Review

Human papillomavirus vaccine as a new way of preventing cervical cancer: a dream or the future?

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Cervical cancer is the major cause of death in women of reproductive age in parts of the developing world. Thanks to the effectiveness of national screening programs, the incidence and mortality rates for cervical cancer have declined dramatically in developed countries. According to many researchers, human papillomavirus (HPV) infection has an important role in the development of cervical neoplasm. The effects of HPV infection on the oncogenesis of cervical carcinoma can be explained to a large degree by the regulation and function of the two viral oncogenes, E6 and E7. About 25 of >80 types infect the genital tract. HPV types are stratified into low, intermediate- and high-risk categories. Today, vaccines are available against many serious human pathogens. It is accepted worldwide that cervical carcinoma is a consequence of infection with HPV viruses. Therefore it is reasonable to assume that vaccine that prevents infection will reduce the incidence of cervical cancer. Virus-like particles are empty viral capsids, and are the leading candidate vaccines for the treatment or prevention of cervical cancer in humans. The HPV type 16 (HPV16) L1 virus-like particle vaccines have been shown to be generally well tolerated and they generate high levels of antibodies against HPV16. Since ~50% of cervical cancers are associated with HPV16 infection, the administration of this type of vaccine to young women could reduce the incidence of HPV16 infection, which is related to cervical dysplasia and cervical neoplasm. Vaccination against HPV infection could reduce the risk of infection and, most importantly, decrease the incidence of cervical cancer. A vaccine for cervical cancer is not a dream in the far future, it is happening today.
Key words: cervical cancer, HPV vaccine, prevention

Introduction

Cervical cancer is a major cause of death in women of reproductive age in parts of the developing world [1]. In developed countries, incidence and mortality rates for cervical cancer have declined dramatically, due to the effectiveness of screening programs that assess cervical cytology by Papanicolau smear [2, 3].

For the period 1990–1994, in the Republic of Serbia, ~1100 women died annually from malignant disease of the genitals; nearly half (45.3%) died from uterine cervical cancer [4]. Despite this disparity, which is related to the absence of effective screening programs, cervical cancer is still a largely preventable disease, with a known causative agent in ~95% of cases—human papilloma virus (HPV). The other 5% of cervical cancer cases may be unrelated to HPV infection [3, 5, 6].

HPV infections, according to many researchers, have an important role in developing cervical neoplasm. There are many papers that suggest and point to a pre-stage of cervical carcinoma, known as cervical intraepithelial neoplasia (CIN), characterized by dysplastic changes showing varying degrees of disordered maturation. CIN is classified as either: (i) CIN I or low-grade squamous intraepithelial lesions (LSIL); or (ii) CIN II/III or high-grade squamous intraepithelial lesions (HSIL). These precursor lesions may last continually for several years until they become an invasive malignant disease [5, 7–11].

The trigger for developing cervical dysplastic changes is HPV infection. This virus infects basal epithelial layers in the region of metaplasia and the transformation zone on the cervix, where the most vulnerable cells are found. As a sexually transmitted disease, HPV infection occurs mostly in younger women, often those <40 years of age [12, 13].

Kjaer et al. [14] conducted a prospective follow-up study of >10000 cytological normal findings in 20- to 29-year-old women. It was found that HPV status at enrollment predicted the future development of HSIL. In a random sample of the women who remained cytologically normal during follow up, only 14% were HPV positive at their first visit, whereas this applied to 80% of the women who were subsequently diagnosed with high-grade lesions. Most women were diagnosed at the second examination, and HPV status at this examination was also strongly associated with the presence of cytological abnormalities, although the outstanding risk factor for incident high-grade lesions in this study was being repeatedly positive for HPV. Cuzik et al. [15] showed that >95% of cervical cancers, 75–95% of HSIL and 25–40% of LSIL are associated with a positive HPV test on exfoliated cervical cells.

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Table 1. Genital tract HPV types, classified by malignant potential

Malignant potential	Low risk	Intermediate risk	High risk
HPV types	6, 11, 40, 42, 43, 44	31, 33, 35, 51, 52	16, 18, 45, 56

As studies have shown, HPV infection precedes the development of both low- and high-grade squamous intraepithelial lesions. High-risk HPV infection is a good predictor of subsequent high-grade lesions in young women [16]. Studies have shown a very high prevalence of HPV infection in approximately one-third of American female college students and in 8% of men between the ages of 15 and 49 years [17]. The former female population must be recruited into a serious screening program because of the role of HPV infection in the development of cervical neoplasia. Most importantly, every doctor must spread knowledge among this population that sexually transmitted disease is a very serious contemporary problem.

