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The Role of Human Papillomavirus in the Malignant Transformation of Cervix Epithelial Cells and the Importance of Vaccination Against This Virus*

Rola HPV w mechanizmie transformacji nowotworowej komórek nabłonka szyjki macicy oraz znaczenie szczepionki przeciwko temu wirusowi

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Abstract

Human papillomavirus (HPV) belongs to the diverse group of sexually transmitted viruses that manifest affinity to the squamous epithelia of the skin and mucous membranes. Over 100 types of HPV have been described and identified in human tissues, and it has been proved that persistent infection with high-risk types of the virus (types 16 and 18 in particular) could lead to cervical cancer. High-risk HPV types have been found in approximately 70% of all cases of cervical cancer worldwide. The aim of this study was to describe the role of HPV in the process of neoplastic transformation in epithelial cells and to emphasize the prophylactic significance of vaccinations against the virus (Adv Clin Exp Med 2012, 21, 2, 235–244).

Key words: human papillomavirus (HPV), neoplastic transformation, cancer of uterine cervix, vaccine against HPV.

Human papillomavirus (HPV) represents a group of widely diversified viruses that are implicated in the etiology of uterine cervix cancer. In 2008, the German scientist Harald zur Hausen was awarded the Nobel prize in medicine for the discovery of the human papillomavirus. The results of his studies confirmed the role of HPV in the etiology of uterine cervix cancer, contributed to the development of new tests permitting an early diagnosis of cervical carcinoma and established new directions for prophylaxis against this cancer. Prof. zur Hausen’s studies made it possible to develop a prophylactic vaccine against HPV infection and therefore lowering the risk of developing cancer of the uterine cervix [1–3].

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HPVs demonstrate a specific tropism to cells of the squamous epithelium of the skin and mucous membranes in humans and several animal species [4]. They may penetrate and infect cells in basal and parabasal layers of the epithelium but the full developmental cycle and production of infectious progeny particles takes place only in differentiated keratinocytes [5]. Over 100 types of HPV are known, of which about 40 infect the mucosal epithelia of the anogenital regions [6, 7]. They have been divided into three groups, depending on their oncogenic potential [8, 9]:

1. HPVs of a low oncogenic potential – HPV types 6, 11, 42, 43 and 44;
2. HPVs of a moderate oncogenic potential – HPV types 31, 33, 35, 39, 45, 51, 52 and 56;
3. HPVs of a high oncogenic potential – HPV types 16 and 18.

Most types of HPV are responsible for the development of benign dermal lesions in the form of dermal warts and condylomas, warts of the genital regions. However, clinical data also indicate a relationship between infection with the virus and pre-neoplastic lesions and tumors of the mucosa of the genital regions and anus. High-risk HPVs (types 16 and 18) are associated with malignant lesions of the genital regions. HPV 16 is the most prevalent of the high-risk types, found in about 54% of cervical cancer cases, while HPV 18 is detected in around 16% of the cases [10].

The HPV Genome Structure and Functioning

Human papillomavirus belongs to the Papillomaviridae family. These are small non-enveloped double-stranded DNA viruses with a genome of approximately 8000 base pairs [3]. All the viral genes are located on a single DNA strand, which is transcriptionally active. They are accumulated in two open reading frames (ORFs) [8]. In the HPV genome three regions can be distinguished:

- the early region (E) – coding for early control proteins (E1, E2, E4, E5, E6 and E7), which are responsible for the persistence of the viral genome in a cell, its replication and for the neoplastic transformation of host cells; they are expressed in all phases of the cell cycle;
- the late region (L) – coding for structural proteins: major and minor capsid proteins (L1 and L2, respectively);
- the long control region (LCR) – not coding for structural proteins, but performing a controlling function in the expression and replication of the viral genome.

