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REVIEW

Carcinogenesis Associated with Human Papillomavirus Infection. Mechanisms and Potential for Immunotherapy

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Abstract—Human papillomavirus (HPV) infection is responsible for approximately 5% of all cancers and is associated with 30% of all pathogen-related cancers. Cervical cancer is the third most common cancer in women worldwide; about 70% of cervical cancer cases are caused by the high-risk HPVs (HR HPVs) of genotypes 16 and 18. HPV infection occurs mainly through sexual contact; however, viral transmission via horizontal and vertical pathways is also possible. After HPV infection of basal keratinocytes or ecto-endocervical transition zone cells, viral DNA persists in the episomal form. In most cases, infected cells are eliminated by the immune system. Occasionally, elimination fails, and HPV infection becomes chronic. Replication of HPVs in dividing epithelial cells is accompanied by increased expression of the E6 and E7 oncoproteins. These oncoproteins are responsible for genomic instability, disruption of the cell cycle, cell proliferation, immortalization, and malignant transformation of HPV-infected cells. Besides, E6 and E7 oncoproteins induce immunosuppression, preventing the detection of HPV-infected and transformed cells by the immune system. HPV integration into the genome of the host cell leads to the upregulation of E6 and E7 expression and contributes to HPV-associated malignization. Prophylactic HPV vaccines can prevent over 80% of HPV-associated anogenital cancers. The vaccine elicits immune response that prevents initial infection with a given HPV type but does not eliminate persistent virus once infection has occurred and does not prevent development of the HPV-associated neoplasias, which necessitates the development of therapeutic vaccines to treat chronic HPV infections and HPV-associated malignancies.

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Abbreviations: AIDS, acquired immunodeficiency syndrome; CIN1, CIN2, and CIN3, light, moderate, and severe cervical intraepithelial neoplasia, respectively; HAART, highly active antiretroviral therapy; (HR) HPV, (high carcinogenic risk) human papillomavirus; HSIL and LSIL, high- and low-grade squamous intraepithelial lesion, respectively; L1 and L2, large and small capsid proteins of HPV, respectively; LCR, NCR, and URR, long control region, noncoding region, and upstream regulatory region of HPV genome, respectively; ORF, open reading frame.

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Up to 15-20% of cancers are caused by viral infections. Human papillomaviruses (HPVs) are responsible for approximately 5% of all cancers. HPVs, first described in 1933 by Shope and Hurst [1], are a large family of small encapsidated double-stranded DNA viruses that cause malignant or benign proliferation of epithelial cells or formation of condylomas. Advances in genomic sequencing have allowed to identify several HPV types with specific tissue tropism for mucosal surfaces or epidermal squamous epithelium. These HPV types can cause both benign warts and carcinomas in the lower genital area, as well as oropharyngeal carcinomas [2, 3]. The incidence of HPV associated oropharyngeal cancer is rising globally at an alarming rate; it has been estimated that by 2020, HPV-associated oropharyngeal cancer may surpass cervical cancer as the leading HPV-associated malignancy [4].

Human and animal papillomaviruses replicate persistently in specific types of host stratified epithelium, causing chronic asymptomatic infections. They are characterized by a long-lasting virion production with limited expression of viral genes, which minimizes the chances of immune clearance. After initial infection, the level of viral genome replication in dividing epithelial cells is low. These cells form a reservoir of infection that can persist for decades. When infected cells differentiate, the level of expression of viral proteins increases by many-fold, with massive production of virions by the surface epithelial cells [5]. To date, more than 200 HPV genotypes isolated from clinical samples have been identified. Their classification is based on DNA sequence analysis; the numbers to the genotypes are assigned as the genotypes are detected and identified (Fig. 1).

HPV genotypes belong to five evolutionary genera (α, β, γ, μ, ν). HPVs of the genus β adhere to the strategy of minimalization of the viral gene expression and their replication is suppressed by the host immune response. HPV of the genus α have developed the strategy of immune escape (avoidance) achieved through down-regulation or suppression of immune response modulated by viral proteins. These strategies reflect significant differences in the expression of viral genes and in the functions of the same viral proteins in different types of HPV. The functions of viral proteins also depend on the type of infected tissues.

HPV TYPES ASSOCIATED WITH CARCINOGENIC RISK

HPVs are divided into two groups according to their clinical significance: low-risk HPV genotypes (types) associated with low risk of carcinogenesis, which mainly cause condylomas, and high-risk (HR) HPV types, which are strongly associated with the development of neoplasias and cancer. The International Agency for Research

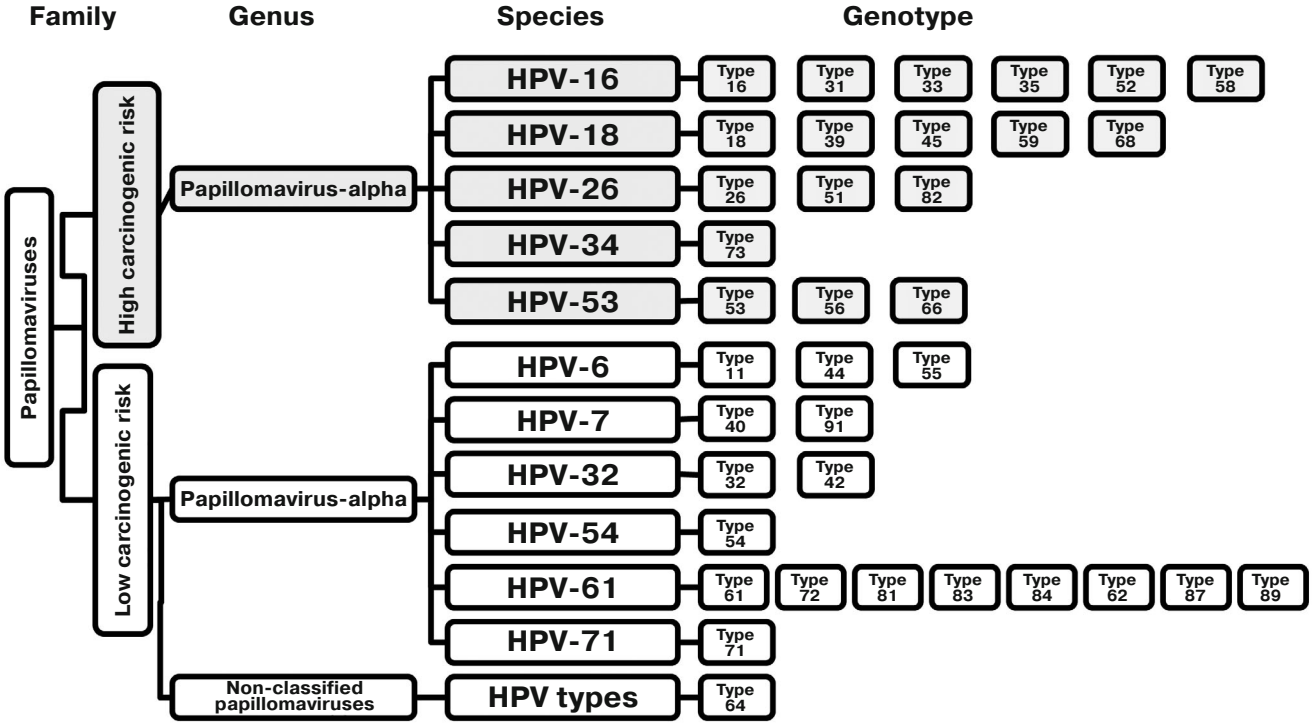

Fig. 1. Taxonomic classification of HPVs developed by the International Committee on the Taxonomy of Viruses (http://cvc.dfci.harvard.edu/hpv/HTML/classification.php).

on Cancer (IARC) has proposed a more detailed classification of HPV types [6]:

– non-carcinogenic types: HPV 6, 11 (2);

– probably carcinogenic types: HPV 26, 53, 66, 67, 70, 73, 82, 30, 34, 69, 85, and 97 (12);

– possibly carcinogenic types: HPV 68;

– high-risk types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (12).

More recently, the list of sexually transmitted HR HPVs associated with carcinogenesis, namely with the development of anogenital and oropharyngeal cancers has been expanded to 20 types [7].

