Published in final edited form as:
Ther Deliv. 2011 April ; 2(4): 427–430. doi:10.4155/tde.11.28.

HPV pseudovirions as DNA delivery vehicles

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Keywords

DNA delivery; DNA vaccine; HPV pseudovirions; human papillomavirus; neutralization assay

Recent advances in human papillomavirus (HPV) biology have enabled the packaging of heterologous DNA plasmids within HPV capsids to generate ‘pseudovirions’ that can efficiently deliver DNA into several cell types and tissues. The ability of HPV pseudovirions to efficiently deliver DNA into cells suggests several potential applications in basic biology, including the characterization of virion biology and measurement of protective neutralizing antibody titers *in vitro* and *in vivo*, as well as their employment for more direct medical applications. Notably, HPV pseudovirions have the potential to deliver therapeutic DNA vectors for gene therapy or for delivering DNA vaccines for the prevention or control of infections and/or cancers. Herein, we discuss the properties and various potential applications of HPV pseudovirions.

One of the major challenges of gene therapy is the delivery of vectors to the appropriate cell type *in vivo*. Papillomaviruses infect epithelia and induce generally benign tumors without detectable viremia. The diversity of more than 100 types of HPVs, with different clades associated with a tropism for mucosal or cutaneous epithelia, creates the opportunity for generating different types of HPV pseudovirions to deliver DNA of interest into different tissues. Recent advances in the development of HPV pseudovirions suggest their promise for delivery of DNA of interest *in vitro* and *in vivo*. HPV has nonenveloped, T = 7 icosahedral capsids that contain a double-stranded, covalently closed circular DNA that is approximately 8000 base pairs in size and bound to histones. The HPV capsid consists of two structural

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Financial & competing interests disclosure
This work was supported by the NCI SPORE in Cervical Cancer P50 CA098252 and the 1 RO1 CA114425–01. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

proteins – the L1 major capsid protein, which represents approximately 80% of the total viral protein, and the L2 minor capsid protein, which is predominantly found on the interior of the viral particle. The expression of L1 in eukaryotic cells has been shown to form virus-like particles (VLPs) that morphologically mimic the native HPV particles and comprise 72 pentameric capsomers. VLPs derived from HPV L1 have been used in commercially available prophylactic HPV vaccines, Gardasil® and Cervarix®, for the prevention of cervical cancer and its precursor lesions [1,2].

The coexpression of L1 and L2 in mammalian cells has been shown to be capable of encapsidating the homologous HPV genomes to produce infectious papillomavirus, or pseudotype heterologous papillomavirus genomes to generate ‘quasivirions’ [3–5]. Moreover, L1 and L2 can encapsidate completely heterologous DNAs of less than approximately 8 Kb without the apparent need for a packaging signal. The resultant ‘pseudovirions’ can efficiently deliver the DNA into numerous cell types, resulting in the expression of the encoded gene. Both capsid proteins are required for efficient DNA packaging.

The ability to generate high titers of infectious papillomavirus pseudovirions suggests their potential utility for vaccination [6]. This technology has greatly improved efficiency over previous methods to generate pseudovirions by amplifying the amount of transfected plasmid to be encapsidated as well as greatly increasing the amount of capsid protein expressed by the L1 and L2 gene-transfected cell. To accomplish this, the production cell line (293 cells) is engineered to express high levels of SV40 large T antigen (293TT) to drive the amplification of the plasmid containing an SV40 origin of replication. These 293TT cells are cotransfected with codon-optomized L1 and L2 capsid genes in an expression vector that is too large for its efficient encapsidation along with a target plasmid (which can contain the SV40 origin of replication but it is not required), allowing for efficient intracellular production of HPV pseudovirions encapsidating DNA. Other approaches have utilized papillomavirus proteins E1 and E2 to replicate the target DNA, but this does not appear to be necessary for the production of pseudovirions. Furthermore, the ability to generate significant quantities of HPV pseudovirions containing heterologous plasmids, not the potentially oncogenic HPV viral genome, in the absence of large T antigen, reduces safety concerns.

There were earlier attempts to use the papillomavirus L1-only VLPs to facilitate delivery of DNA [7,8], which require either the conjugation of DNA to the VLPs or the *in vitro* assembly of DNA within the VLPs. However, since this approach does not include the L2 minor capsid protein, which is essential for efficient papillomavirus infection [9–11], the efficiency of DNA delivery by L1 VLP is significantly reduced compared with the delivery of DNA using pseudovirions.