Proteins, of the E1 and E2 regions are responsible for the initiation of viral DNA replication, due to their ability to bind transcription factors. They play key roles in the transcriptional regulation and replication of viral DNA [3, 7]. Proteins of the E2 region bind to DNA sequences in the control region and perform the function of activator or repressor of viral gene transcription. E4 protein has ability to bind keratin filaments, inducing changes in keratinocytes; and to release viral progeny particles from cells, resulting in the formation of koilocytes (cells with a characteristic bright perinuclear halo and a clearly marked cell membrane, resulting from the proliferation of HPV). E5, E6 and E7 are viral proteins with oncogenic properties, responsible for the neoplastic transformation of cells. E6 in particular manifests a high oncogenic potential and mediates the degradation of the p53 antioncogenic protein. E7, in turn, has the ability to bind and degrade another antioncogene, the cellular RB protein [11, 12].

Products of the late region (L) include two structural proteins of HPV, L1 and L2. They form the viral capsid and are synthesized exclusively in differentiated keratinocytes. The most important of these proteins is coded in L1 region, while L2 region codes additional proteins of the viral capsid [3].

The LCR is a significant non-coding fragment of viral DNA, accounting for about 10% of the entire genome. It performs regulatory functions, affecting the transcription of the E6/E7 genes, which are particularly involved in the process of neoplastic transformation [13]. The region contains a promoter sequence (p97), which binds RNAII polymerase, and binding sequences for all regulatory transcription and replication factors of viral genetic material. All the LCR regions of HPV that have been examined so far contain specific enhancers that provide the virus with specific tropism to stratified squamous epithelial cells [14].

Epidemiology and Risk Factors in HPV Infection

Human papilloma virus is the most common sexually transmitted infection, particularly in young individuals. It is estimated that over 50% of sexually active women become infected with one or several HPV types in their lifetimes [15]. The prevalence of HPV depends on the geographical region and the prevailing standard of living [16]. Around 20 types of HPV have been found to be associated with the development of cervical cancer HPV 16 is identified as being the most oncogenic type, detected in 53% of cervical cancer followed by
HPV 18, HPV 45, HPV 31 and HPV 33 and HPV 56. Infections with the oncogenic types represent over 50–75% of all HPV infections [15, 16].

The most significant risk factors in HPV infection include age and sexual activity [17]. Infection is most frequent in women between 20 and 40 years of age, with the peak of incidence between 15 and 25 years of age. As women's age progressed the rate of infection decreases, and after age 40 it remains at a stable low level. In post-menopausal women HPV infection occurs seldom, but it is always associated with a very high risk of developing cervical cancer [18]. The risk of HPV infection significantly increases with the number of sexual partners. Another factor associated with increased risk of HPV infection is prolonged use of hormonal contraception, due to the effects of estrogens and progesterone, which stimulate transcription and cell proliferation and overexpression of E6 and E7 oncogenes [19]. Some investigators also note an increased risk of HPV infection in women who smoke cigarettes [20, 21]. Other factors that increase the risk of HPV infection and cervical cancer are pregnancy and multiparity. Incidental or persistent infections with high-risk HPVs are detected in 30% of pregnant women; however, the virus is transmitted in the perinatal period in only 2% of newborn infants whose mothers were infected with HPV [22, 23].

Persistent infection with a high-risk type of HPV is the most significant factor in the neoplastic transformation of mucosal cells of the uterine cervix [15, 19]. In women between 15 and 25 years of age most infections are transient, and in around 70% of the patients they spontaneously vanish around one year following the detection of the virus [24]. On the other hand, a protracted infection, lasting over one year, is associated with infection with the highly oncogenic virus types, particularly with HPV 16 [15, 19, 25].

The Mechanism of HPV Penetration and Infection

Infection with human papilloma virus is usually sexually transmitted. Infection follows micro-injuries of the mucosa that uncover the basal layer of stratified squamous epithelium [26]. This allows the virus to penetrate to deeper epithelial layers with epidermal growth factor receptors (EGFRs), integrins α-6 and also receptors for HPV. The penetration of viral particles to deeper layers of the epithelium is followed by an incubation period lasting from around 3 weeks to 8 months, with subsequent expression of viral proteins [7]. At the beginning, the early proteins of the virus are expressed in the basal and parabasal layers of the epithelium [11]. Subsequently, the later, structural proteins of the virus are expressed in the cells of higher layers. Expression of the late proteins increases as the distance from the surface of mucosal epithelium decreases. At this stage, synthesis of structural proteins takes place and mature viral particles are produced [5, 7, 11].