Infection with HR HPVs is strongly associated with the development of anogenital cancers (cervical cancer, in the first place), as well as nasopharyngeal and head and neck cancers and high-grade intraepithelial neoplasias (cancer precursor lesions). The Bethesda classification of neoplasias for cervical cytology [8] includes the following categories:

– atypical squamous cells of undetermined significance (ASC-US);

– atypical squamous cells, cannot exclude HSIL (ASC-H);

– low-grade squamous intraepithelial lesion (LGSIL or LSIL);

– high grade squamous intraepithelial lesion (HGSIL or HSIL);

– squamous cell carcinoma;

– atypical glandular cells, not otherwise specified (AGC-NOS);

– atypical glandular cells, probably adenocarcinoma *in situ* (AIS) or neoplastic (AGC-neoplastic);

– adenocarcinoma *in situ* (AIS).

Low-grade squamous intraepithelial lesion (LSIL) corresponds to the mild cervical intraepithelial neoplasia (CIN1); HSIL corresponds to the moderate or severe intraepithelial neoplasia (CIN2/3) [9]. Up to 75% of all squamous cell cancers and 94% of all adenocarcinomas are caused by infection with HPV types 16, 18, 31, 33, and 45 [10]. The most common types of HR HPV responsible for the development of more than 80% of all cervical cancers are HPV 16 and 18 [11]. HPV 16 also dominates in the cases of anal cancer [12].

PREVALENCE OF HPV AND HPV-ASSOCIATED FORMS OF CANCER

According to the US Center for Disease Control and Prevention (CDC), more than 90% of sexually active men and more than 80% of women during their lifetime become infected with at least one type of HPV [13]. Approximately 50% of those infections are HR HPVs (https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer). The most common HR HPV types worldwide are 16, 18, 31, 33, 35, 45, 52, and 58 (https://www.who.int/bulletin/volumes/85/9/06-038414/en/). The frequency of occurrence (prevalence) of different types of HR HPVs varies between the countries, reflecting differences in the resources available to their healthcare systems. Prevalence of the most common type, HPV 16, is 77% in Germany, 71% in South America, 59% in USA, and 33-39% in Japan. The second common type is HPV 18 with a worldwide prevalence of 8%, but in the USA, it is ranked fifth after HPVs 16, 52, 51, and 31. In general, HPV 33 is prevalent in Europe, while HPVs 52 and 58 dominate in Asia (in Japan, HPV 52 is detected in 20% cervical cancers, while in USA, it occurs only in 2% of cases) [6, 14, 15]. In South Africa, the most common HPV types are 16 (11.7%), 58 (10.3%), 51 (8.9%), 66 (8.6%), and 18 (7.6%). Sub-Sahara is a region with one of the highest prevalences of HPV infection; in this region, prevalence of HR HPVs 16, 18, and 45 among HIV-infected women with cervical cancer is 3 to 4 times higher than among HIV-uninfected women [16-18].

While in Europe, USA, Australia, and New Zealand the prevalence of cervical cancer is decreasing, in the Russian Federation it is increasing. In 2007-2016, the annual incidence of HPV-associated neoplasias among the male population of the Russian Federation was 33.7, and among the female population – 715.5 per 100,000 [19]. Since 2007, the incidence of cervical cancer among the female population of the Russian Federation has increased on average by 2.26% per year: in 2007, it was 12.48, and by 2017, it has increased to 15.76 per 100,000 women. The number of new cases in 2017 was 17,587, and the overall and standardized incidence rates of the cervical cancer were 22.3 and 15.76 cases per 100,000, respectively [20]. Furthermore, in certain regions of the Russian Federation, the standardized incidence rate of cervical cancer surpasses the national average by three or more times. The contribution of cervical cancer to the mortality due to the development of malignant neoplasias significantly varies in different age groups: it is the cause of death for 7.1% of women younger than 30 years and for 23.1% of women in the 30-39-year age group. According to other data, cervical cancer is the most common cause of cancer mortality in the Russian Federation in women under 45 years [19]. In the older age groups, its significance decreases: among 40-49-year-old women, it ranks second (17.3%) after the breast cancer (23.5%), and in 50-59-year-old women, cervical cancer is the cause of death in 7.2% of cases. In 2017, cervical cancer was the cause of death of 6480 Russian women, which manifests an increase in the cervical cancer death rate between 2007 and 2017 by 4.31% [20].

In 2012, Rogovskaya et al. published a detailed analysis of the HPV types prevalent in different regions of the Russian Federation in various population groups. The prevalence of HR HPV infection in the general population ranged from 13 to 40% and correlated with the sexual behavior. In most regions of the Russian Federation, HPVs

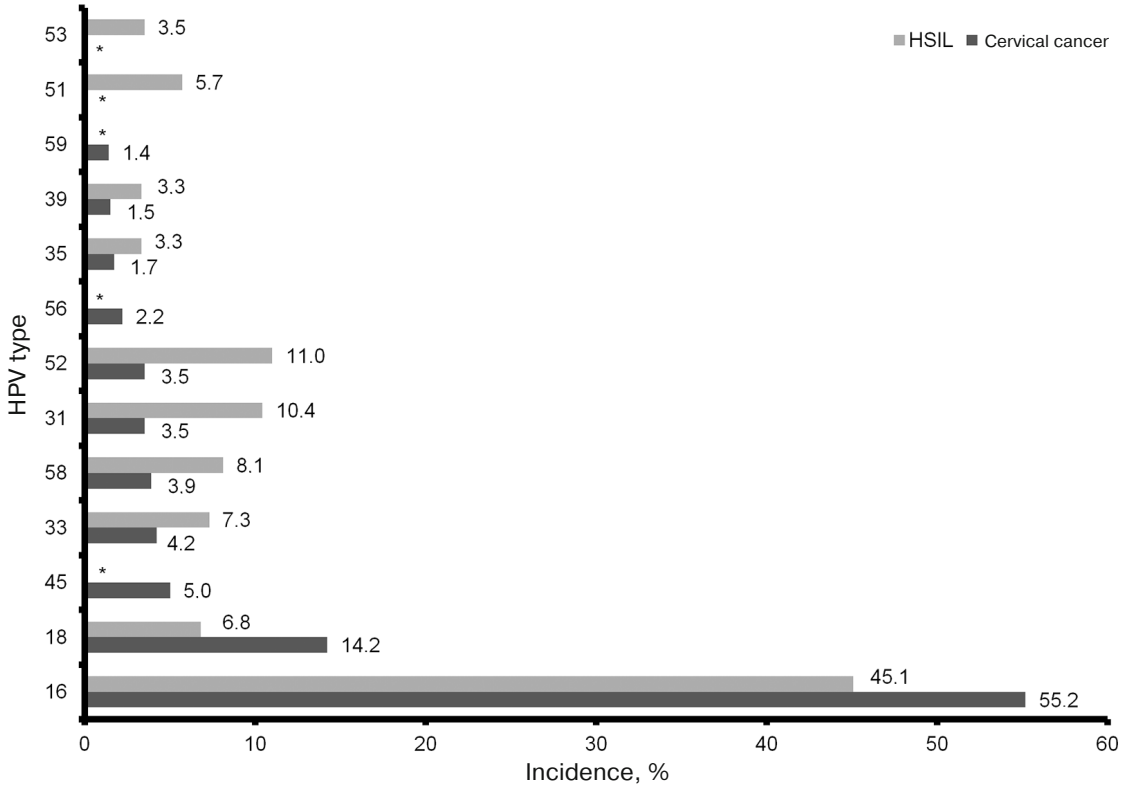

Fig 2. Incidence of HR HPV in women with HSIL and cervical cancer; *, no data. The frequency of occurrence is ranked by the frequency of detection in the cases of cervical cancer (Bruni et al., 2019 [11]).

