradermal administration of DNA encoding antigen linked to VP22 does lead to an increase in antigen-loaded DCs in the draining lymph nodes, resulting in potent antigen-specific immune responses in vaccinated mice [[29].

The number of antigen-loaded DCs can also be enhanced by linking the antigen to molecules that can bind with surface molecules on DCs in the context of DNA vaccines. For example, the linkage of antigen to heat shock protein 70, which binds to scavenger receptors on the surface of DCs such as CD91, may target and concentrate the linked antigen to DCs, thus enhancing antigen-specific immunity [30-32]. DNA vaccines encoding heat shock protein 60 linked to HPV16 E6 or E7 tumor antigens have also been shown to generate more potent immunotherapeutic effects than E6 or E7 tumor antigens alone [33]. Furthermore, the linkage of antigen to Fms-like tyrosine kinase 3-ligand (Flt-3L-E7) has been shown to target antigen to DCs and significantly enhance CD8+ T immune responses in mice compared to wild-type E7

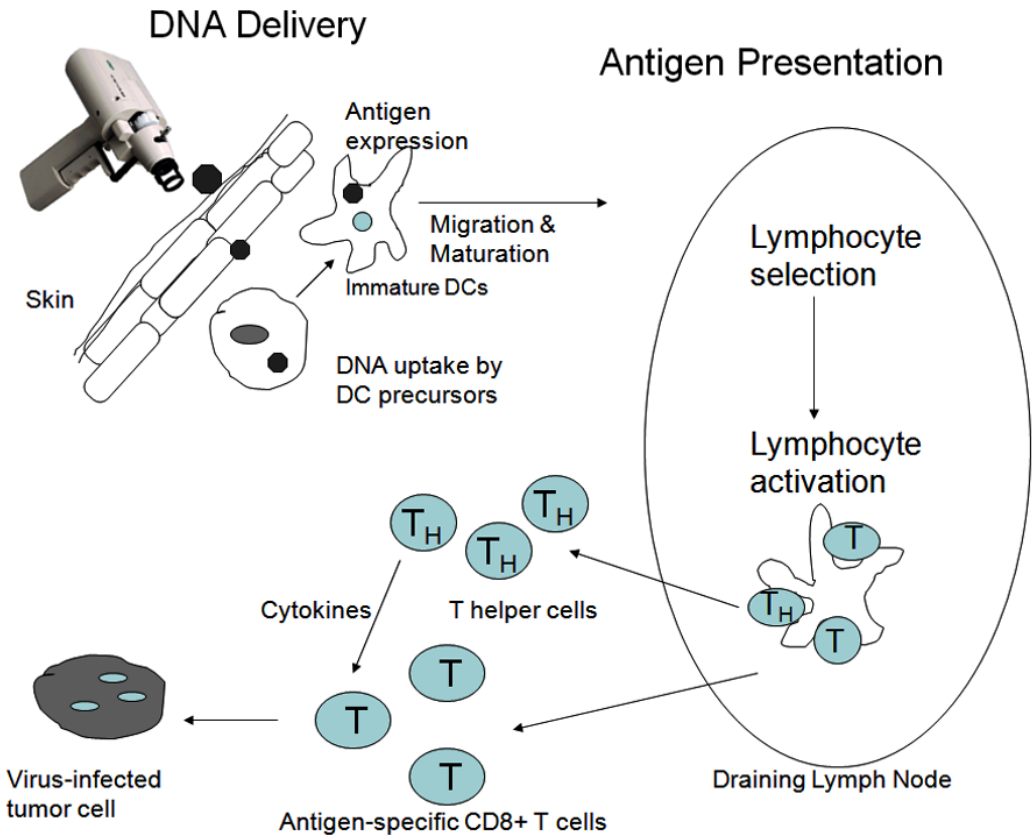

Figure 1. Schematic diagram depicting the delivery of DNA into the skin leading to an antigen-specific systemic immune response. Intradermal administration of DNA via gene gun allows direct delivery of DNA into the skin. This will lead to uptake of DNA by immature DCs in the skin. The antigen-expressing DCs then migrate to the draining lymph nodes where they mature and present the antigen to the CD8+ and CD4+ T cells. This leads to activation of antigen-specific CD8+ T cells that then migrate to the tumor site and generate antigen-specific immune responses against the tumor. Furthermore, the CD4+ T cells may contribute to humoral immunity. The T cells also release cytokines which in turn activate the innate immune system including NK cells and macrophages which also play a role in tumor killing.