As noted above, chronic infection with a high-risk type of HPV is the most significant factor in the progression of pathological lesions into cancer of the uterine cervix [15, 19]. However, only a few of the infections and lesions induced by the presence of HPV lead to neoplastic transformation. Most HPV infections undergo regression within 12–18 weeks after infection. This suggests that a number other factors (such as immune system imbalances, treatment with immunosuppressants, coexistent infections) occurring along with viral particles are decisive for the neoplastic transformation of epithelial cells in the uterine cervix [19].

The Mechanism of HPV-Induced Neoplastic Transformation of Cells

The process of cell neoplastic transformation is complex and multistage. Even if infection with highly oncogenic types of HPV is decisive for the process, on its own it is not sufficient to induce the development of a malignant tumor [7, 27, 28]. Other significant factors include the duration of the infection, environmental factors, cigarette smoking and the age and health of the infected person [24]. The development of a tumor requires mutations in genes controlling cell division, cell growth and differentiation [5].

Integration of HPV with the Genome of the Host Cell

Particles of HPV are present in cells of the mucosal epithelium of the uterine cervix in episomal or integrated forms. The episomal form is present in primarily infected, undifferentiated epithelial cells, while in tumor lesions the viral DNA is detected in the integrated form with the genome of the host cells [28].

The process of neoplastic transformation induced by high-risk HPV types (e.g. HPV 16 and HPV 18) can be initiated only when the viral ge-
The integration of HPV with the host cell genome leads to disruption of viral DNA in the E1 and/or E2 region, and also to a loss of E2-adjacent ORFs, i.e. E4, E5 and a part of L2. However, ORFs E6 and E7 remain intact during the integration process [30]. Disruption of E2 and/or E1 readout frames leads to the inhibition of the gene expression [7]. In HPV-induced neoplastic transformation of cells, the loss of correctly functioning E2 regulatory protein is of key importance, since that protein controls expression of the E6 and E7 genes through the sequences located in the LCR region. A lack of repression by the E2 protein results in overactivation of viral E6 and E7 genes, leading to synthesis of the two oncogenic proteins [7, 30]. The oncoproteins exhibit the ability to bind and to degrade anti-oncogenic cell products, p53 and pRB proteins [28]. In this way, the DNA repair process is disrupted in the host cells, promoting their proliferation and growth and delaying their terminal differentiation [5].

Only early genes undergo expression in the epithelial basal layer, while the E6 and E7 proteins are expressed in higher layers of the epithelium, which stimulates the replication of DNA in the host cells and allows for replication of viral DNA [7, 31]. In the initial phase of the infection, most infected cells in the basal layer of the epithelium contain HPV DNA in the episomal form (not integrated with the DNA of the infected cells). Neoplastic progression is accompanied by the integration of viral DNA with the cellular DNA. The presence of the E6 protein promotes the integration of viral DNA with the host cell genome [9]. The transformed cells contain an insignificant amount of viral DNA; only the E6 and E7 genes are expressed, while the late capsid proteins, L1 and L2, cannot be detected [32].

The Effect of the E6 Protein on the Neoplastic Transformation of Cells

E6 binds to the tumor-suppressor p53 protein, leading to its proteolysis by the ubiquitination of proteasomes [11]. In normal cells p53 manifests a low level but in response to DNA damage and viral infections its expression increases. The p53 protein is involved in controlling the cell cycle, DNA repair and apoptosis [7]. In the presence of the E6 protein, the activity of p53 becomes suppressed, which may disrupt the cell division cycle, promoting cell proliferation and increasing the frequency of spontaneous mutations. This may consolidate chromosomal instability in cells infected with high-risk types of HPV [5, 7].