16 and 18 were detected in more than 82% cases of cervical cancer and severe cervical neoplasia [21]. During implementation of the 7th framework program of the European Commission, extensive statistics has been collected on the cervical cancer, other anogenital cancers, head and neck cancers, including the frequencies of HPV detection, factors contributing to cervical cancer, screening practices for cervical cancer, and introduction of HPV vaccination (projects PREHDICT, HPV AHEAD, HEALTH-F3-2010-242061, and HEALTH-F2-2011-282562). The prevalence of HPVs 16 and 18 among women with normal cytology was 2.9 and 1.2%; with LSILs – 19.3 and 6.5%; with HSILs – 45.1 and 6.8%; and with invasive cervical cancer – 55.2 and 14.2%, respectively [11]. According to our data, the prevalence of HR HPV among women of childbearing age in the central part of Russia (n = 2783) in 2018 was 10%, of which 2% was HPV 16, 0.4% was HPV 18, and the rest were other HR HPV types (unpublished data). This data is similar to the global data on the prevalence of the main HR HPV types (Fig. 2).

HPV TRANSMISSION ROUTES

HPV is one of the most common sexually transmitted infections. HPV is spread via direct skin-to-skin contact during vaginal, anal, or oral sex with an infected individual even in the absence in the latter of the clinical manifestations. There are also other modes of horizontal and vertical virus transmission [22, 23], as evidenced by frequent detection of HPV, including HR HPV, in the foreskin of children and adolescents [24]. Vertical HPV transmission from mother to fetus has been also documented [25, 26]. HPV DNA has been found in the oral cavity of newborns [26], breast milk [27], amniotic fluid, placenta, and umbilical cord blood [28]. HPV causing laryngeal papillomatosis can be transmitted to the newborns during birth and in utero; it was also found in children born by caesarean section [22, 23]. A study on the presence of HPV in the amniotic fluid in women who have genital warts and in the nasopharyngeal aspirates in their children detected concordant HPV types, indicating transplacental and intranatal transmission of HPV (in particular, HPV types 6 and 11). Intranatal HPV infection can lead to juvenile recurrent respiratory papillomatosis, the prevalence of which is 1.7-2.6 per 100,000 children and one per 1500 births among women with genital HPV infection [29].

Meta-analysis of 15 studies performed at the healthcare centers and public institutions and 36 patients studies showed multiple horizontal pathways of HPV transmission, including ones related and non-related to sexual

contacts, e.g., HPV transfer from hands to genitals or from genitals to hands. Infectious HPV virions have also been detected on the surfaces and equipment in medical facilities and public places [30]. A number of reports have shown intra-family routes of HPV transmission [31]. A retrospective study was conducted in Spain in which HPV-positive and HPV-negative pregnant women and their children have been observed for 14 months; 16.9% of children of HPV-negative women were diagnosed with HPV infection consistent with the horizontal HPV transmission within the family [32].

Thus, both horizontal and vertical routes of HPV transmission, including transmission from infected parents to children, have been documented. This demonstrates the importance of prophylactic vaccination against HPV, not only to protect the health of women and men, but also their children, until they reach the age of recommended prophylactic HPV vaccination.

HPV AND HPV-ASSOCIATED CANCERS IN THE BACKGROUND OF HIV INFECTION

The frequency of HR HPV detection among patients infected with the human immunodeficiency virus (HIV) is extremely high [33, 34]. Impaired immune function leads to the loss of control over HPV infection and increases the risk of developing HPV-associated cancers. The course of somatic and secondary HPV-associated diseases is directly related to the development of HIV-induced immunosuppression and depends on the disease duration, the level of CD4+ T lymphocytes, HIV replication, patient's individual characteristics, and the efficacy of highly active antiretroviral therapy (HAART) [34]. Surprisingly, in HPV-induced carcinogenesis, immunosuppression is associated with the event of HPV infection and with the early stages of dysplasia, but not with the development of cancer. A correlation was shown between the immunosuppression, indicated by a decrease in the level of CD4+ T lymphocytes (<500 cells/mm^3), the prevalence of cervical and anal HPV infection, and the level of HPV DNA in mucosal cells in adult HIV-positive men and women. A pronounced manifestation of this relationship among patients with low CD4+ cell content (<200 cells/mm^3) is consistent with the role of immune system in the control over HPV replication [35]. There is also a direct correlation between the decrease in the content of CD4+ T cells and increase in the number of HPV types found in anogenital samples in HIV-infected individuals. A broader range of co-infecting HPV types and a higher viral load correlate also with the number of sexual contacts; however, the decisive factor is the loss of immune control over viral replication [35].

Despite the above-described correlations, no direct relationship has been found between immunosuppression and incidence of anogenital cancer. Comparison of the registries of patients with acquired immunodeficiency syndrome (AIDS) and cancer does not reveal any correlation between the low CD4+ cell counts (e.g., below 500 cells/mm^3 or even less than 200 cells/mm^3) and the incidence of cervical or anal cancers in women and men with AIDS. There is also no apparent increase in the incidence of anogenital cancer after AIDS diagnosis [36]. Thus, neither the regression, nor the progression of neoplasias appear to be related to the immune status.

The success of HAART in the control of HPV replication in people co-infected with HIV and HPV is debatable. The incidence of HPV-associated cancers in the era of HAART is increasing [37]. Although some studies on the HAART impact on the natural history of HPV-associated intraepithelial neoplasias have shown a positive effect of this therapy [38], other studies have failed to reveal any effect [39, 40]. Also, studies demonstrating positive effect of HAART manifested as a decrease in the HPV level found no regression of neoplasias in most of women with HSIL even after normalization of their CD4+ cell counts [41, 42].

This phenomenon is explained by the fact that restoration of the immune status may be "useless" at the later disease stage of progression to cancer because of the advanced genetic changes in the HPV-infected cells. Chromosomal instability caused by HPV (to be discussed below) leads to the accumulation of irreversible genetic alterations. The failure of the cell cycle control prevents apoptotic death of the affected cells, and they are not exterminated by the immune system affected by HPV. Therefore, the cause of persistent intraepithelial lesions is not the viral infection per se, but irreversible genetic changes that eventually lead to the development of invasive cancer [39]. Understanding the mechanisms of emergence of HPV-associated cancer, especially on the background of immunosuppression, is of paramount importance for the development of immunotherapies, including therapeutic vaccines, as it provides a basis for the rational choice of vaccination strategy and vaccine components.

HPV GENOME AND PROTEOME

HPV virions have a conservative icosahedral morphology. Viral particles have a diameter of about 50-55 nm and molecular weight of $5 \cdot 10^6$ Da. The genomic organization of all papillomaviruses is very similar: the viral genome represents a 6-8 kb double-stranded circular DNA associated with histone-like proteins [5] (Fig. 3). The genome contains three functional regions: the early region (E) with the open reading frames (ORFs) for encoding E1, E2, E4, E5, E6, and E7 proteins; the late region (L) encoding structural large (L1) and small (L2) capsid proteins; and the non-coding regulatory region [long control region (LCR) also known as NCR or URR]

located between the L1 and E6 ORFs. LCR contains a set of *cis*-elements controlling replication and transcription of the viral DNA, in particular, the origin (*ori*) of replication [5, 43].

The main proteins encoded by the viral genome are either structural, as capsid proteins L1 and L2, or functional as E1 and E2 proteins involved in the replication of viral DNA. The remaining proteins (E4, E5, E6, and E7) are not encoded by all papillomaviruses and can be considered as "evolutionary embellishments" [5]. The main functions of HPV proteins are described in the table.

L1 and L2 proteins. L1 is the large capsid protein (molecular weight 55-57 kDa) through which a viral particle binds to the heparin sulfate receptors on the basement membrane [44]. L1 is encoded by ORF L1, the most conserved nucleotide sequence in the genome used for phylogenetic classification of HPV types and subtypes. Small capsid protein L2 (molecular weight 43-53 kDa) is responsible for packaging of the viral DNA. L1 and L2 proteins are expressed at the late stages of viral life cycle; they participate in the virion assembly and infection and can be detected mainly in the differentiating epithelial cells [44].

