ACTA FAC MED NAISS

UDC 616.988:618.146-006.6

Review article

ACTA FAC MED NAISS 2009; 26 (1): 3-9

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HUMAN PAPILLOMAVIRUS

SUMMARY

The term Human papillomavirus (HPV) refers to a group of viruses which cause warts on the skin and the genital region. HPV is a very common genital infection, with the prevalence rates of 30% to 40% in young adults.

Genital HPV infections represent the most common sexually transmitted disease. Their numbers have been ever increasing in the last three decades, being three times more common compared to genital herpes. These are mostly transient infections, with mild, asymptomatic course. Most of them spontaneously heal in a period of eight months to two years. Around 50 HPV types infect the anogenital region and are predominantly transmitted by sexual contacts. The association of HPV infection and cervical cancer led to the universal recognition that HPV is the required causal agent for cervical cancer to occur. Cancer of the cervix uteri is the most common female cancer in developing countries. Worldwide, about 500.000 women acquire the disease annually and about 75% are from developing countries. About 300.000 women die of the disease annually.

Lately, more and more attention has been focused on HPV-related intraepithelial neoplasms of the external genitals in both sexes, with already well- studied association of HPV and cervical intraepithelial neoplasms.

Key words: **human papillomavirus, oncogenesis, cervical cancer**

INTRODUCTION

Human papillomavirus (HPV) belongs to the family of *Papillomaviridae.* It is a very heterogenous group of DNA viruses which can be etiologicaly associated with numerous benign and malignant tumours of the squamous epithelium. There are more than 100 known HPV types.

Papillomaviruses were first identified in 1932, when Richard Edwin Shope showed that skin warts (medically known as papillomas) could be transmitted between rabbits as a filterable infectious agent. It is now recognized that papillomaviruses are a diverse group of non-enveloped DNA viruses that infect vertebrate species ranging from birds, manatees to people (1,2). Papillomaviruses replicate exclusively in body surface tissues such as the skin, or the mucosal surface.

Some HPV types have evolved to be transmitted through sexual contact. Many sexually-transmitted HPV types do not cause easily discernible symptoms in most infected individuals. However, a minority of individuals who are persistently infected with a subset of sexually-transmitted HPVs, such as types 16 and 18, can go on to develop cervical cancer or anal/genital cancer.

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STRUCTURE OF THE VIRUS

Virions are approximately 55 nm in diameter and are composed of 72 capsomers. Papillomaviruses are non-enveloped, meaning that the outer shell or capsid of the virus is not covered by a lipid membrane. A single viral protein, known as L1, is necessary and sufficient for formation of a 60 nanometer capsid composed of 72 star-shaped capsomers. The papillomavirus virion also contains a minor capsid protein, known as L2. Although it is not clear how L2 is arranged within the virion, it performs several important functions, including facilitating the packaging of the viral genome into nascent virions and facilitating the infectious entry of the virus into new host cells.

Genetic organization

Genomic organization is a well-conserved feature among papillomaviruses. We can distinguish the three main regions: early, late and the long control region of all papillomaviruses. In the early region (E) resides the transformation and immortalization potential of HPVs and consists of a number of regulatory genes for viral transcription and replication and cell cycle control. The late region (L) codes for the two capsid genes and the long control region (LCR) contains all the cis-regulatory elements necessary for HPV transcription including the early promoter and the origin of replication (Figure 1).

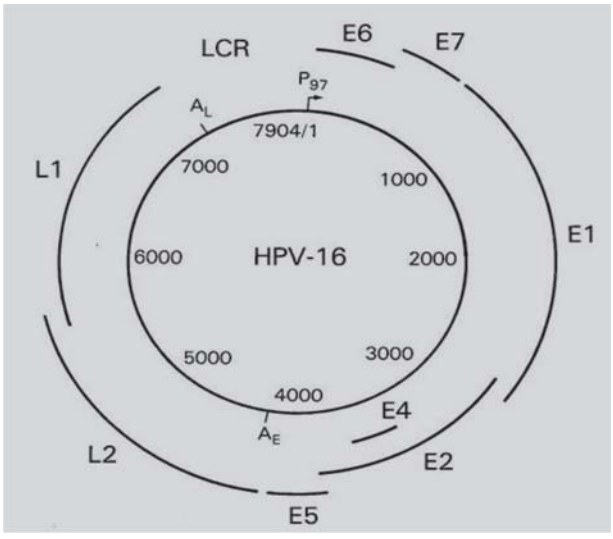

Figure 1. HPV genome

Early region

E1 gene: Encodes a protein that binds to the viral origin of replication in the long control region of the viral genome. E1 uses ATP to exert a helicase activity that forces apart the DNA strands, thus preparing the viral genome for replication by cellular DNA replication proteins.