Human papillomavirus

HPV is a virus that belongs to the papovavirus family. It is a double-strand DNA tumor virus [18]. More than 80 human types are specific for the infection of epithelial cells, including those in the skin, the respiratory mucosa and the genital tract. Genital tract HPV types are classified by their relative malignant potential as low-, intermediate- and high-risk types (Table 1). Low-risk varieties, such as types 6 and 11, are commonly associated with either viral condyloma or mild dysplastic changes in cervical epithelium (CIN I), which do not usually progress to invasive disease [19–21] and are almost never present in women with cervical cancer. High-risk types of HPV, however, are often observed in association with moderate dysplasia (CIN II) and severe dysplasia or carcinoma *in situ* (CIN III). These high-risk types are also observed in the majority of patients with cervical cancer [13, 19, 22].

Syrjanen et al. [23] observed that progression rates from HPV detection to dysplasias ranged from 15% to 30% for HPV types 18 and 16, compared with 5% to 25% for types 6 and 11. Regression rates were ~30% for all viral types.

Stanimirovic et al. [13] also showed that the absence of HPV infection and the presence of benign types 6 and 11 were significantly more common in LSIL-type lesions. Oncogenic HPV types were detected more in severe lesions of HSIL type.

Kjaer et al. [14] showed that HPV-positive women had a significantly increased risk of developing atypical cells [odds ratio (OR) = 3.2, 95% confidence interval (CI) 1.3–7.9], low-grade lesions [OR = 7.5 (95% CI 4.8–11.7)] and high-grade lesions [OR = 25.8 (95% CI 15.3–43.6)]. They divided women who were HPV positive into groups of low risk or unknown HPV types, or high risk or oncogenic types. The oncogenic HPV types were associated with the highest risk, especially for high-grade lesions, when compared with the HPV-negative group.

HPV16 is most commonly linked with cancer, since it is present in 50% of cervical cancers and high-grade CINs [24, 25].

Oncogenetic effects of HPV

The oncogenetic mechanism of HPV viruses can be explained by the regulation and function of the two viral oncogenes E6 and E7. HPV genome is usually present in an episomal (circular and non-integrated) configuration in CIN, whereas in invasive cervical cancer the genome is commonly integrated into the host DNA [22]. Genes E6 and E7 are regulated by the E2 gene product. A characteristic event in malignant transformation is the integration of circular viral genome into the patient's genome. Gene E2 is often the site for integration, resulting in disruption of the E2 gene and subsequent de-repression of E6 and E7 [26]. The product of gene E6 binds to the resident p53 tumor suppressor gene and induces p53 degradation. E7 targets another tumor suppressor, the retinoblastoma gene product (pRb) [27, 28]. By binding to it and altering its phosphorylation state, it functionally inactivates this protein, which, like p53, functions in cell cycle control. Since p53 and pRb are tumor-suppressor proteins that inhibit cell-cycle progression, their inactivation by viral proteins E6 and E7 leads to deregulated entry of cells into S phase.

Vaccine: a new prevention method

Today, vaccines are available for many serious human pathogens such as bacteria and viruses, and for about half of all human parasites. Traditionally, attenuated vaccines were made by repeated passaging of the infectious agent in tissue culture or animal hosts until its virulence was greatly decreased but its immunogenicity was retained. Alternatively, chemicals such as formalin were used to destroy infectivity. More recently, parts of an infectious agent, usually a surface antigen, have been used as a subunit vaccine. The current vaccines against hepatitis B virus and Lyme disease rely on recombinant DNA technology [29].

Today, it is universally accepted that cervical carcinoma is a consequence of HPV infection. It is therefore reasonable to assume that a vaccine that prevents infection will reduce the incidence of cervical cancer. In mouse studies, HPV vaccines produce a cytotoxic T-cell response that not only eradicates HPV-bearing tumors, but also protects against subsequent challenge by tumor [30]. Several groups will soon begin phase III trials of vaccines targeted against HPV types 16 and 18, which account for at least 70% of cancer cases [31].

The L1 capsid protein has been targeted for neutralizing-antibody formation using a DNA or polynucleotide vaccine in the Cottontailed Rabbit Papilloma Virus model [32]. Empty viral capsids, termed virus-like particles (VLPs) are synthesized using microbial or cellular expression systems [33], and represent the leading candidate vaccine for the treatment or prevention of cervical cancer in humans. Vaccination with L1 VLPs derived from species-specific papillomaviruses neutralizes virus and, in animal models, protects against the development of lesions [34, 35].