E1 protein. Highly conserved E1 protein encoded by ORF E1 is required for papillomavirus replication. It is the second most conserved sequence in the HPV genome [45, 46]. E1 protein contains three functional domains – *N*-terminal, central, and the most important *C*-terminal domain. The *N*-terminal domain carries signal sequences for nuclear localization and nuclear export, cyclin E/A-binding motif, and two phosphorylation sites for the cyclin-dependent kinase CDK2. The central domain is responsible for the complex formation with the E2 protein and binding to *ori*. A part of the *C*-terminal domain acts as a helicase of the AAA+ ATPase superfamily; it binds and unwinds viral DNA, making it accessible to the cell replication complex. All three domains are critical for viral replication. E1 and E2 form a complex which binds to the replication initiation site. The presence of one E1-binding site and one E2-binding site in the *ori* is sufficient for replication; however, a higher number of E2-binding sites promotes the replication efficiency. After binding, E2 is expelled from the complex, and E1 forms a dihexamer, which recruits topoisomerase I, DNA polymerase α and replication protein A (RPA), required for HPV replication. E1 protein also contributes to the formation of DNA breaks in the chromatin of the host cell, facilitating viral integration [47, 48].

E2 protein. E2 protein is encoded by the ORF E2. E1 and E2 are necessary and sufficient for the replication of papillomaviruses [45, 46]. E2 contains the *N*- and *C*-terminal transactivating domains and acts as the E1 helicase "loader" [47]. The *C*-terminal domain binds to the BRD4 protein, the bromodomains of which interact with lysine residues in the histones, leading to chromatin remodeling [49]. In addition, E2 connects viral DNA with chromatin

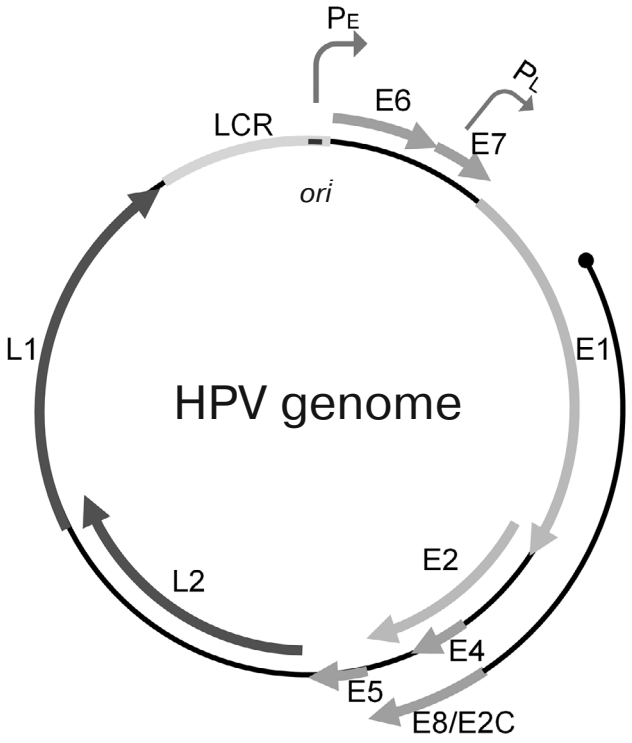

Fig. 3. Human papillomavirus (HPV) genome on the example of the double-stranded circular DNA of the alpha family of HPV. LCR, long control region; P_E, early promoter; P_L, late promoter; *ori*, replication initiation site. The major proteins involved in virus replication are E1 and E2; the major structural proteins are L1 and L2.

of the cell: the E2–BRD4 complex binds to mitotic chromosomes, transferring episomal copies of the viral DNA to the daughter cells after cytokinesis [47]. E2 acts as a transcriptional regulator for the ORFs E6 and E7 [9]. When expressed in larger amounts, E2 binds to the palindromic sequence 5′-ACCG$(N)_4$CGGT-3′ in in the LCR region of the P97 HPV 16 promoter [43]. This impedes promoter binding of RNA polymerase II and suppresses expression of E6 and E7 proteins. Even at low levels, E2 is capable of recruiting transcription factors required for viral replication [49]. Hence, E2 acts as a regulator of HPV reproduction. E2 is also an epigenetic regulator capable of interacting with the global transcription regulator, the p300/CBP-p/CAF complex, which leads to a decrease of the transcriptional activity of p53 due to chromatin hypoacetylation [47]. Interestingly, HPV can replicate using a combination of E1 and E2 from viruses of different types, even a combination of human and animal papillomaviruses, which indicates a high degree of conservation of their functions.

E4 protein. ORF E4 encodes a family of proteins generated as a result of RNA splicing and subsequent post-translational modification. E4 is synthesized in large quantities in the suprabasal and granular epithelial layers

Main proteins encoded by the HPV ORFs and their functions (compiled from literature data discussed below)

HPV protein	MW, kDa	Main functions
E1	68-85	helicase; participates in the virus replication, in particular, unwinding of viral DNA
E2	48	transcription factor; essential for viral replication, genome segregation, and viral DNA packaging in the capsid; transcription regulator of the ORFs E6 and E7
E3	no data	function(s) unknown, exists in few types of HPV
E4	10-44	binds to the cytoskeleton proteins, facilitates release and distribution of virions
E5	14	viroporin; ensures penetration of the viral genome into the cell; enhances the transforming activity of E6 and E7 by regulating expression of growth factors and inhibiting apoptosis; modulates expression of genes involved in cell adhesion and cell motility; reduces expression of MHC class I and II molecules and suppresses the antiviral interferon response
E6	16-18	transcription factor; interacts with a number of cellular proteins; induces p53 degradation and cytokine production
E7	~10	inhibits pRb activity by targeting it to proteasome degradation; disrupts transcriptional regulation of genes coding for proteins involved in DNA synthesis; disrupts epigenetic regulation; stimulates progression of cell cycle phases and genomic instability
E8/E2C	20	E8 is present only in some HPVs; fused to the C-terminal part of E2; functions as a repressor of transcription and replication of HR HPV
L1	57	large capsid protein; organized into pentameric capsomers forming icosahedral virions
L2	43-53	small capsid protein; participates in the encapsidation of viral DNA; provides the entry and transport of virions

and forms keratin-associated amyloid filaments in the cells of middle and upper epithelial layers, which increases cell fragility and facilitates release and spread of HPV virions [50].

E5, **E6**, and **E7** are the HPV oncoproteins responsible for the immortalization and transformation of HPV-infected cells. The main oncogenic HPV proteins are E6 and E7. Differences in the oncogenic potential of HPV belonging to different genus, are believed to be associated with the functional properties of these proteins: E6 and E7 of β genus HPVs are less effective in promoting oncogenesis and cellular transformation than E6 and E7 of α genus HPVs [51]. The main oncogenic activity of E6 and E7 is based on their ability to inactivate tumor suppressor proteins p53 and pRb, respectively (Fig. 4).

E5 protein. E5 oncoprotein is a hydrophobic transmembrane protein with two cysteine residues in the *C*-terminus which stabilize the E5–E5 homodimer. The molecular weight of E5 ranges from 8.3 kDa for HPV 18 to 9.4 kDa for HPV 16. HR HPVs encode E5 isoform (E5α) with high pro-carcinogenic activity, whereas low carcinogenic risk HPV variants either contain E5 isoforms with a reduced carcinogenic activity (E5β, -γ, -δ) or do not carry ORF E5 at all [52]. It was proposed to classify E5 as a viroporin (protein which forms pores in the cell membrane) capable of modulating ion homeostasis, vesicle transfer, virion production, and penetration of the viral genome into the cell [53]. The oncopotential of E5 was identified relatively recently. E5 plays a key role in cell growth by interfering with a number of signaling pathways. As an oncoprotein, E5 enhances the transforming activity of E6 and E7 by regulating growth factors (e.g., epithelial growth factor receptor EGFR), Bcl-2, Bax, Fas, and calnexin and is involved in the control of cell survival, growth, and differentiation [54]. The pro-carcinogenic activity of E5 also includes inhibition of apoptosis induced by tumor necrosis factor ligand (TNFL) and CD95 ligand (CD95L) [54]. E5 can also modulate the expression of genes involved in cell adhesion and motility [55]. E5 actively interferes with the immune system functions. It reduces surface expression of the major histocompatibility complex (MHC) class I and II molecules on the surface of the infected cells, inhibits the antiviral interferon response and the activity of natural killer (NK) cells [56]. Taken together, this leads to the disruption of presentation of viral antigens, suppression of the inflammation, and weakening of the antiviral immune response, contributing to the survival of infected and transformed cells [57].