HPV pseudovirions for *in vitro* neutralization

It has proven impossible to grow HPV in culture without laborious organotypic culture technologies, and the native virions lack a readily detectable phenotype in culture because they do not induce cell lysis or rapid transformation. Thus, HPV pseudovirions containing a reporter plasmid have been used for the development of *in vitro* neutralization assays to detect protective humoral responses generated by preventive HPV vaccines. The *in vitro* neutralization assays using a specific type of HPV pseudovirion can be used to characterize the humoral immune responses to preventive HPV vaccine candidates. HPV pseudovirions encapsidating marker genes, such as luciferase, green fluorescent protein or secreted alkaline phosphatase, can be utilized for *in vitro* neutralization assays to detect neutralizing antibodies generated by preventive HPV vaccines or to determine the effectiveness of drugs

or other approaches to block infection. The presence of neutralizing antibodies would abolish the successful infection of cells by the HPV pseudovirion, reducing the expression of the marker gene in the cells. With the increasing number of successful cloning and sequencing of HPV types, it has now become possible to generate a wide panel of different types of HPV pseudovirions. HPV pseudovirions containing mutations in viral capsid proteins have also been used to examine the mechanisms of HPV infection and neutralization by antibodies *in vitro* [12,13].

HPV pseudovirions for *in vivo* characterization of protective immunity

The availability of HPV pseudovirions carrying a marker gene has been used to create challenge models for HPV in mice and rabbits to facilitate drug and vaccine testing. Infection upon vaginal challenge of mice with HPV pseudovirions carrying a luciferase or RFP reporter can be visualized by bioluminescence or fluorescence of infected tissues. Vaginal challenge of rabbit using HPV quasivirions containing a cottontail rabbit papillomavirus genome produces cutaneous warts.

Since challenge with HPV pseudovirions can recapitulate the initial phases of papillomavirus infection, they have been used to characterize the protective humoral immunity using *in vivo* imaging in animal models. For instance, a model of cervicovaginal infection with HPV-16 pseudovirion was developed in mice to model the establishment of papillomavirus infection [14]. Furthermore, HPV pseudovirions carrying of a marker gene have been used in a skin challenge murine model, which has significantly simplified the characterization of the protective immunity against HPV pseudovirion infection *in vivo* [15]. Different types of HPV pseudovirions have also been used to characterize the cross-protection mediated by L2-specific neutralizing antibodies using *in vivo* imaging systems with different types of HPV pseudovirions carrying a marker gene [15,16]. In addition, adoptive transfer of sera containing anti-L1/L2 neutralizing antibodies has been used to demonstrate protective immunity against the different types of HPV pseudovirion infection *in vivo* using the imaging systems [17].

The use of HPV pseudovirions carrying a marker gene can be used for the study of pathogenesis of infection by HPV. HPV pseudovirions have been found to bind preferentially to the cervicovaginal basement membrane at sites of trauma [14]. The application of HPV pseudovirions in a murine cervicovaginal tract model further facilitated a detailed analysis of the mechanism required for *in vivo* HPV pseudovirion infection by mucosal type HPV-16, involving conformational changes at the basement membrane, leading to protease digestion of L2 and exposure of the N terminus, which were found to be critical steps in the papillomavirus life cycle before infection of keratinocytes *in vivo* [18]. Thus, HPV pseudovirions have enabled the greater understanding of the biology of HPV infection.

HPV pseudovirions for the delivery of DNA vaccines

HPV pseudovirions have also been used to deliver DNA vaccines. The major obstacle to DNA vaccination is the delivery of the DNA into cells. Hence, HPV pseudovirions to package DNA vaccine present many potential advantages compared with naked DNA and other routes of administration. The encapsidation of the DNA vaccine protects the DNA from nucleases and provides efficient targeted delivery with great stability. In addition, because HPV pseudovirions contain a DNA construct with genes of interest, but not the natural HPV viral genome, they are nonreplicative and lack many of the safety concerns associated with live viral vectors. Furthermore, since neutralizing antibodies against one type of papillomavirus pseudovirion are usually not crossreactive to other types of

papillomavirus pseudovirions, the spectrum of over 100 different types of papillomavirus pseudovirions allows repeated boosting without concern for pre-existing immunity.