E2 gene: The E2 protein serves as a master transcriptional regulator for viral promoters located primarily in the long control region. The protein has transactivation domain linked by a relatively unstructured hinge region to a well-characterized DNA binding domain. E2 facilitates the binding of E1 to the viral origin of replication and utilizes a cellular protein known as Brd4 to tether the viral genome to cellular chromosomes (3). This tethering to the cell's nuclear matrix ensures faithful distribution of viral genomes to each daughter cell after cell division. It is thought that E2 serves as a negative regulator of expression for the oncogenes E6 and E7 in latently HPV-infected basal layer keratinocytes. Genetic changes, such as integration of the viral DNA into a host cell chromosome, that inactivate E2 expression tend to increase the expression of the E6 and E7 oncogenes, resulting in cellular transformation and possibly further genetic destabilization.

E3 gene: This small gene exists only in a few papillomavirus types. The gene is not known to be expressed as a protein and does not appear to serve any function.

E4 gene: Although E4 proteins are expressed at low levels during the early phase of viral infection, expression of E4 increases dramatically during the late phase of infection. The E4 of many papillomavirus types is thought to facilitate virion release into the environment by disrupting intermediate filaments of the keratinocyte cytoskeleton. Viral mutants incapable of expressing E4 do not support high-level replication of the viral DNA, but it is not yet clear how E4 facilitates this task. E4 has also been shown to participate in arresting the cell in the G2 phase of the cell cycle.

E5 gene: The E5 protein of some papillomavirus types functions as an oncogene primarily by activating the cell growth-promoting signaling of platelet-derived growth factor receptors. E5 has also been shown to down-regulate the surface expression of major histocompatibility complex class I proteins, possibly to protect the infected cell from destruction by killer T cells.

E6 gene: The primary function of the E6 protein is to inactivate the tumor suppressor protein P53. E6 also interacts with a large number of other cellular proteins and is a major focus of research. Since the expression of E6 is strictly required for maintenance of a cancerous phenotype in HPV-induced cancers, it is an appealing target of therapeutic HPV vaccines, which seek to eradicate established cervical cancer tumors.

E7 gene: In most papillomavirus types, the primary function of the E7 protein is to inactivate members of the pRb family of tumor suppressor proteins. Together with E6, E7 serves to promote cell cycle progression, thus priming the cell for replication of the viral DNA. E7 also participates in immortalization of infected cells by activating cellular telomerase. Like E6, E7 is the subject of intense research interest and is believed to exert a wide variety of other effects on infected cells. As with E6, the ongoing expression of E7 is required for survival of cancer cell lines, such as HeLa, that are derived from HPV-induced tumors (4).

E8 gene: Only a few papillomavirus types have the capacity to express a short protein from the E8 gene. In the case of BPV-4 (genus *Xi*), the E8 open reading frame may substitute for the E6 open reading frame, which is absent in this papillomavirus genus (5).

Late region

L1 and L2 genes: The L1 gene encodes the 56-60 kD major capsid protein. L1, the most antigenic of papillomavirus proteins, is weakly phosphorylated and does not bind DNA. It can be glycosylated and cross-linked through disulfides, but the implications of these changes are unclear. (Glycosylated forms have not been reported in virions). It is relatively well-conserved among all papillomaviruses. L1 has self-assembly capacity and is the overwhelmingly predominant molecule in the viral capsid.The L2 gene encodes the 49-60 kD minor capsid protein, which is highly phosphorylated and binds DNA. L2 migrates in a gel as if it were a 73 kD protein. Unlike L1, L2 does not self-assemble nor does it link to itself.

Long Control Region (LCR): sometimes referred to as upstream regulatory region or noncoding region. Operationally defined as the region from the termination of the L1 gene to the first methionine of the E6 gene. The LCR is the less conserved region among papillomaviruses. It contains the early promoter and various transcriptional regulatory motifs.

LIFE CYCLE

Keratinocyte stem cells in the basal layer are thought to be the target of productive papillomavirus infection. Papillomaviruses are thought to gain access to basal layer cells through small wounds or microtraumas in the skin or mucosal surface.