E6 protein. E6 oncoprotein is composed of 151 to 158 amino acid residues (a.a.). It does not belong to structural proteins and has no enzymatic activities. The main cellular target of E6 of HR HPVs is the tumor suppressor protein p53. The level of p53 in the cell is regulated by the Mdm2 ubiquitin ligase containing the RING

finger domain. Under stress, e.g., in the case of DNA damage or viral infection, this mechanism is impaired, p53 is stabilized and activated by phosphorylation. In HPV-positive cancer cells, the Mdm2-dependent regulation of p53 is completely turned off, and p53 level is regulated by E6 [58]. To promote p53 degradation, E6 recruits E6AP ubiquitin-protein ligase (E6-associated protein), also known as ubiquitin-protein ligase E3A (UBE3A), the latter binds to E6 through the LXXLL motif forming a stable E6/E6AP complex. This complex ubiquitinates p53, targeting to its proteasomal degradation. E6 has also other anti-apoptotic properties. A number of studies have shown that E6 interacts with other components of the proteasomal degradation pathway, which further contributes to malignization [59]. In particular, E6 is involved in the proteasomal degradation of Bak, which plays an important role in the terminal differentiation of epithelial cells, and other factors involved in cellular apoptosis, such as the tumor necrosis factor receptor 1 (TNFR1), Fas-associated protein with death domain (FADD), and pro-caspase 8 (Fig. 4).

A characteristic feature of E6 is the presence at the *C*-terminus of the PDZ class I domain (PSD-95/Dlg/ZO-1) and two zinc fingers formed by two pairs of CXCS motifs. These motifs are strictly conserved in all E6 proteins; their integrity is crucial for the functional activity of this oncoprotein [60]. E6 acts as a transcription factor; its zinc finger domains interact with a wide range of cellular substrates, altering gene expression profiles and disrupting intracellular regulation and signaling, which contributes to the loss of cellular polarity [61]. In particular, E6 is involved in the regulation of p53 gene transactivation. This is a unique auxiliary mechanism of p53 activity regulation, independent of the proteasomal degradation. Mutant E6 is unable to target p53 to proteasomal degradation, however, it can block the transactivation properties of p53. E6-mediated repression of p53-dependent activation correlates with the inhibition of acetylation of p53 and core histones, which prevents their incorporation into the chromatin and interferes with tumor suppressor functions. E6 also controls p53-dependent gene regulation by interacting with the p300/CBP co-activator, thereby contributing to the inhibition of the p53-dependent transcription [59].

Besides, E6 interferes with the repair of DNA single-strand breaks by binding of the X-ray repair cross-complementing protein 1 (XRCC1) and O^6-methylguanosine DNA transferase [47]. Furthermore, E6 deregulates the process of DNA replication by activating telomerase and human reverse transcriptase (hTERT), as well as by

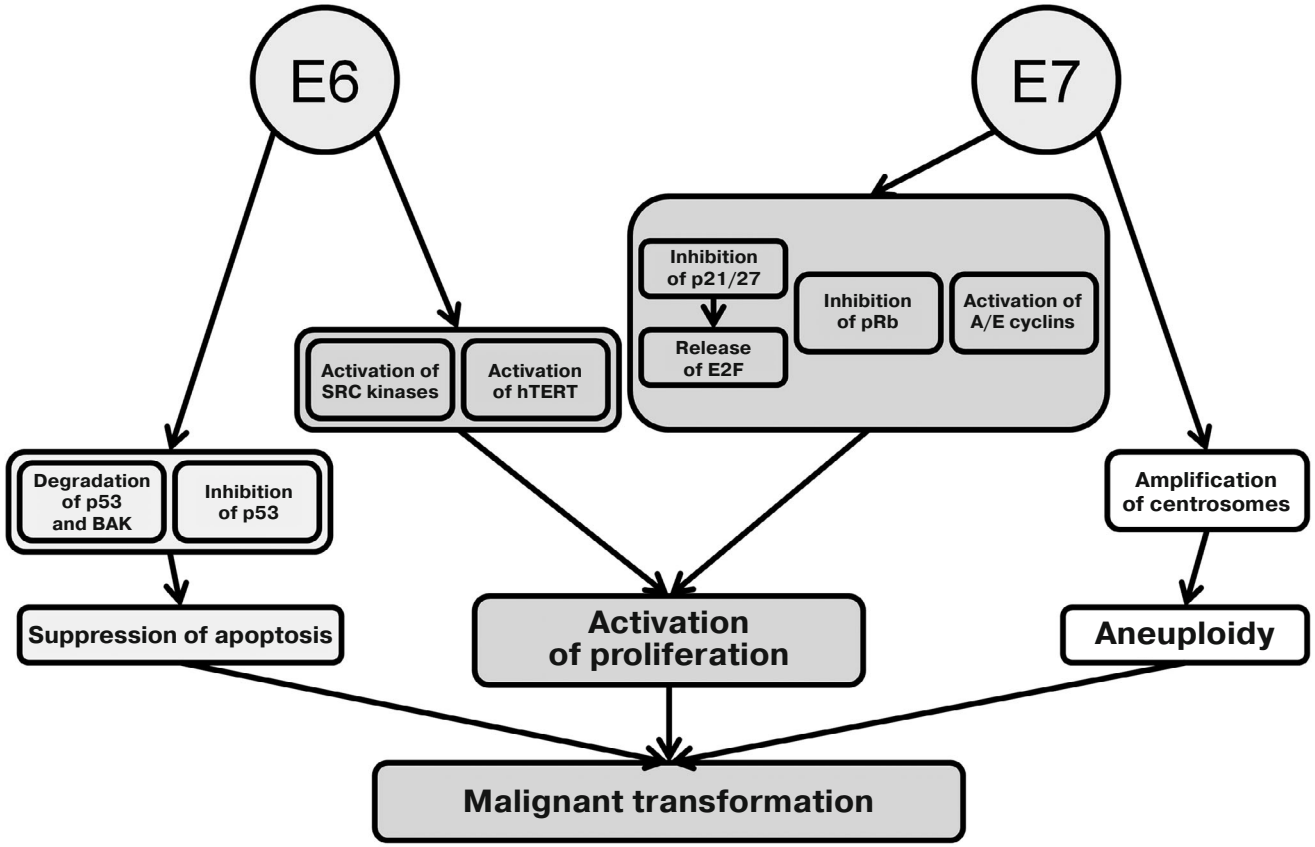

Fig. 4. The main mechanisms of HPV E6/E7-induced carcinogenesis.

affecting a number of cellular factors that control telomere shortening and cell aging [62]. This combination of properties makes E6 necessary and sufficient for the HPV-driven carcinogenesis, as evidenced by spontaneous induction of carcinomas in E6-transgenic mice [63].

E7 protein. HR HPV ORF E7 encodes the E7 protein (up to 127 a.a.) containing CD1, CD2, and CD3 conserved domains (the latter is homologous to the large SV40 T-antigen). The most important E7 functions determining its oncogenicity are associated with the CD2 and CD3 domains. The CD2 domain contains the protein kinase CKII phosphorylation site and the LXCXE retinoblastoma suppressor protein pRb binding motif. pRb plays a key role in the cell cycle by regulating cell transition from the G1 to S phase. Under normal conditions, pRb is not phosphorylated at the onset of G1 and undergoes phosphorylation as the cell moves toward the S phase. Unphosphorylated pRb interacts with the E2F transcription factors and represses the transcription from the E2F-regulated promoters. Members of the E2F family are involved in the cell-cycle-dependent transcriptional regulation of proteins involved in DNA synthesis [64]. E7 binds to unphosphorylated pRb through the LXCXE motif and directs it to ubiquitination and subsequent proteasomal degradation. Disruption of the pRb–E2F complex releases E2F, leading to the E2F-induced transcription, which results in the increase in the levels of cyclin-dependent kinase CDK2 and cyclins A and E [59]. Upregulation of the CDK2 activity by E7 promotes amplification of centrosomes and their clustering, a characteristic feature of malignant cells [65]. E7-dependent suppression of pRb activity is considered to be a critical factor in stimulating the transition between the cell cycle phases in differentiating epithelium, which provides optimal conditions for the viral DNA replication and, at the same time, contributes to malignant transformation of the cells.