DNA [34].

The induction of apoptotic tumor cell death in animals with HPV-associated tumors is another strategy to increase the number of HPV antigen-loaded DCs. Co-administration of therapeutic HPV DNA vaccines with chemotherapeutic agents has been shown to lead to release of HPV E6/E7 antigen from apoptotic tumor cells, which may potentially facilitate antigen uptake by local DCs, resulting in enhancement of DNA vaccine potency. Several drugs, including EGCG (epigallocatechin gallate) [35], cisplatin [36], bortezomib [37], DR5 (death receptor 5) [38] and apigenin [39] have been shown to enhance antigen-specific $CD8^+$ T cell-mediated antitumor immunity induced by coadministration with therapeutic HPV DNA vaccination. Future studies should focus on whether other chemotherapeutic agents could exhibit similar synergistic effects when combined with DNA vaccines.

Strategies to enhance antigen expression, processing, and presentation in DCs

Once the DNA vaccine is taken up by DCs, the efficiency of antigen expression, processing and presentation by DCs significantly impacts the ability of DCs to present the antigenic peptide to prime the antigen-specific T cells. Several strategies have been developed to improve the antigen expression, processing and presenta-

tion of DCs, including i) codon optimization and demethylating agents to improve antigen expression, ii) intracellular targeting strategies to improve MHC I and II presentation of antigen in DCs, iii) strategy to enhance the expression of MHC class I/II molecules iv) MHC class I single-chain trimer (SCT) technology to bypass antigen processing and presentation in DCs.

Codon optimization is a commonly used strategy to enhance the expression of antigen encoded by the DNA vaccine. Codon optimization refers to the modification of antigenic gene sequences by replacing codons that are rarely recognized by cellular protein synthesis machinery with codons that are more commonly recognized. For example, mice immunized with codon-optimized HPV-16 E6 DNA were shown to generate enhanced antigen-specific CD8+ T cell immune responses compared to mice immunized with wild-type E6 DNA [40]. In addition, a DNA vaccine containing codon-optimized modified E7 gene was also shown to be effective in generating antigen-specific T cell immune responses and protective antitumor immunity in vaccinated mice [41].

Another strategy to improve the gene expression of the encoded HPV antigen is the employment of demethylating agents. It has been demonstrated that demethylating agent 5-aza-2'-deoxycytidine co-delivered with an E7 DNA vaccine can overcome gene silencing by methylation of CpG islands in the cytomegalovirus (CMV) promoter region and thus increase the level of expression since CMV promoter is commonly used in DNA vaccines [42].

Antigen processing and presentation may be enhanced by intracellular targeting strategies. Strategies to facilitate MHC class I antigen processing in DCs have been shown to activate large numbers of antigen-specific $CD8^+$ T cells. The linkage of antigen to proteins that target the antigen for proteasomal degradation or entry into the endoplasmic reticulum (ER) can improve MHC class I presentation of the linked antigen in DCs. For example, antigen linked to *Mycobacterium tuberculosis* heat shock protein 70 (HSP70) [43], heat shock protein 60 (HSP60) [33], γ-tubulin [44], calreticulin (CRT) [45-48] or the translocation domain of *Pseudomonas aeruginosa* exotoxin A (ETA(dII)) [49] has been shown to significantly improve MHC class I presentation of the encoded antigens. Several of these studies have resulted in encouraging results which have led to clinical translation.

$CD4^+$ helper T cells have been shown to play an essential role in $CD8^+$ T cell priming and memory T cell generation [50]. Thus, the potency of the DNA vaccines can be significantly improved using strategies to improve MHC class II presentation. For example, it has been shown that the linkage of HPV-16 E7 with the sorting signal of lysosomal-associated membrane protein type 1 (LAMP-1) can target the E7 antigen to cellular endosomal/lysosomal compartments leading to enhance II presentation of E7 antigen [51]. DNA vaccines encoding Sig/E7/LAMP-1 generated stronger E7-specific $CD4^+$ as well as CD8+ T cell immune responses in mice compared to DNA vaccines encoding wild-type E7 alone [52].