To establish a wart or papilloma, the virus must infect a basal epithelial cell. Our knowledge is limited about the initial steps in the replication cycle such as attachment, uptake, endocytosis, and transport to the nucleus and uncoating of the viral DNA. Early-region transcription, translation of the early proteins, and steady-state viral DNA replication all occur in the basal cell and in the infected suprabasal epithelial cell. Events in the viral life cycle leading to the production of virion particles occur in the differentiated keratinocyte: vegetative viral DNA replication, transcription of the late region, production of the capsid proteins L1 and L2, assembly of the virion particles, nuclear breakdown, and release of virus.

HPV DNA tends to exist in an unintegrated, circular episomal form. Although genomic integration is usually considered to be an important step in malignant transformation, it may not be essential. HPV DNA has been found to be integrated, as a partially deleted viral genome, into host chromosomal DNA in many high-grade dysplastic and most invasive cancer cervical specimens.

HPV DNA TYPES

Thanks to the development of modern molecular medicine methods, recombinant DNA technology above all, more that 100 HPV types were identified. Among them, > 80 HPV types have DNA genomes that have been well characterized by sequencing.

The classification of HPV types is based on nucleotide and amino acid sequence data. By definition, the nucleotide seguences of E6, E7 and L1 ORFs of a new type should carry no more than 90% homology to the corresponding sequences of known HPV types. HPVs have been further classified based on sequence similarity to the prototype:"subtypes" (90-98% similarity), and "variants" (> 98% similarity).

Based on their malignant potential, HPV DNA types can be divided into three groups: high risk HPV types; „medium“ risk HPV types; and low risk HPV types (Table 1).

Table 1. Genital HPV types

High risk HPV types	16, 18, 31, 45, 33, 35, 39, 51 52, 56, 58, 59, 68, 73, 82
Medium risk HPV types	26, 53, 66
Low risk HPV types	6, 11, 40, 42, 43, 44, 54, 61,70 72, 81, 89
HPV with unclear oncogene potential	34, 55, 57, 62, 64, 67, 69, 71, 74, 83, 84

The most prevalent types in cervical carcinoma are the HPV-16 and HPV-18, making up as much as 80 % of the cases. On the other hand, HPV types 6 and 11 are more commonly found in benign genital warts (condylomata). Other genital HPV types such as HPV-31, HPV-33 and HPV-35 are also associated with cervical cancer, although they are far less common than types 16 and 18.

The International Agency for Research on Cancer (IARC) has classified the HPV into three groups: carcinogenic (HPV types 16 and 18); probably carcinogenic (HPV types 31 and 33) and possibly carcinogenic (other HPV types except 6 and 11).

ONCOGENESIS

The host cell genes p53 and Rb (the retinoblastoma gene) are responsible for repairing damages (mistakes, or mutations) during cell replication. If the damage is irreparable, the cell is destroyed by a process called apoptosis. In persistent HPV infection the anti-oncogenic activity of p53 and Rb is blocked by the production of proteins by E6 and E7 from the HPV resulting in uninhibited host cellular growth and lack of repair of damaged cells. Abnormal cell proliferation then results in the integration of the viral DNA into the host cellular DNA with resultant immortalisation of the host cell which then becomes capable of invasion (6). Some of the wildly growing cells may develop irreparable, permanent changes in the genetic structure (mutation). This eventually results in the production of cancerous cells.

As the HPV cells occupy the host cells, various cellular changes occur in the epithelial squamous cells detectable by cytology. The first to occur is the koilocytic atypia in which the host cellular nucleus is displaced to the side with a 'hollow' appearance of the cytoplasm (perinuclear cavitation) (7).

The squamous cells, as the disease advances, then begin to show signs of change in size and shape with sometimes nuclear changes. About 60% of such cases may regress spontaneously; 20-35% would persist unchanged and the remaining 10% are likely to develop High Grade Intra-epithelial Lesion with increased cellular changes, reduced nuclear/cytoplasmic ratio and mitotic elements. As the whole epithelium is replaced with mutant cells cervical intraepithelial neoplasia (CIN) III or carcinoma in situ develops.

Today, the degree of cervical intraepithelial neoplasia (CIN I-III) is one of the fundamental cytologic and histologic indicators of (pre)malignant uterine cervix condition. The presence of HPV DNA is registered in 44-77% of CIN I cases; in 69-91% of CIN II cases; and in 86-100% of CIN III cases (8,9). Based on the association of presence of particular cervical HPV genotypes with cervical cancer, determination of oncogene risk of HPV types is a standard procedure.

Lately, more and more attention has been focused on intraepithelial neoplasms of external genitals in both sexes associated with HPV, together with relatively well-studied association of HPV with cervical intraepithelial neoplasms (Table 2).