HPV pseudovirions carrying DNA vaccines have been tested using different routes of administration. HPV-16 pseudovirions containing a DNA vaccine encoding model antigen, ovalbumin, which was subcutaneously injected was demonstrated to generate significantly stronger ovalbumin-specific $CD8^+$ T-cell immune responses in a dose-dependent manner [11]. HPV pseudovirions carrying DNA expressing M and M2 antigen of respiratory syncytial virus has also been delivered via mucosal immunization [19]. Intravaginal delivery of HPV pseudovirions encapsidating M and M2 elicited local and systemic M–M2-specific $CD8^+$ T-cell and antibody responses in mice that were comparable to an approximately 10,000-fold higher dose of naked DNA. HPV pseudovirions carrying therapeutic DNA have been demonstrated to be significantly more efficient than vaccination with naked DNA [11,19]. Thus, HPV pseudovirions represent an innovative and promising delivery system for DNA vaccines to trigger potent systemic and local antigen-specific immune responses.

HPV pseudovirions for targeted gene therapy

The ability of HPV pseudovirions to deliver therapeutic DNA has potential implications for gene therapy since many of the advantages mentioned above for the encapsulation of DNA vaccines are also applicable to the delivery of a gene of interest (not for vaccination purposes). We speculate that, for example, they might be used to complement defective genes in epithelial cells, or to deliver modifying genes at the site of wounds since it was found that HPV pseudovirions do not infect intact normal epithelium without wounding. HPV pseudovirions may also infect many human tumor-derived cell lines *in vitro*. Thus, the ability for HPV pseudovirions to target epithelial cells, sites of trauma and tumors has important implications for the delivery of a therapeutic gene to these sites.

Future perspective

The promise of HPV pseudovirions as DNA delivery vehicles will likely be further explored in several capacities. The next 5–10 years will likely see the increasing exploration of the tissue-specific tropism of different types of HPV for gene therapy. For instance, the employment of skin-tropic HPV pseudovirions (e.g., HPV-2 and -4) may be used to deliver a gene of interest for the treatment of skin disease in which the gene is defective, thereby addressing a potential medical need. HPV DNA is typically in an episomal form in natural infections. This episomal nature of papillomavirus genomes may confer advantages in safety, since there is a lack of integration, as well as an advantage in copy number, thus leading to maintenance of a high amount of therapeutic DNA to be delivered. As papillomaviruses are also epithelial-tropic and do not enter the bloodstream, they may allow for a degree of immune privilege by expression of the transgene only in the skin. Hence, HPV pseudovirions represent promising vectors for the delivery of therapeutic genes to the skin.

The potential advantages of HPV pseudovirion technology for carrying DNA vaccines warrant consideration for future clinical translation. As the skin has abundant numbers of Langerhans cells, which are immature dendritic cells, as well as many dermal dendritic cells, intradermal administration would likely be a route of administration to be explored for the application of HPV pseudovirions carrying DNA vaccines for cancer immunotherapy and for triggering mucosal immune responses. It will be important to find the optimal route of administration for maximal vaccine potency of DNA vaccine delivered by HPV pseudovirions.

