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CHAPTER 1

Biology of genital human papillomaviruses

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HPV genome;
Transformation;
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Immunity

Abstract Human papillomaviruses (HPVs) are small DNA viruses that infect various epithelial tissues. The more than 100 types of HPV described share a circular DNA genome of about 8000 base pairs organized into an early, a late, and a long control region. The products of 2 genes from the early control region, *E6* and *E7*, are essential in the HPV-induced processes of cellular transformation and immortalization, and 2 genes from the late control region, *L1* and *L2*, encode the viral capsid proteins. A few high-risk types of HPV types, including HPV-16 and HPV-18, are associated with more than 99% of cervical carcinomas. Continuous expression of the E6 and E7 oncoproteins by high-risk types of HPV often leads to genomic aberrations, a step toward malignant conversion. Although in most cases innate and adaptive immune responses control HPV infection, the high-risk types of virus have the ability to subvert immune defenses, which explains persistent infection and progression to neoplasia.

1. Introduction

Papillomaviruses are small DNA viruses that infect epithelial tissues. Whether cutaneous or mucosal, the more than 100 types of HPV described have in common a circular DNA genome of about 8000 base pairs [1]. These small genomes are organized into an early, a late, and a long control region. The products of 2 genes from the early control region, genes *E6* and *E7*, are essential in the HPV-induced processes of cellular transformation and immortalization [2], and 2 genes from the late control region, genes L1 and L2, encode the viral capsid proteins. Figure 1 shows the general organization of a papillomavirus genome.

2. Mechanism of neoplastic transformation in cervical cells

Carcinoma of the uterine cervix is the sixth most common cancer among women worldwide, with very high mortality rates in developing countries. It was observed more than 20 years ago that some types of HPV were more frequent in malignant than in benign lesions, and infection with high-risk types of HPV is now considered the major risk factor for the development of cancer of the uterine cervix [3]. It is now established that these risk categories reflect the viruses' ability to promote the proliferation of infected cells by disrupting the stability of the cells' DNA [4], and the types were classified into low and high risk according to viral ability to promote malignant transformation [5,6] (Figure 2).

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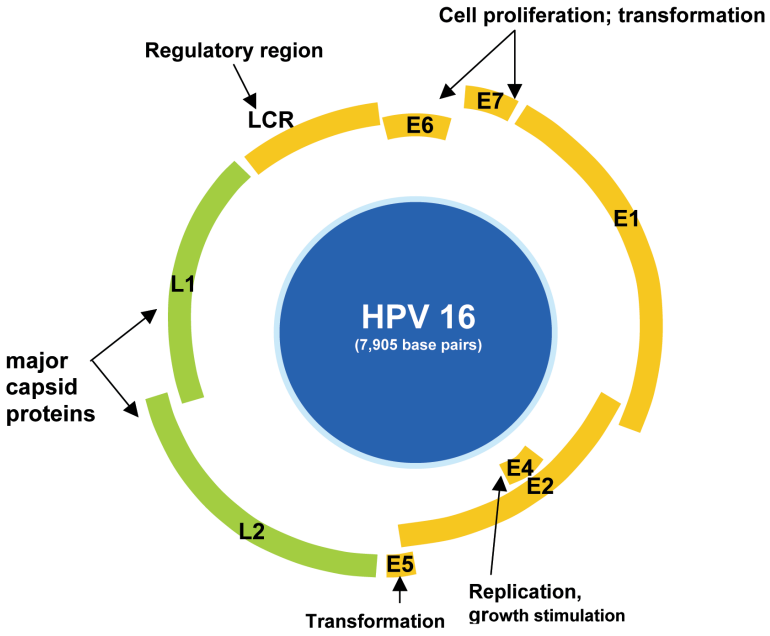

Figure 1 General organization of the HPV genome.

High-risk types:	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Low-risk types:	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
Potentially high-risk types:	26, 53, 66

Figure 2 Main low- and high-risk types of HPV.

A subset of mucosotropic HPV types belonging to the alpha genus, which includes high-risk HPV-16 and HPV-18, are associated with more than 99% of cervical carcinomas [7]. In these cancers, the viral genome is integrated into host cell chromosomes [8]. In vitro, cervical epithelial cells with integrated HPV-16 genes multiply faster than those with extrachromosomal HPV-16. An explanation for this is that the expression of viral genes *E6* and *E7* is increased in cells with where the HPV-16 genome is integrated [2], and these gene products, oncoproteins E6 and E7, respectively bind and inactivate cell tumor suppressor proteins p53 and pRB.