Table 2. HPV DNA types associate with genital malignancies

Carcinoma of vulva	6, 11, 16, 18
Carcinoma of vagina	16
Carcinoma of cervix	**16, 18**, 31, 45, 33, 35
Carcinoma of anus	16, 31, 32, 33
Carcinoma in situ of penis (erythroplasia of Queyrat)	16
Carcinoma of penis	16, 18

CLINICAL MANIFESTATIONS OF HPV INFECTION

Clinical manifestations of genital HPV infections most commonly correspond to the following entities: condylomata acuminata, condylomata plana, gigantic condylomata (Buschke-Lovenstein) and bowenoid papulosis.

Condylomata acuminata is the most common form of genital condylomas. These are papulous or nodal entities, with papillomatous or veruccoid appearance, most frequently located on the external genitals – typically on the distal part of the corpus of penis or on the preputium, or the vulva in women, or in the anal region in both genders. They, however, may be located in the inner portions of the vagina, intraurethrally, inguinally or perineally. The changes may be solitary or, more commonly, multiple. Confluent changes are more common in intertriginous regions (inguinal, scrotal, perianal), where maceration has an important pathogenetic role.Condylomata acuminata is most commonly associated with HPV 6 and 11, but some papers report other HPV types as well, such as HPV 16, 18, 31, 42 (10).

Condylomata plana is the form separately described in recent literature. In the past, they were described as a variant of condylomata acuminata. These are plane papillomatous lesions most commonly caused by HPV 16, 18, 31 or 33 types.

Most authors consider them separately not only because of their shape but also because they are more inconspicuous and with higher oncogenic potential compared to „classic“ condylomata acuminata. Most HPV-induced genital infections of the uterine cervix as well as a number of „asymptomatic“ changes in males belong to this clinical entity.

Buschke-Lewenstein (BL) gigantic condylomas is a form characterized by a massive tumour lesion of anorectal region, within which histologic signs of malignancy cannot be found despite the impressive clinical presentation. HPV 6 and 11 are most commonly isolated from this tumor. As it seems, immune suppression has an important role in BL pathogenesis (11). However, the results of the latest studies have to be taken into account, suggesting the clinical and histologic signs of malignancy and detection of high risk HPV types (HPV 16,18) (12).

Bowenoid papulosis is characterized by the changes composed of multiple papulas most commonly localized on the external genitals, histologic signs of cellular atypia resembling morbus Bowen or spinocellular carcinoma *in situ*. HPV 16 is the most frequent isolate from Bowenoid papulosis.

DIAGNOSIS

HPV infection is detected by observation of visible lesions or microscopic changes in cells, by detection of HPV DNA, or by serological tests (assays to detect antibodies to HPV antigens in the blood).

Condylomata acuminata are genital lesions visible to the naked eye; they have a fleshy red appearance and a raised surface that usually extends in finger-like projections (papillae). Condylomata plana are flat, nonpapillary lesions; they are more difficult to detect and may be apparent only after swabbing with acetic acid and colposcopic examination, in which they appear as white, flat, shiny lesions. The Papanicolaou smear, which involves microscopic examination of stained exfoliated genital cells, detects koilocytosis and other signs of CIN; it is used to screen for cervical cancer by detecting high-grade CIN.

The most sensitive and specific method for detecting HPV infection is to test for HPV DNA. DNA testing can be used to detect a broad spectrum of HPV genotypes. Detection of HPV DNA signifies present exposure or persistent infection resulting from a past exposure. The most sensitive HPV DNA tests are those based on the polymerase chain reaction (PCR) and the hybrid capture assay.

The most commonly used serological tests for HPV infection measure antibodies (IgG) against capsid antigens.The presence of serum antibodies to the HPV capsid has been proposed as an indicator of lifetime cumulative HPV exposure. The serum antibody response to the HPV particle is stable over time, and persists after clearance of HPV infection. Thus, it can provide a marker of cumulative HPV exposure that can be used to compare trends over time. Because most HPV infections are cleared spontaneously within weeks to months, many people testing negative for HPV DNA may have had a previous infection. In some subjects, antibody seroconversions can be delayed many months after the detection of viral DNA. The major isotypes of serum antibodies against HPV capsids are lgG1 and IgA. Other IgG subclasses are only occasionally detected. IgG response may be more stable over time, compared to IgA.