Strategies to enhance the expression of MHC class I/II molecules have also been used to improve therapeutic HPV DNA vaccine potency. It has been shown that cells transfected with DNA encoding MHC CIITA, a master regulator of MHC class II expression, can lead to higher expression of MHC I and II molecules on transfected cells, leading to enhanced antigen presentation through the MHC I/II pathways [53]. Furthermore, coadministration of CIITA DNA with the therapeutic HPV DNA vaccines has been shown to enhance the antitumor effects and prolong survival in HPV antigen-expressing TC-1 tumor-bearing mice [53].

MHC class I single-chain trimer (SCT) technology has emerged as an innovative strategy to bypass the antigen processing and presentation. DNA vaccines encoding SCT composed of an HPV-16 E6 CTL epitope linked to the β2-microglobulin and heavy chain of $H\text{-}2K^b$ MHC class I were shown to enhance the E6-specific $CD8^+$ T cell responses in vaccinated mice compared to wild-type HPV-16 E6 DNA alone [54]. These strategies have also been applied in other antigenic systems to enhance DNA vaccine potency [55].

Strategies to increase the interaction between DCs and T cells

The interaction between DCs and T cells may be enhanced by i) employing cytokines and costimulatory molecules to enhance T-cell activation, ii) prolonging the life of DCs, iii) inducing $CD4^+$ helper T cells to augment $CD8^+$ T-cell

response and iv) eliminating immunosuppressive regulatory T cells to increase the number of activated T cells.

DNA vaccines encoding IL-2 linked to HPV-16 E7 antigen have been shown to generate enhanced E7-specific CTL responses and antitumor activity [56]. A DNA vaccine encoding IL-6 linked to (HPV-16) E7 has also been shown to enhance DNA vaccine potency [57]. A recent study showed the enhancement of immunogenicity of a therapeutic cervical cancer DNA-based vaccine by co-application of sequence-optimized genetic adjuvants including DNA encoded cytokines (IL-2, IL-12, GM-CSF, IFN-gamma) and the chemokine MIP1-alpha [58].

Another strategy to prolong DC survival involves employment of DNA encoding antiapoptotic proteins. We have also shown that co-administration of a DNA vaccine encoding HPV-16 E7 with siRNA or shRNA targeting the key proapoptotic proteins Bak, Bax and Fas ligand or coadministration of the DNA vaccine with the antiapoptotic protein Bcl-x_L can all prolong the survival of transfected DCs and enhance E7-specific $CD8^+$ T cell responses against E7-expressing tumors in mice [59-62]. A recent study showed that connective tissue growth factor linked to the E7 tumor antigen could generate potent antitumor immune responses mediated by an antiapoptotic mechanism [63].

Inducing $CD4^+$ helper T cells to augment $CD8^+$ T-cell response may also lead to enhanced DC and T cell interaction. CD4+ T helper cells are known to play an integral role in the generation of CD8+ T-cell immune responses. Based on our understanding of the MHC class II processing pathway involving the MHC class II-associated invariant chain (Ii) (for reviews, see [64, 65]), a novel DNA vaccine was developed, which encodes Ii with the class II-associated invariant chain peptide (CLIP) region of Ii replaced with the pan HLA-DR binding epitope (PADRE) (Ii-PADRE). We have previously shown that vaccination with Ii-PADRE DNA was capable of generating potent PADRE-specific CD4+ T cell immune responses. Furthermore, we demonstrated that co-administration of DNA encoding HPV E6 or E7 antigen with Ii-PADRE DNA led to significantly stronger E6- or E7-specific CD8(+) T -cell immune responses and more potent protective and therapeutic anti-tumor effects [66]. The observed enhancement of the antigen-specific immune responses and antitumor effects are likely due to IL-2 derived from PADRE-specific CD4 (+) T cells [67]. In addition, it has been shown that the potency of HPV-16 E7 DNA vaccines combined with a strategy to prolong the life of dendritic cells (DCs) and/or a strategy to bypass antigen processing could be further enhanced by the addition of a DNA vaccine capable of generating high numbers of pan -HLA-DR reactive epitope (PADRE)-specific CD4+ T cells [61, 62].