The CD3 domain of E7 protein contains four highly conserved cysteine residues and is involved in the interactions with numerous cellular proteins, including p21 and p27 inhibitors of CDKs. E7 suppresses their activity, allowing the cells to avoid cell cycle arrest activated by DNA damage. In addition, E7 directly binds and activates DNA methyltransferase, which leads to the uncontrolled increase in DNA methylation level and disrupts epigenetic regulation of cellular processes [66].

Oncoproteins E6 and E7 act synergistically by inducing genetic instability, destabilizing transcriptional complexes, and remodeling chromatin, thereby promoting uncontrolled cell proliferation (Fig. 4). Hyperproliferation stimulated by these oncoproteins can lead to replicative stress and clastogenesis, which also destabilizes the genome [9]. Studies of the last thirty years culminating with the work by Mirabello et al. [67] have shown a unique genetic stability of HR HPV oncoproteins in neoplasias and tumors. The few observed variations in the E6 and E7 nucleotide sequences have no correlation with the severity of cervix intraepithelial lesions [67, 68]. The unique carcinogenic properties of E6 and E7, their high degree of conservation, and constitutive expression in precancerous and malignant tissues make these proteins ideal targets for vaccine immunotherapy of HPV-associated precancer and cancer [69].

INFECTION AND VIRAL REPLICATION

Phases of HPV life cycle are presented in Fig. 5. HPV is an obligate epithelial pathogen – both infection and vegetative reproduction of the virus totally depend on the keratinocyte differentiation [70]. It has been shown that the virus is able to infect "young" keratinocytes of the basal layer, presumably, activated keratinocytes in the areas of damaged epithelium [5, 9]. Epithelial damage makes it possible for HPVs to access keratinocytes of the basement membrane and bind to the extracellular proteoglycan heparan sulfate via the L1 capsid protein. This results in the primary attachment and binding of the virus to the basal layer cells. The binding leads to the changes in the capsid conformation though the furin-mediated cleavage of the *N*-terminal fragment of L2 protein. After the proteolysis, the conformation of capsid undergoes another change, which leads to capsid binding to the cell receptors, in particular, to $\alpha 6$ and $\alpha 6\beta 4$ integrins, laminin, and collagen-binding integrins involved in the adhesion of keratinocytes to the dermis and dissemination of the epithelial cells during wound healing and carcinogenesis. Subsequent virus internalization into vacuolar structures occurs through the clathrin- or caveolin-mediated endocytosis [71].

Development of HPV-induced neoplasias had been for a long time attributed to the viral infection of basal stem cells. However, a number of studies have revealed a critical role in the HPV-induced pathogenesis of a discrete population of cells in the transition zone of squamous epithelium, the squamo-columnar (SC) junction [72]. The microenvironment of the ecto-endocervical transition zone (EESP) harbors unique epithelial cells residing inside or in close proximity to this area. The SC junction cells exhibit intriguing phenotypic similarities to approximately 90% of cervical squamous cell carcinomas (SCCs) and high-grade precursors and could serve as a reservoir for the latent infection and subsequent formation of cervical intraepithelial neoplasias (CIN) [72].

HPV is incapable of self-replication due to the lack of its own polymerases; this makes its life cycle totally dependent on the host cell machinery. After infection of basal cells in the damaged area, viral DNA undergoes the first replication cycle (independently of the cell cycle) which produces up to 50-100 copies of the viral genome per cell. During this infection phase, the viral genome is maintained in the episomal form; no further DNA repli-

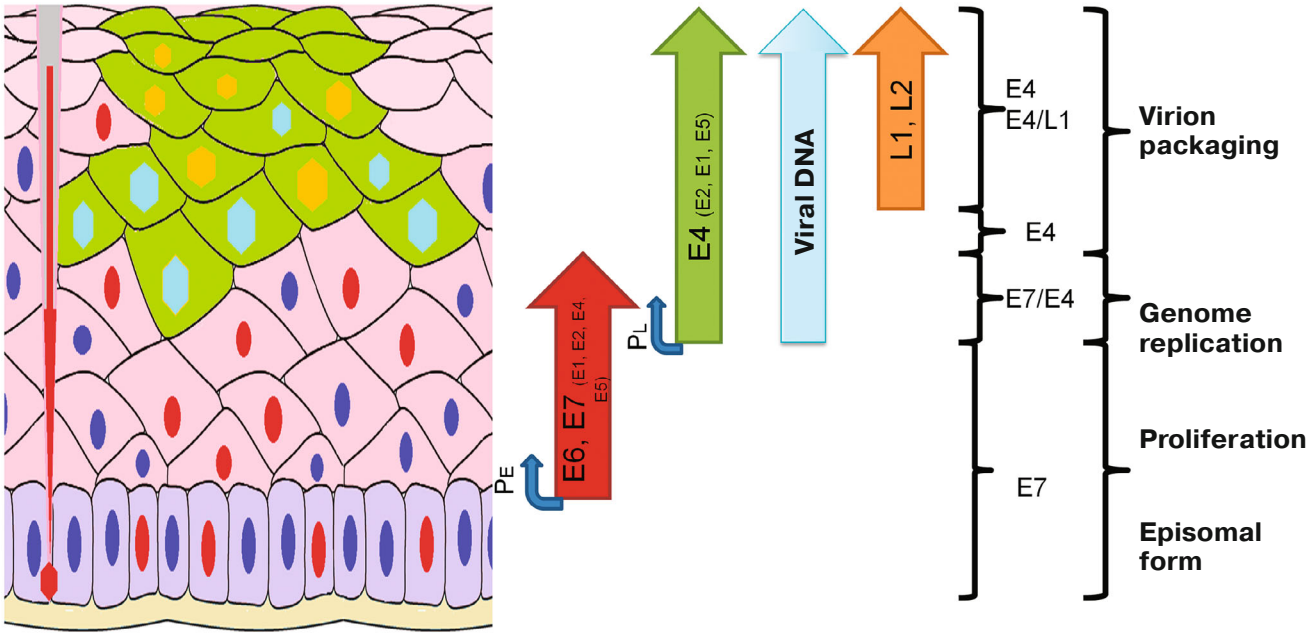

Fig. 5. The course of HPV infection. On the left, differentiated epithelium (scheme); on the right, HPV proteins expressed in the infected cells. After infection of the basal layer keratinocytes, the virus is maintained in a low-copy episomal state. Proteins E6 and E7 (red arrow) are expressed from the early promoter in the lower layers of the epithelium (red nuclei); blue nuclei designate non-infected cells. Expression of the E4 protein, and, possibly, some other proteins (green arrow), the expression of which is controlled by the late promoter, starts before the termination of expression of E6 and E7. Cells expressing E4 (green) and E7 most likely contain all early proteins necessary for replication of viral DNA. Expression of viral capsid proteins L1 and L2 (orange arrow) occurs after the completion of amplification of genomic DNA in cells expressing E4 (from [105] with modifications).

cation occurs, the number of genomes remains constant. Expression of viral proteins is minimal and tightly controlled; E6 and E7 as transcription products of the viral genes in the epithelium proliferative compartment are mostly undetectable.

Proteins encoded by the early region of the HPV genome induce cell transition to the S phase, thereby providing the virus with required polymerases [73]. When an infected keratinocyte enters the differentiating compartment, expression of both early and late viral genes and viral DNA replication gets strongly activated, the number of viral genomes increases to many thousands of copies per cell [74]. Virions are formed only in fully differentiated cells of the outer epithelial layer [75]. The release of virions is accompanied by the death of differentiated cells [44], leading to the formation of koilocytes [46].

FORMS OF HPV INFECTION

HPV infection can occur in an acute (non-persistent or transient), latent, or chronic forms. Acute, latent, and chronic infections differ in the virus activity, expression of viral and cellular genes, impact on cell proliferation, ability to induce local immunosuppression, and oncogenicity.