Several validation studies have estimated the sensitivity of such serological tests to be approximately 50%, using detection of HPV DNA as a standard. Because of their low sensitivity, serological assays are not recommended for diagnostic use, but they are useful for comparison of groups in epidemiological studies, which also commonly use HPV DNA testing. Clinical diagnosis of HPV most commonly is based on the polymerase chain reaction and the hybrid capture assay.

TRANSMISSION

HPV genital infections are most common in young, fertile population. It is believed that condylomas are usually transmitted by direct sexual contact, though other transmission routes cannot be excluded (such as unhygienic depilation, shaving etc.). Condylomas are not transmitted via blood (transfusion) or as droplet infections. Two prerequisites should be satisfied for HPV infection: microtraumas of skin/mucosa, i.e. „entry“ and direct contact of skin/mucosa with infectious skin/mucosa or infectious secretions.

Genital HPV infections are ubiquitous and their rates are constantly rising. The results of comprehensive U.S. studies demonstrate six-fold increase of HPV genital infections in the last three decades, being three times more common than genital herpes (13,14). Some epidemiologic studies demonstrate HPV in the cervical smear of 60% of sexually active women. Signs of HPV disease can be observed in 40-60% of male partners of women with proven genital HPV infection. Genital HPV infection incidence is highest in the age range of 20 to 24 years. Epidemiologic data on genital HPV infections have been usually studied related to cervical cancer. Around 500.000 of new cases of cancer of the uterine cervix are diagnosed a year and that malignancy is the second most common in female sex (15).

Incubation of HPV genital infection is relatively long and lasts 2-9 months, though some literature data suggest the range of 6 weeks up to several years. Infectious individuals can be unidentified infection sources during such long intervals, and are the probable cause of difficult detection of infection sources and surveillance of HPV genital infection.

VACCINE

Many researchers worldwide try to design an anti-HPV vaccine. In the U.S., the vaccination has already started with the vaccine specific for highly oncogene HPV types. A recombinant vaccine is applied which contains capside protein (L1). It induces virus-neutralizing antibodies. The intention is to administer the vaccine in virgo girls, i.e. the individuals without infection with some oncogene-potent HPVs. The vaccine is not effective in sexually active women and possible prevention in this population is dismissed. The aim of such a concept of vaccination is the reduction of infection rate and reduction of incidence of cervical malignancies. The first results of anti-HPV vaccines regarding the reduction of cervical cancer rate should be expected in 10-30 years since the vaccine is applied in very young persons.

CONCLUSION

Genital HPV infections have more and more influence on the sexual and reproductive health of people, especially having in mind their undisputable association with carcinoma of the uterine cervix.

It is clear today that cervical cancer resulting from HPV infection is a preventable disease. Most important elements in that regard are the existance of a national prevention program, together with education and information of the general public on the problem of HPV infection and its consequences. Widely available early diagnosis of HPV infections and immunization programs are necessary. Such a concept aims above all to reduce infections with high risk HPV genotypes and thus reduce the rate of cervical malignancies. Until then, regular systematic gynecologic examinations (Pap-test and colposcopy) and detection and HPV genotypization three years at the latest of the start of sex life.

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HUMANI PAPILOMA VIRUS

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SAŽETAK

Humani papiloma virus (HPV) obuhvata viruse uzročnike bradavica na koži i genitalnoj regiji. HPV infekcija je veoma česta genitalna infekcija sa prevalencom od 30% do 40% kod mladih ljudi.

Genitalna HPV infekcija predstavlja najčešću polno prenosivu bolest. Njihov broj je u stalnom porastu u poslednje tri decenije i sada je tri puta veći u poređenju sa genitalnim herpesom. Ove infekcije su uglavnom prolaznog karaktera, blagog, asimptomatskog toka. Većina infekcija završi spontanim izlečenjem u periodu od osam meseci do dve godine. Oko 50 HPV tipova inficira anogenitalnu regiju i dominantno se prenose seksualnim kontaktom.

Zbog povezanosti HPV infekcije i cervikalnog karcinoma HPV je danas priznat kao uzročnik potreban za razvoj karcinoma cerviksa. Karcinom cerviksa je najčešće maligno oboljenje žena u zemljama u razvoju. Širom sveta, oko 500 000 žena oboli godišnje, a od toga 75% su žene iz zemalja u razvoju. Oko 300 000 žena umre godišnje od ove bolesti.

Pored dokazane povezanosti HPV i cervikalne intraepitelne neoplazije, u poslednje vreme, velika pažnja poklanja se intraepitelnim neoplazijama spoljašnjh genitalija u oba pola povezanim sa HPV.

Ključne reči: **humani papiloma virus, onkogeneza, karcinom cerviksa**