The clinical translation of HPV pseudovirions as DNA delivery vehicles will likely require further developments in regards to its production. The current production cell line used for the generation of pseudovirions contains SV40 large T antigen, which is an oncoprotein, and thereby raises some possible safety concerns. A possible area of exploration would be the use of a packaging cell line that would avoid the potential transforming ability associated with the usage of large T antigen but maintain the DNA in episomal form. For instance, HPV E1 and E2 in the packaging cells may avoid the risk of transformation but still facilitate packaging of DNA. Alternatively, an approach for producing pseudovirions in yeast has been described [20]. The mass production of HPV pseudovirions will also likely require efficient standardized protocols to generate large titers of infectious HPV pseudovirions for mass vaccination programs. The continual development of applications for HPV pseudovirions as DNA delivery vehicles has promise for the control of chronic viral infections and cancers.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Roden R, Wu TC. Preventative and therapeutic vaccines for cervical cancer. Expert Rev Vaccines. 2003; 2(4):495–516. [PubMed: 14711335]
2. Harper DM, Williams KB. Prophylactic HPV vaccines: current knowledge of impact on gynecologic premalignancies. Discov Med. 2010; 10(50):7–17. [PubMed: 20670593]
3▪▪. Roden RB, Greenstone HL, Kirnbauer R, et al. *In vitro* generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. J Virol. 1996; 70(9):5875–5883. First paper depicting the generation of human papillomaviruses (HPV) pseudovirions. [PubMed: 8709207]
4. Zhao KN, Sun XY, Frazer IH, Zhou J. DNA packaging by L1 and L2 capsid proteins of bovine papillomavirus type 1. Virology. 1998; 243(2):482–491. [PubMed: 9568045]
5. Brendle SA, Culp TD, Broutian TR, Christensen ND. Binding and neutralization characteristics of a panel of monoclonal antibodies to human papillomavirus 58. J Gen Virol. 2010; 91(Pt 7):1834–1839. [PubMed: 20181746]
6▪▪. Buck CB, Pastrana DV, Lowy DR, Schiller JT. Efficient intracellular assembly of papillomaviral vectors. J Virol. 2004; 78(2):751–757. Reports an efficient method of generating high titers of infectious HPV pseudovirions. [PubMed: 14694107]
7. El Mehdaoui S, Touze A, Laurent S, Sizaret PY, Rasschaert D, Coursaget P. Gene transfer using recombinant rabbit hemorrhagic disease virus capsids with genetically modified DNA encapsidation capacity by addition of packaging sequences from the L1 or L2 protein of human papillomavirus type 16. J Virol. 2000; 74(22):10332–10340. [PubMed: 11044077]
8. Touze A, Coursaget P. *In vitro* gene transfer using human papillomavirus-like particles. Nucleic Acids Res. 1998; 26(5):1317–1323. [PubMed: 9469843]
9. Campos SK, Ozbun MA. Two highly conserved cysteine residues in HPV16 L2 form an intramolecular disulfide bond and are critical for infectivity in human keratinocytes. PLoS ONE. 2009; 4(2):E4463. [PubMed: 19214230]
10▪. Gambhira R, Jagu S, Karanam B, Day PM, Roden R. Role of L2 cysteines in papillomavirus infection and neutralization. Virol J. 2009; 6:176. Addresses the critical role of L2 for efficient infection by HPV. [PubMed: 19860897]
11▪▪. Peng S, Monie A, Kang TH, Hung CF, Roden R, Wu TC. Efficient delivery of DNA vaccines using human papillomavirus pseudovirions. Gene Ther. 2010; 17:1453–1464. Reports the delivery of therapeutic DNA vaccine using HPV pseudovirions and supports that vaccination with HPV pseudovirions is a significantly more efficient method of delivery compared to other methods of DNA delivery. [PubMed: 20668481]

12. Day PM, Thompson CD, Buck CB, Pang YY, Lowy DR, Schiller JT. Neutralization of human papillomavirus with monoclonal antibodies reveals different mechanisms of inhibition. J Virol. 2007; 81(16):8784–8792. [PubMed: 17553881]

13. Day PM, Gambhira R, Roden RB, Lowy DR, Schiller JT. Mechanisms of human papillomavirus type 16 neutralization by L2 cross-neutralizing and L1 type-specific antibodies. J Virol. 2008; 82(9):4638–4646. [PubMed: 18305047]

14▪. Roberts JN, Buck CB, Thompson CD, et al. Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. Nat Med. 2007; 13(7):857–861. Describes a mouse model depicting infection with HPV pseudovirions *in vivo*. [PubMed: 17603495]

15. Alphs HH, Gambhira R, Karanam B, et al. Protection against heterologous human papillomavirus challenge by a synthetic lipopeptide vaccine containing a broadly cross-neutralizing epitope of L2. Proc Natl Acad Sci USA. 2008; 105(15):5850–5855. [PubMed: 18413606]

16▪. Jagu S, Karanam B, Gambhira R, et al. Concatenated multitype L2 fusion proteins as candidate prophylactic pan-human papillomavirus vaccines. J Natl Cancer Inst. 2009; 101(11):782–792. Describes the development of broadly-protective L2-based preventive HPV vaccines using HPV pseudovirions to characterize L2-specific neutralizing antibodies. [PubMed: 19470949]

17. Day PM, Kines RC, Thompson CD, et al. *In vivo* mechanisms of vaccine-induced protection against HPV infection. Cell Host Microbe. 2010; 8(3):260–270. [PubMed: 20833377]

18. Kines RC, Thompson CD, Lowy DR, Schiller JT, Day PM. The initial steps leading to papillomavirus infection occur on the basement membrane prior to cell surface binding. Proc Natl Acad Sci USA. 2009; 106(48):20458–20463. [PubMed: 19920181]

19▪. Graham BS, Kines RC, Corbett KS, et al. Mucosal delivery of human papillomavirus pseudovirus-encapsidated plasmids improves the potency of DNA vaccination. Mucosal Immunol. 2010; 3:475–486. Reports the employment of HPV pseudovirions carrying DNA encoding M and M2 proteins of respiratory syncytial virus for mucosal immunization. [PubMed: 20555315]

20. Rossi JL, Gissmann L, Jansen K, Muller M. Assembly of human papillomavirus type 16 pseudovirions in *Saccharomyces cerevisiae*. Hum Gene Ther. 2000; 11(8):1165–1176. [PubMed: 10834618]