Viral genetic material can be detected several days after sexual intercourse with an infected partner even in the absence of visible infection in the latter. After infection with HPV, viral DNA persists in the episomal form and, in most (up to 90%) cases, is eliminated by the immune system. This can be called “transient infection”, although the term “transient detection” may be more accurate. HPV can replicate in the infected epithelial cells and produce virions; however, spontaneous elimination of the virus (the so-called “acute infection”) ultimately occurs [76]. In some cases, viral genome is retained in the infected cells in an inactive form (“latent infection”) [77]. During the period of latent infection, HPV genetic material is identified only occasionally. Reactivation of the latent infection can occur a long time after the initial infection, possibly as a result of immunosuppression, although it often happens without obvious triggers. Differentiation between regressing cervical lesions and transient virion-producing infection can be made by serial consecutive type-specific measurements of virus load [76].

Infections, in which viral activity is retained over long periods of time, are classified as persistent or “chronic” (although, they can still be spontaneously eliminated). HPV is often successful in establishing chronic infection, since the virus does not kill the host

cell but rather uses it as a reservoir to periodically (for weeks and months) produce a large number of infectious viral particles, which are further transmitted to uninfected people.

Basal cells infected with HPV can divide in three possible ways with: (i) division of one infected cell into two non-dividing differentiated cells; (ii) asymmetric division into one parabasal and one basal cell; (iii) division into two basal cells, both retaining the ability to divide (clonal pathway of HPV-induced transformation). Cytological detection of LSIL in the cervical cytology or biopsies is the hallmark of HPV virion production; the halo observed using optical microscopy indicates accumulation of HPV virions inside the differentiated non-dividing cells. Production of large quantities of new virions in non-dividing cells guarantees HPV infection of the new target cells. In all these cases, HPV DNA is present in the episomal form and can be packaged into the virions with subsequent release from the cell and expansion of the infected area [78].

In 10-12% of cases, HR HPV infection leads to the integration of viral genome into chromosomes of the host cells [79]. Integration is observed in more than 70% of HPV-associated cancers and considered to be a key event in carcinogenesis [80]. Although viral genome integration is important for cell transformation, it is not a part of the normal virus life cycle [81]. Interestingly, integration is largely determined by the presence of microhomologous HPV sequences in the human genome. HPV integration sites in the human genome are distributed unevenly: from 3667 sites identified during the study of 135 clinical samples, more than 50% were located in the integration hot spots, in the *POU5F1B* (9.7%), *FHIT* (8.7%), *KLF12* (7.8%), *HMGA2* (7.8%), *KLF5* (6.8%), *LRP1B* (5.8%), *LEPREL1* (4.9%), *DLG2* (4.9%), and *SEMA3D* (4.9%) genes [82] (gene names are given in accordance with the GeneCards database, https://www.genecards.org/). HPV integration may influence the functioning of the genes located in the region(s) of integration, up- or downregulating/abrogating their expression.

After integration, which involves a loss of a part of viral genome, HPV DNA loses its infectivity and can no longer be packaged into the infectious virions. At the same time, oncoproteins E6 and E7 retain their activity, as their coding sequences are integrated into the host genome. E6 and E7 modulate gene expression, inactivate tumor suppressor genes, activate oncogenes, and induce cell proliferation, further increasing genomic instability [83-85]. Altogether, this leads to the development of LSIL or HSIL and/or cancer in the infected anatomic region (vulva, vagina, penis, anal canal, and oropharynx). HPV DNA sequences found in the HPV-related cancer lesions and tissues demonstrate a high level of homology, which indicates a common source (origin) of the integrated fragment derived from one integrated virus with further propagation of the infected cell, i.e., tumor clonality (although formally it may appear as an indication of HR HPV sequence conservation). In view of this, high homology of HPV DNA sequences can be considered as one of the markers of cell transformation. Indeed, elevation of a single HPV type viral load in female patients infected with multiple HPV types was taken for a prognostic sign of the development of cervical cancer [76].

MOLECULAR MECHANISMS OF HPV-INDUCED CARCINOGENESIS

The conditions required for the formation of irreversible neoplasias (malignancies) include:

1) active initial expression of the HR HPV E6 and E7 genes, causing genetic instability in the infected cell;

2) cell cycle dysregulation induced by E6 and E7 oncoproteins, suppression of apoptosis of cells with damaged DNA, immortalization and transformation of these cells;

3) stable longitudinal expression of E6 and E7 proteins protecting immortalized and transformed cells from apoptosis [86]. In this scenario, temporal suppression of expression E6 and E7, for example, during hypoxia, does not prohibit the carcinogenic effects of E6 and E7. Hypoxia causes only a temporary arrest of cell proliferation and tumor growth, eventually leading to chemotherapy resistance and loss of E6/E7-specific immune control over the tumor growth, with cell proliferation and tumor growth restored with restoration of tissue oxygenation [87];

4) hormonal regulation of the formation and "maintenance" of the transformed/malignant tissues.

HPV oncoprotein-induced alterations of cell genetic machinery are manifested already at the early stages of pre-neoplastic changes, when the virus is still in the episomal form [81]. HR HPV E6 and E7 induce genomic instability characterized by the genome rearrangements and gene amplification, characteristic to cancer cells. Genomic instability normally induces apoptosis, but in the HPV-infected cells it is inhibited via several parallel independent mechanisms mediated by the E5, E6, and E7 proteins (see the relevant sections of this review).

Viral integration is a direct result of the genomic instability induced by oncoproteins [9]; in turn, it leads to a series of negative consequences. Integration is often accompanied by the deletion of early (E1, E2, E4, and E5) and late (L1 and L2) ORFs with the retention of early E6 and E7 ORFs. The levels of L1 and L2 expression in HSIL and cancer cells are low even when L1 and L2 ORFs are intact. After their deletion, HPV-infected cells can no longer be recognized by the immune system of individuals subjected to prophylactic vaccination. This, together with insufficient induction of cellular immune response, makes useless the attempts to use preventive vaccines for therapeutic purposes. Furthermore, deletion

during integration of the ORF for E2, which is a negative regulator of E6/E7 expression [88], results in the upregulation of E6/E7 expression, contributing to the malignant transformation of the cells [89]. In addition, HPV integration often occurs in the promoter region of the *TERT* gene (5q15) or in the 3q26 locus, which leads to the increased expression of TERC telomerase [82], immortalization of cells carrying fragments of the HPV genome, and uncontrolled amplification. It should be noted that integrated nucleotide sequences of the HPV genome have been detected only in 62% of all cervical carcinoma samples [90]. Although the pronounced pre-neoplastic changes are known to be associated with the increased frequency of HPV genome integration, virus integration is still considered as a consequence of chromosomal instability caused by the oncoproteins, rather than the driving force of malignant transformation [9].

The basic molecular mechanisms of the first three conditions for the irreversible neoplasia formation regulated by E6 and E7 were discussed above. The last one, hormonal regulation, may also play an essential role in the HPV-induced carcinogenesis. The cervix consists of estrogen-sensitive tissues, which is important in the neoplastic processes regulated by estrogens, including estradiol. Estradiol has high affinity to estrogen receptors, and its interaction with these receptors modulates cell proliferation and metabolism. The EPIC study of 308,036 women, including 261 patients with cervical cancer and 804 patients with CIN3 (HSIL), showed a significant increase in the estradiol content in the serum of patients with cervical cancer [91]. It is interesting that cells in the HPV-associated tumors are not sensitive to estrogens, since they do not express the corresponding receptor. However, estrogen expression is significantly increased in the peritumoral tissues that supports the tumor growth [92].

PROPHYLACTIC HPV VACCINES AND THE POSSIBILITY OF CANCER PREVENTION

HPV is often detected in the genital tracts of young sexually active people. Most of those who develop benign HPV-associated tissue changes ultimately develop an efficient cellular immune response, leading to the regression of condylomas, LSIL, and HSIL and to the control over viral replication. However, in many cases, especially in patients with a history of immunosuppression (for example, due to HIV infection), HPV is not eliminated. HPV persistence is associated with its ability to effectively downmodulate innate immune response, which delays the development of adaptive immunity and prevents viral elimination during the stages preceding neoplastic transformation. Alterations in the functions of immune system induced by the HPV infection include differentiation of tumor-associated macrophages (TAMs) and suppression of activation and maturation of dendritic cells. This leads to the dysfunctional cellular immune response, including an imbalance between the T-helper cells of types 1 (Th1) and 2 (Th2) and induction of regulatory T cells [93]. The failure to develop an efficient cellular immune response against HPV results in the establishment of persistent viral infection. Delayed immune response to HR HPV increases the probability that even transient viral infection will last long enough to transform epithelial cells. At this stage, elimination of the virus by the antiviral immune response will no longer be able to prevent cancer development. This indicates the need for timely preventive HPV vaccination, as well as for development of therapeutic HPV vaccines, which can induce cellular immune response against HPV oncoproteins and eliminate malignant cells expressing HPV proteins.