In normal cells, the ubiquitin-dependent degradation of p53 involves, among other factors, Mdm2 ligase [33]. In cells with viral DNA, the degradation is fully dependent on E6 HPV and develops with the participation of E6AP ligase [34]. The oncoprotein E6 forms a stable complex with E6AP ligase. The complex binds p53 and leads to its proteolysis [35].

Effective degradation of p53 depends on the type of HPV that infected the cell. The efficiency of p53 protein degradation in various HPV types depends on the ability of the oncoprotein E6 to bind the p53 protein and E6AP ligase. The E6 protein in both low- and high-risk mucosal types binds p53, but it is only in the high-risk types that it effectively degrades the protein. This reflects the ability of the E6 protein in high-risk virus types to form a strong complex with E6AP ligase and to bind a distinct domain of the p53 protein. Dermal types of HPV, on the other hand, are completely unable to bind with E6AP and p53. High-risk viruses vary in their efficacy in p53 degradation: E6 of HPV 16 binds the ligase with a higher affinity and the degradation of p53 is more efficient than that of E6 in HPV 18; the E6 protein of HPV 11, on the other hand, forms a very weak association with ligase [36, 37].

Apart from the p53 degradation-dependent mechanism, HPV has developed several other mechanisms that prevent programmed cell death. The various pathways in which the viral E6 protein interacts with the host cell are presented in Figure 1. In this way the survival and proliferation of cells with damaged DNA and the accumulation of mutations in the cellular genome are promoted. This leads to the neoplastic transformation of host cells with integrated HPV DNA. The E6 protein of HPV also prevents apoptosis by degrading factors other than p53. Bak is one of proapoptotic proteins that undergoes ubiquitin-dependent proteolysis when affected by HPV E6/E6AP: A high level of Bak is found in the upper layers of the epithelium, but the E6 protein of HPV inhibits Bak-dependent apoptosis [38]. The Fas-associated death domain protein (FADD) is another peptide degraded by HPV E6/E6AP: The peptide binds to the death domain (DD) of the Fas receptor, activating caspase 8 and mobilizing the apoptosis pathway. Procaspase 8 is also degraded, which prevents its conversion to active caspase 8 [39]. Another pathway through which the virus prevents programmed epithelial
cell death involves elevated expression of the inhibitor of apoptosis-2 protein (IAP-2): HPV E6 activates the transcription factor NF-κβ, which induces increased expression of IAP-2 [40]. The E6 proteins of highly oncogenic viruses may also immortalize host cells by their ability to prevent shortening of telomere length [41]. In somatic cells every consecutive division of a cell results in abbreviated telomeres. Telomere length reflects the age of the cells. Elevated telomerase activity, which adds additional telomere sequences, has been documented in human neoplastic cells, and it has been suggested that this may be of significance in the development of tumors [41]. The activity of the telomerase enzyme is dependent on the human telomerase catalytic subunit (hTERT). The E6 and E6AP proteins of high-risk HPV prevent telomere shortening due to increased expression of the hTERT gene. Moreover, the E6/E6AP complex induces degradation of NFX1–91 (a recently discovered hTERT repressor) and thus increases expression of the telomerase subunit [42].

Host cell neoplastic transformation is affected by the highly conservative domain at the C-terminal of the E6 peptide, present exclusively in the E6 of highly oncogenic viruses. This domain contains the XT/SXV motif, which interacts with proteins containing the PDZ domain [38]. Proteins with the PDZ domain are responsible for the adhesion and polarity of cells, as well as for intercellular signalling, and their degradation leads to cell transformation [35] Proteins with the PDZ domain include (among others) human homologues of the tumor-suppressor proteins Dlg and Scrib, manifested in Drosophila melanogaster; the postsynaptic protein PSD 95; the multi-PDZ-domain protein (MUPP1); MAGI-1, -2, -3, which are proteins forming intercellular junctions; PTPN13 phosphatase; and PATG [35].

Neoplastic cells infected with the virus are able to avoid the immune response. Overexpression of the E6 protein leads to the blocking and inactivation of p300/ CBP, which is a co-activator of the NF-κβ transcription factor. The NF-κβ transcription factor controls (among other things) promoters of the interleukins IL-6, IL-8 and IRF β [43]. E6 also inactivates the co-activator ADA3, which stabilizes the p53 protein [44].