HPV VLPs and chimeric VLPs are immunogens that are able to elicit potent anti-viral/tumor B- and T-cell responses. VLPs were found to bind very well to human and mouse immune cells that expressed markers of antigen-presenting cells (APCs) such as MHC class II, CD80 and CD86, including dendritic cells, macrophages and B cells. Monoclonal antibody blocking studies iden-

tified FcγRIII (CD16) as one of the molecules to which the VLPs can bind, both on immune cells and foreskin epithelium [36].

Dendritic cells, as the most potent inducers of immune responses, play a central role in VLP-induced immunity. It was clearly demonstrated that immature human dendritic cells were fully activated by chimeric HPV16 VLPs and were subsequently capable of inducing endogenously processed, epitope-specific human T-cell responses *in vitro* [37, 38].

Koutsky et al. [39] performed a controlled trial of an HPV16 vaccine. In a double-blind study, 2392 randomly assigned young women (females aged 16–23 years) received three doses of placebo or HPV16 VLP vaccine, given at day 0, month 2 and month 6. Among 2392 women enrolled in the study, 1194 received vaccine and 1198 received placebo. Altogether, 1533 women (64% of the study cohort) were included in the primary analysis. These women were followed for a median of 17.4 months after completion of the vaccination regimen. The most common reason for exclusion was evidence of HPV16 infection at enrollment or antibodies in samples collected during the visit at day 0 or month 7. The reason for these exclusions is based on the fact that the vaccine was designed to prevent HPV16 infection. If patients were infected before vaccination, the vaccine would not have been able to protect these patients from infection.

This controlled trial showed evidence of a highly efficacious prophylactic vaccine against HPV infection. A three-dose regimen of HPV16 vaccine reduced the incidence of persistent HPV16 infection; all 41 cases of new HPV16 infection, including nine cases of HPV16-related CIN occurred among placebo recipients (vaccine efficacy of 100%). Only six cases in which tests were positive at a single visit occurred among vaccine recipients, whereas 27 cases were expected on the basis of the observed rate in the placebo group. Among women who received HPV16 vaccine, 99.7% seroconverted. At month 7, geometric mean titer of HPV16 antibodies in these women was 58.7 times as high as the geometric mean titer among women with serologic evidence of natural HPV16 infection at enrollment [39].

In an early study, HPV16 L1 VLP vaccines were shown to be generally well tolerated and generated high levels of antibodies against HPV16 [40]. Since ~50% of cervical cancers are associated with HPV16 infection, the administration of this type of vaccine to young women could reduce the incidence of HPV16 infection that is related to CIN and cervical neoplasm. With this type of immunization the incidence of cervical carcinoma should decrease; however, while this HPV16 vaccine is monovalent, other types of HPV with high malignant potential should be evaluated using multivalent vaccines.

Fausch et al. [41] explored the interaction of HPV with Langerhans cells. These cells are the first APCs that virus comes into contact with during infection. Some HPV-infected women do not have protective antiviral immunity. In contrast to dendritic cells, Langerhans cells are not activated by HPV VLPs, which is illustrated by the lack of up-regulating activation markers, secreting IL-12, stimulating T cells in a mixed lymphocyte reaction, inducing HPV-specific immunity and migrating from epidermal tissue. The possible immune escape mechanism used by HPVs is discussed in this study, which points to the fact that such a mechanism could decrease the effects of vaccination. The questions that must now be answered are: what does the future hold for an HPV vaccine?; what is going on in women that are infected with these viruses?; and is there any chance for a therapeutic vaccine?

There are efforts focused on targeting the oncogenic E6/E7 proteins as therapeutic vaccines. E6- or E7-based vaccine strategy is designed to stimulate T-cell immunity. These peptide vaccines are designed to stimulate cytotoxic T-lymphocytes against specific E7 epitopes that have been shown to be conserved and constitutively expressed in cervical cancers [42].

At the University of Wales, eight patients with advanced cervical cancer received a recombinant vaccinia virus with a mutant HPV18 E6/E7 fusion gene with no toxic side effects. Three of the eight developed an HPV-specific antibody response, one of whom developed HPV-specific cytotoxic T-lymphocytes [43].

Conclusions

HPV infection is a common sexually transmitted disease in young women. Most infections are benign, but persistent infections of HPV types with high malignant potential are associated with the development of cervical cancer. Vaccination against HPV infection reduces the risk of infection and, most importantly, decreases the incidence of cervical cancer.

Young women who are sexually active must be recruited to a screening program. HPV typing is important in women with positive tests for HPV infection so that adequate control of HPV viruses with high malignant potential and better control of women with CIN caused by HPV viruses may be exerted.

The education of young people regarding sexually transmitted disease is one of health care's primary goals, as the results of such infections lead to very serious contemporary problems, affecting the patient, their family and society as a whole.

Vaccination against HPV infection as a new method of prevention no longer lies in the future; it is happening right now.

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