The interaction between DCs and T cells may also be enhanced by eliminating immunosuppressive regulatory T cells. It has been demonstrated that using an anti-CD25 monoclonal antibody, PC61, to eliminate the amount of regulatory T cells before E7/HSP70 DNA vaccination potentiate the effects of HPV DNA vaccines [68]. Thus, it may be important to consider strategies to eliminate the suppressive regulatory T cells to improve the effect of therapeutic HPV DNA vaccines.

DNA vaccine clinical trials

Several therapeutic HPV DNA vaccine clinical trials have been completed or are currently ongoing. A microencapsulated DNA vaccine termed ZYC-101 which encodes multiple HLA-A2-restricted E7-specific CTL epitopes (ZYCOS, Inc., now acquired by MGI Pharma), has been tested in patients with CIN-2/3 lesions [69] and in patients with high-grade anal intra-epithelial lesions [70]. The vaccine was found to be well tolerated in both trials. A new version of the vaccine that encodes HPV-16 and HPV-18 E6- and E7-derived CTL epitopes (termed ZYC-101a) was tested in a multicenter, double-blind, randomized, placebo-controlled trial conducted in a group of women with biopsy confirmed CIN2/3. The subjects received 3 intramuscular doses of ZYC-101a or placebo drug. The proportion of subjects whose lesions resolved was higher in ZYC101a groups compared to the placebo but the difference was not statistically significant. Nevertheless, a significantly greater number of CIN2/3 lesions were resolved in women younger than 25 years receiving the DNA vaccine compared to those who received the placebo [71].

Several therapeutic HPV DNA vaccine trials have been tested in patients with HPV-associated lesions. For example, phase I trials

have been conducted using the Sig/E7detox/HSP70 DNA vaccine which encodes a signal sequence linked to an mutated form of HPV-16 E7 with an abolished the Rb binding site (E7detox) and fused to heat shock protein 70 in patients with high-grade CIN (CIN2/3) lesions. The vaccination was considered to be feasible and tolerable in patients with CIN2/3 lesions and no adverse or dose-limiting side effects were observed at any dose level. The patients in the highest dose cohort generated stronger $CD8^+$ T cell immune responses to E7 in peripheral blood mononuclear cells (PBMCs) than patients in lower dose cohorts. Disease regression was observed in 3 of 9 patients in the highest dose cohort post-vaccination [72]. Another phase I trial using the same naked DNA vaccine (Sig/E7detox/HSP70) has recently been completed in HPV-16 positive patients with advanced head and neck squamous cell carcinoma (Dr. Maura Gillison, personal communication). No significant adverse effects were observed in the study and some of the patients developed appreciable E7-specific immune responses.

Another candidate DNA vaccine that is currently being prepared for clinical trials at University of Alabama at Birmingham in collaboration with Johns Hopkins is a DNA vaccine encoding calreticulin (CRT) fused to HPV-16 E7 (E7detox) (Drs. Warner Huh and Cornelia Trimble, personal communication). Intradermal administration of the CRT/E7 DNA vaccine was found to generate significant E7 antigen-specific immune responses in preclinical models (see above). This therapeutic HPV DNA vaccine trial will be performed in HPV-16 positive patients with CIN2/3 lesions using a PowderMed/Pfizer proprietary gene gun device ND-10, an individualized gene gun device suitable for clinical trials. This study aims to investigate whether intradermal CRT/E7 DNA vaccination is safe and able to generate E7-specific $CD8^+$ T cell immune responses in patients with CIN2/3 lesions. Another phase I therapeutic HPV DNA vaccine trial is currently open employing a DNA vaccine encoding modified E6 and E7 proteins of HPV 16 and 18 delivered via intramuscular injection followed by electroporation in patients with CIN2/3 lesions (See http://clinicaltrials.gov/ct2/show/NCT00685412).