The studies of natural HPV infections in animals have shown that neutralizing antibodies to the L1 capsid protein are protective, their induction serves as a basis for the strategy of preventive vaccination. The evidence on how antibodies against HPV prevent infection of the basal epithelial cells provide a mechanistic explanation for the efficacy of such vaccines [94]. Prophylactic HPV vaccines should elicit protective antibodies, which accumulate in high amounts and bind the virus before it enters epithelial cells of the basal layer, as well as generate B-cell memory to ensure the long-term presence of antibodies for local neutralization of the virus during sexual activity of the immunized person. Interestingly, however, there are no immune correlates of the efficacy of prophylactic vaccines – the minimal level of antibodies required for protection against HPV infection (as in the case of infection with the hepatitis B virus) [94]. Unlike prophylactic vaccines, therapeutic HPV vaccines must be directed against HPV E6 and E7 proteins expressed in already HPV-infected, particularly, in HPV-transformed cells, and should induce a cellular immune response able to identify and effectively eliminate such cells from the epithelium.

Currently available commercial prophylactic vaccines against HPV are based on the large capsid protein L1, which self-assembles into the virus-like particles (VLPs) [95]. In 2006, the US Food and Drug Administration (FDA) registered the world's first quadrivalent vaccine, Gardasil® (Merck & Co., Germany). Gardasil® has been shown to be effective in preventing infections with HR HPV 16 and 18, as well as HPV 6 and 11, the causative agents of genital papilloma and recurrent respiratory papillomatosis. This vaccine offers partial cross-protection against other HR HPV types, including HPV 31, 33, 35, 45, 52, and 58, which are phylogenetically related to HPV 16 and 18 [96]. The viral proteins in this vaccine are manufactured in *Saccharomyces cerevisiae* yeast using recombinant DNA technology. The VLPs are then mixed with aluminum hydroxyphosphate sulfate,

which acts as an adjuvant [97]. Already by the second quarter of 2007, this vaccine was approved for the use in 80 countries around the world. When used in accordance with the manufacturer's recommendations, Gardasil® provides 98% protection from infection with these types of HPV, greatly reducing the risk of HSIL, squamous cervical carcinomas, adenocarcinomas, and other complications caused by HPV infection [98].

Another, bivalent vaccine Cervarix®, containing two types of L1 capsid proteins of HPV 16 and 18 (GlaxoSmithKline, UK), was registered in 2008. The vaccine is produced in *Spodoptera frugiperda* (SF9) and *Trichoplusia ni* (Hi 5) cells infected with recombinant baculovirus encoding the L1 protein. Vaccine is adjuvanted by AS04t, which includes aluminum hydroxide and 3-deacetylated monophosphoryl lipid A (a detoxified form of liposaccharide agonist TR-R4). The protective effect of Cervarix® in the prevention of HPV-associated CIN3+ lesions lasts for >10 years. However, the vaccine is ineffective, if at the time of vaccination the person was already infected with HPV 16 [99].

In 2015, the Advisory Committee on Immunization Practices (ACIP) recommended the use of the nonavalent Gardasil® 9 vaccine (Merck & Co.). Gardasil® 9 provides protective immunity to nine types of HPV: 6, 11, 16, 18, 31, 33, 45, 52, and 58. As in the case of Gardasil®, the vaccine contains recombinant capsid protein L1 manufactured in *S. cerevisiae* yeast cells with aluminum hydroxyphosphate sulfate as an adjuvant. It is believed that the use of Gardasil® 9 will reduce the risk of genital papillomas by 90% and of HPV-associated cervical and anal canal cancers by 89%. HPV has developed multiple mechanisms to bypass the immune surveillance in the skin; not all of them have been identified yet. To circumvent this potential limitation, all prophylactic HPV vaccines are given intramuscularly.

Undoubtedly, HPV vaccines will become the second most effective antiviral cancer vaccines after hepatitis B virus vaccine. HPV vaccines have already demonstrated their capacity to protect against HPV-associated neoplasias. The meta-analysis study of the results of trials of 26 monovalent ($n = 1$), bivalent ($n = 18$), and tetravalent vaccines ($n = 7$) in altogether 73,428 participants showed the efficacy of HPV vaccines in protecting adolescent girls and young women aged 15-26 against development of precancerous lesions. The protective effect was higher for the lesions associated with HPV 16/18 infection than for those caused by HPV of undefined types. Reliable evidence was obtained that HPV vaccines reduce the risk of developing HSIL in older women with no prior evidence of exposure to HPV16/18 [3].

Prophylactic HPV vaccination is a major step in preventing other HPV-induced neoplastic changes, in particular, in the penis and anal canal in men, both through collective (population) immunity (herd effect) and through expanding the practice of vaccination of boys and young men [100, 101]. However, studies conducted to date are not long enough to reliably assess the level of reduction of cervical cancer and other forms of HPV-associated cancer [3]. It is also difficult to adequately assess the indirect effects of vaccination, in particular, protection against HPV infection and HPV-associated neoplasia in men who are in contact with HPV-vaccinated women, and in children born to HPV-vaccinated women.

In the countries which haven't implemented screening for cervical and anal cancers or prophylactic HPV vaccination for adolescents, the incidence of cancers associated with HR HPV infection remains high. This specifically affects individuals with various forms of immunodeficiency. The Children's Oncology Group (COG) has developed recommendations for HPV vaccination of all women with immune system defects who have had cancer in childhood [102]. Up to 70% of cancers in HIV-infected individuals are caused by HR HPV infection. Activation/depletion of T cells and myeloid cells increases in HAART-receiving HIV patients infected with HR HPV types, indicating the contribution of HPV to the immune system dysfunctions [103]. As noted above, HAART does not reduce the risk of developing neoplasias and cancer [37, 103]. Despite the success of HAART, the prevalence of HPV infection, LSIL, HSIL, and cervical cancer remains consistently high, and the incidence of anal cancer is increasing [37]. There is an urgent need for vaccination against HR HPV infection in young HIV patients, as well as in individuals with other forms of immunosuppression. A pharmacoeconomic study showed that prophylactic vaccination against HPV in the Russian Federation at a cost of 3.4 billion rubles could prevent a loss of 19.4 billion rubles associated with disability and costs of treatment of patients with various forms of HPV-associated cancers [104]. Vaccination against HPV in children and adolescents living with HIV, one of the most vulnerable groups, would prevent up to 50,000 cases of cancer. Therefore, prophylactic vaccination against HPV could have a huge socioeconomic benefit.

Infection with HPV is a cause of 5% of human cancers and of 30% of all cancers caused by infectious agents. This review discusses in detail the classification of HPVs, organization of the viral genome, functions of viral proteins, mechanism of viral replication, forms of HPV infection, and molecular mechanisms of HPV-driven carcinogenesis. The review also covers the prevalence of HPV types and HPV-associated cancers, routes of HPV infection and viral transmission, and clinical course and outcome of HPV infection in immunosuppressed individuals.

Special attention is given to the prophylactic vaccination and possibilities to prevent the development of HPV-associated cancers. Prophylactic vaccination

against HPV must be performed in early adolescence, before the HPV infection. Late vaccination has no effect on the HPV persistence in already infected individuals and can neither prevent the development of HPV-associated disorders, nor the vertical and horizontal infection of children before they reach the age for HPV vaccination. Even a mass vaccination would hardly eradicate HPV infection either in the nearest or even more distant future, which urgently necessitates the development of therapeutic vaccines against HPV and HPV-associated preneoplastic states and malignancies.

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