### The Effect of the E7 Protein on the Neoplastic Transformation of Cells

The E7 protein interacts with proteins of the retinoblastoma family, such as pRb and homologous proteins, p107 and p130. E7 binding to pRb releases the E2F transcription factor from the pRb complex, activating the transcription of genes that control cell
proliferation this leads to the expression of proteins engaged in the synthesis of cellular DNA, and the cell enters the S phase of the cell cycle [45].

Viral E7 may bind other host cell proteins, causing the cell to enter subsequent phases of the cell cycle [46]. This is how HPV controls the proliferation of the infected cells and promotes oncogenesis. HPV E7 interacts with histone deacetylases (HDAC), a class of enzymes that act as co-repressors of gene transcription: Their interactions with the E2F transcription factor prevent the activation of several genes, the products of which are involved in cell proliferation. The binding of E2F by viral E7 makes this repression impossible and promotes transcription of the genes [46].

The E7 protein may also bind to the complex of cyclin A/CDK2 and cyclin E/CDK2. This activates kinases, leading to the phosphorylation of pRb and the transcription of genes involved in cells entering the S phase of the cell cycle [47, 48]. Another way in which the virus promotes the amplification of its genes involves interactions with a group of proteins called CDK inhibitors (CKIs). The HPV E7 protein binds to such proteins as p21 or p27. This allows the checkpoints that control the progression of cells from G1 phase to the S phase of the cell cycle to be bypassed [49, 50].

It has also been found that the E7 protein affects the expression of interleukin 6 (IL-6) and the pro-apoptotic factor Mcl-1. Overexpression of IL-6 and Mcl-1 induced by HPV E7 inhibits the development of apoptosis in cells with integrated viral DNA [51]. Tumor cells with HPV are able to avoid the immune response due to the E7 protein binding with the interferon regulatory factors IRF1 and IRF9, which results in the inactivation of the factors and blocking of the the interferon α (IFNα) signalling pathway [52, 53]. Various interactions of the viral E7 protein are presented in Figure 2.

**Vaccines Against HPV**

As noted earlier, infection with HPV is the most common sexually transmitted viral infection all over the world; and infection with high-risk types of HPV may lead to carcinoma of the uterine cervix or other urogenital organs [54]. Viruses of HPV types 16 and 18 are responsible for around 70% of all cervical cancer cases and for most pre-neoplastic intraepithelial lesions [25, 55]. Cancer of the uterine cervix is the second most frequent malignant tumor in females [25, 31]. Since the presence of oncogenic HPV types correlates with the development of neoplastic lesions in the uterine cervix, the application of a prophylactic vaccine against oncogenic genital types of HPV is the most effective form of prophylaxis against the development of such tumors [15, 31].

Commonly used prophylactic vaccines against various viral diseases are directed against infectious agents that induce systemic diseases, rather than against a local infection such as infection with papilloma virus. HPV’s are viruses that take advantage of cell proliferation in their life cycle but do not induce cell lysis, which is reflected by the poor immune response of the host. In contrast to lytic viruses which are rapidly detected by the immune system (causing host cell destruction at the terminal stage of infection), antibodies directed against HPV capsid proteins arise as late as 6–12 months following the infection [56]. Moreover, in recent years studies have proved that viruses may develop mechanisms shielding them from the immune system or slowing down its reaction [28, 56].