Combination vaccines

The effect of therapeutic HPV DNA vaccines may be enhanced through a combination approach using heterologous prime-boost strategies. Prime-boost regimens have proven to be one of the most effective strategies for vaccination against HPV. Since DNA vaccines generate only modest immune responses, combination approaches are used to circumvent this limitation. DNA prime followed by viral vector-based vaccine boost has been shown to result in enhanced immune responses compared to single modality vaccinations. Vaccination with DNA followed by vaccinia boost was found to generate a significantly higher antigen-specific immune response compared to DNA vaccination alone [73]. A phase I clinical trial using heterologous E7 DNA prime (Sig/E7(detox)/HSP70) followed by recombinant vaccinia boost (TA-HPV) in combination with topical treatment with imiquimod is currently ongoing in HPV-16 associated CIN2/3 patients at Johns Hopkins (See http://clinicaltrials.gov/ct2/show/NCT00788164). TA-HPV is a vaccinia construct derived from the Wyeth strain of vaccinia, obtained from Xenova/Cantab/Celtic Pharma and has been shown to be less neurovirulent than the parental virus. TA-HPV was engineered to express the E6 and E7 genes from HPV types 16 and 18 [74]. The proposed phase I clinical trial will also include the topical administration of Toll-like receptor agonist imiquimod, to enhance access of the effector immune cells to the intraepithelial compartments of lesions. Toll-like receptor agonists, such as imiquimod, have been shown to activate the immature DCs and contribute to the direct killing of tumor cells [75]. Thus, the clinical trial design will test whether the combination of pNGVL4a-Sig/E7(detox)/HSP70 DNA prime-TA-HPV vaccinia boost vaccination with or without imiqimod treatment is safe and well-tolerated in patients with HPV-16 associated CIN2/3 lesions. Furthermore, the trial will determine if the combination of DNA prime-TA-HPV vaccinia boost vaccination with imiqimod treatment will generate significantly stronger E7-specific immune response and better therapeutic effects compared to prime-boost vaccination alone or imiquimod treatment alone.

The employment of chemotherapy, radiation or other biotherapeutic agents in combination with HPV therapeutic vaccination may also serve to enhance the potency of therapeutic HPV vaccines. The successful results of several preclini-

cal studies have led to the planning of a phase I clinical trial at Johns Hopkins involving the combination of chemoradiation with cisplatin with intramuscular administration of CRT/E7 DNA vaccination in conjunction with electroporation in patients with advanced HPV-associated head and neck cancer (Dr. Sara Pai, personal communication).

The therapeutic effects of HPV vaccines may be further enhanced by blocking the factors that inhibit T cell activation, such as CTLA-4 and PD-1. Thus, antibody-mediated blockade of CTLA-4 and PD-1 can potentially be used to prolong antitumoral T cell responses (for review, see [76, 77]). HPV therapeutic vaccines may be used in combination with agents that influence the tumor microenvironment to generate enhanced therapeutic effects against HPV-associated malignancies. Several factors in the tumor microenvironment including B7-H1 [78], STAT3 [79] and MIC-A and B [80], indoleamine 2,3-dioxygenase (IDO) enzyme [81], and galectin-1 [82] on tumor cells, immunosuppressive cytokines such as IL-10 [83] and TGF-b [84], T regulatory cells [85], myeloid-derived suppressor cells [86] may negatively influence the immune responses. Thus, the inhibition of these molecules may be used as an approach to enhance the therapeutic effects of the HPV vaccines.

Conclusions

The identification and characterization of high-risk human papillomavirus as a necessary causal agent for cervical cancer provides a promising possibility for the eradication of HPV-related malignancies. In the development of therapeutic HPV DNA vaccines, the focus has been on enhancing DNA vaccine potency to augment vaccine-elicited T cell immune responses by: 1) increasing the number of antigen-expressing DCs; 2) improving antigen expression, processing, and presentation in DCs; and 3) enhancing DC and T cell interaction. These strategies can potentially be combined to further enhance DNA vaccine potency. Furthermore, it is important for HPV therapeutic DNA vaccines to consider using strategies such as prime-boost regimens and/or combination strategies using molecules that are capable of blocking the negative regulators on T cells to further enhance the T cell immune responses. A better understanding of the molecular mechanisms that obstruct the immune response in the tumor microenvironment may aid in the identification of novel molecular targets that can be blocked in order to enhance the therapeutic effect of HPV DNA vaccines. With continued endeavor in the development of HPV therapeutic vaccines, we can foresee that HPV therapeutic DNA vaccines will emerge as a significant approach that can be combined with existing forms of therapy, such as chemotherapy and radiation, leading to effective translation from bench to bedside for the control of HPV-associated malignancies.

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