Cellular genes p53 and pRB are not inactivated in cell lines derived from HPV-positive cervical cancers, whereas they are mutationally inactivated in cell lines derived from HPV-negative cervical cancers. The expression of viral genes E6 and E7 is required for the continued growth of cell lines derived from HPV-induced cervical cancer [2], which supports the hypothesis that these proteins are causally related to the onset and maintenance of human cervical cancer. In addition, continuous expression of these early proteins can lead to an accumulation of mutations in the cellular DNA, which in turn promotes malignant conversion [9]. Both E6 and E7 cooperate to induce the transformation of epithelial cells. However, a fully malignant phenotype is observed in vitro only after prolonged cultivation of the transformed cells because of the multistep nature of HPV-induced malignant transformation. In low-risk mucosal types of HPV, E6 and E7 have a very low or no transforming activity in vitro. The integration of HPV DNA affects gene expression in both virus and host. In HPV, gene expression is regulated by viral and cellular transcriptional activators and repressors. This normal regulation, however, is altered by the integration of viral DNA into cell chromosomes, leading to the continuous expression of E6 and E7 proteins and consequently to cell proliferation.

Thus, continuous DNA expression of high-risk HPV often leads to aberrations in cell DNA that are considered a necessary step towards malignant conversion [3,9]. An accumulation of genetic alterations is frequently observed in epithelial tumors, including cervical cancer. Chromosomal instability has been shown to occur in epithelial cells expressing E6 and E7 oncogenes from high-risk HPV collected in the human genital mucosa [10]. These events may occur early in HPV-mediated cell transformation but are better characterized in invasive cancers. Described abnormalities include monosomies and trisomies, chromatid gaps and breaks, double minutes, and aberrant chromosomes. Structural changes are more commonly detected in chromosomes 1, 3, and 5, and less frequently in chromosomes 7, 8, 12, 13, 16, and 22. Moreover, cells expressing HPV-16 E6

and E7 genes have been shown to contain an abnormal number of centrosomes, multipolar mitotic spindles, anaphase bridges, chromosome lagging, aneusomy, and polyploidy [9]. These in-vitro studies suggest that chromosomal abnormalities originating during mitosis lead to an increased risk of mutations, whose accumulation may lead to malignant transformation. In fact, allelic losses have been associated with particular genes that could be involved in malignant conversion and/or progression. Among these, losses in 3p and 10p have been associated with telomerase activation, a crucial step for cell immortalization mediated by high-risk HPV types [8]. However, little is known about the temporal relationship between chromosomal integration of high-risk HPV DNA and the onset of cell genomic instability, particularly in vivo. Progress in this area has been hampered by a lack of experimental models and methods suitable for population samples large enough to investigate HPV integration.

Current evidence strongly suggests that the expression of oncoproteins E6 and E7 is essential for cellular immortalization. It is clear, however, that other factors are required for the acquisition of a fully transformed phenotype. Epigenetic events involving the methylation of viral oncogenes or of the long control region (LCR) of high-risk HPV types have been described by Badal and colleagues [11]. These authors have shown that the LCR of HPV-16 is hypermethylated in normal and low-grade cervical smears and is gradually less methylated in high-grade smears. This correlates with an increased transcriptional activity of the early region, and therefore with a greater availability of the E6 and E7 oncoproteins. In addition, a series of cellular gene hypermethylation promoters has also been observed in cervical carcinomas [5]. In this case, the down-regulation of tumor suppressor gene expression could be considered important for tumor progression.

3. Immune responses to HPV

The control of HPV infection or the development of cervical neoplasia depends on both innate and adaptive immune responses. The available data about humoral immune responses indicate that only about half of the women naturally exposed to HPV will develop immune responses to the virus [12]. Women with transient infections are less likely to develop antibody responses than those with persistent infections, which is indicative of a role for memory responses. Consistently, serum antibodies against peptides derived from different HPV early proteins are more often present in women with cervical cancer than in healthy women [13].