Despite such limitations, recent research has resulted in the production of a vaccine which is 100% effective in preventing the development of precancerous lesions, non-invasive carcinoma of the uterine cervix and pointed condylomas, which

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**Fig. 2.** Interactions of E6 in high-risk HPV types leading to neoplastic transformation of host cells – adapted from Ganguly and Parihar [35]

**Ryc. 2.** Oddziaływanie E7 HPV wysokiego ryzyka prowadzące do transformacji nowotworowej komórek gospodarza (wg Ganguly i Parihar [35] zmodyfikowano)
are linked to infection with HPV types 6, 11, 16 and 18 [19, 57]. The development of the vaccine was advanced by the studies of Professor Harald zur Hauser, who was awarded the 2008 Nobel prize in medical sciences. Production of the prophylactic anti-HPV vaccine was announced by two pharmaceutical companies [1, 24]. These proved to be virus-like vaccines, consisting of the paraviral protein L1 (a virus-like particle – VLP), which represents the principal viral capsid protein. The vaccines contain no viral DNA and therefore have no ability to infect cells, to stimulate proliferation or to induce pathological lesions [31]. Similarly to the course of natural infection, VLPs induce antibody production [31, 58]. In a natural infection the antibodies are directed against epitopes of the L1 capsid protein of HPV. The L1 and L2 proteins of HPV manifest the ability to self-assemble and to form virus-like particles of viral capsid [31, 54].

Large quantities of HPV L1 protein can be obtained using genetic engineering techniques: The HPV L1-coding gene is introduced to plasmid vectors or recombinant baculoviruses. Subsequently, it undergoes translation to L1 protein in yeast cells or insect cells cultured in vitro. Following export from the cells, L1 protein particles self-assemble into VLPs [59]. The VLPs obtained closely resemble the structure of native HPV virions and they induce the production of neutralizing antibodies [56]. Since they do not contain viral DNA, they are not infectious and have no oncogenic potential to induce neoplastic transformation of cells in the vaccinated individual. The viral-like particles of genital HPV types are highly immunogenic and application of even small doses of L1 VLP results in the production of large numbers of antibodies against the protein [58]. The vaccination results mainly in the production of IgG1 class antibodies, which effectively neutralize HPV particles [60].

Currently, two vaccines against HPV are available on the market. The bivalent vaccine is directed against HPV types 16 and 18, while the tetravalent vaccine is targeted at the four so-called genital types: HPV 16, HPV 18, HPV 6 and HPV 11. The efficiency of the tetravalent vaccine was proven in the clinical trials FUTURE I and FUTURE II, conducted all over the world [57, 61, 62]. Over 12,000 women from 13 countries (including Poland) took part in the latter trial. The participants were from 16 to 23 years of age and received three intramuscular doses of the tetravalent vaccine or placebo [62]. In every case gynecological and cytological examinations were conducted both before vaccination and on a few occasions following vaccination. Clinical examinations and observations conducted in the study’s second phase (which lasted 5 years) and third phase (which lasted 2–2.5 years) demonstrated the tetravalent HPV vaccine’s 100% effectiveness in the prevention of uterine cervix carcinoma, cervical intraepithelial neoplasias types CIN2/3 and preneoplastic lesions of the vulva and vagina [57, 63]. However, the vaccine manifests no therapeutic activity: It cannot be used to treat already existing genital warts or developing carcinoma of the uterine cervix [25]. Tests continue on vaccines that might offer treatment of diseases induced by HPV infection [18, 31], but the results of preliminary studies are not promising. This is explained by the fact that during long-term infections linked to the presence of viral DNA in epithelial cells, no expression of L1 protein occurs [31]. In experimental studies conducted on animals with pathological lesions in the epithelium, the administration of VLP induced no therapeutic effects even if it provided protection when applied prophylactically [26]. Thus, administration of an HPV vaccine is justified to prevent infection. According to a statement by Polish Gynecological Society experts, vaccinations against HPV should be administered to girls at the age of 12 to 13, before the start of their sexual activity and before their bodies have contact with HPV [64]. The vaccine may also be effective in women who have started sexual activity but have had no contact with HPV.

The effects of chronic HPV infection, with sequelae linked to treatment and medical expenditures, as well as the psychological aspect of uterine cervix carcinoma, represent a very serious social problem. There is therefore a real need for routine administration of the vaccine to women, particularly in countries with very irregular or no screening examinations. Widespread application of anti-HPV vaccines might significantly reduce the incidence of uterine cervix carcinoma. Whether or not the vaccine will be widely used and promoted, the key problem remains: making women aware of the need to conduct regular cytological monitoring examinations [9].

References


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