Several studies have shown that serologic diagnosis of HPV infection using genetically engineered HPV capsids (also known as virus-like particles [VLPs]) correlates well with HPV DNA presence in cervical smears. The antibodies produced recognize type-specific conformational epitopes present on VLPs, particularly against the viral capsid protein L1, and the humoral response against HPV, i.e., the production of IgG, is stable over time. Neutralizing antibodies are generated, although frequently in low titers, and are considered to be host protective against further infection with that virus type. Moreover, the sensitivity of enzyme-linked immunosorbent assays to detect HPV VLPs ranges between 50% and 60%; their specificity is very high (>90%); and they have shown good interlaboratory agreement [13].

It has been demonstrated that VLPs for each HPV type almost always induce genotype-specific serum antibody response. The exceptions concern HPV-6 and HPV-11, which are considered to be cross-reactive, and HPV-31 and HPV-45, which produce low levels of cross-reactive antibodies against HPV-33 and HPV-18, respectively. Since variants of HPV-16 are considered to belong to the same serotype [20], VLP serology, along with sexual behavior, has been used to determine whether a patient has a history of cumulative exposure to HPV — even though seroconversion may be delayed or never occur in a subset of women testing positive for HPV DNA. So far, no clinical correlation has been observed between HPV antibody titers and disease control.

Cellular immune responses are most important to control lesions caused by HPV [12]. This is demonstrated by several studies showing lesion persistency and progression in both animals and humans with genetic, iatrogenic, or acquired cell-mediated immune deficiencies. There is sufficient data to indicate the central role of CD4+ and CD8+ lymphocytes; however, natural history studies have not consistently used standardized assays to measure immune responses, making the interpretation of the results obtained so far very difficult.

To make possible the persistence of infections and progression to neoplasia, high-risk HPV types directly subvert immune responses by interfering with the interferon pathway, modulating antigen presentation, and down-regulating the expression of major histocompatibility complex (MHC) class I genes [14]. Moreover, the ability of HPV to circumvent the immune system and thus persist can be related to its life cycle, during which high levels of viral proteins are not readily available for pick-up and recognition (Figure 3). A different scenario occurs in women

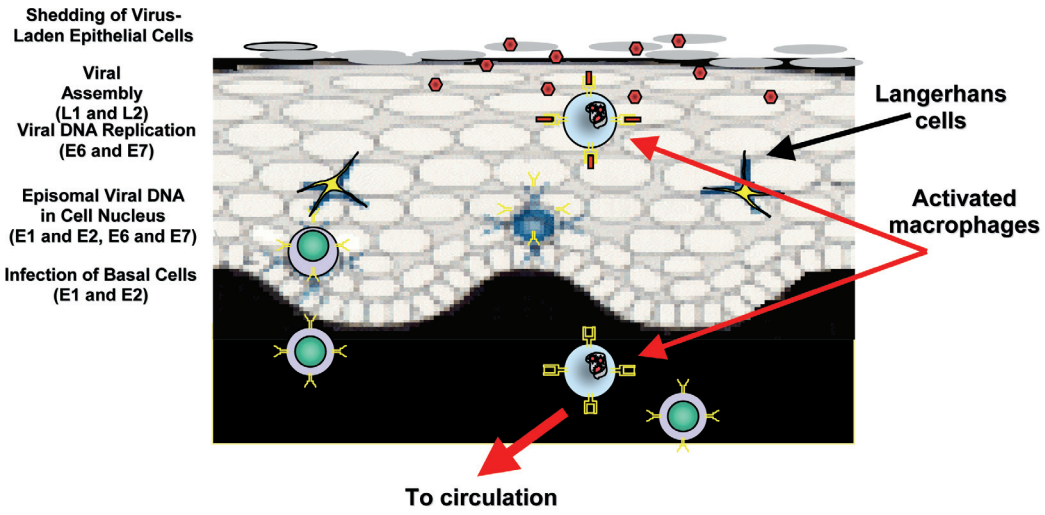

Figure 3 Life cycle of HPV in squamous epithelium. Virions have to reach cells at the basement membranes, possibly through microtraumas, to be able to engage their life cycle.

vaccinated with prophylactic HPV vaccine. In these women, the levels of antibodies against each VLP contained in the vaccine are 10 to 100 times higher than the levels observed in women who are naturally infected with the corresponding viral type. Results from several ongoing clinical trials may help unravel the clinical significance of such findings.

4. Natural history of other HPV-associated anogenital tumors

Compared with HPV infection of the uterine cervix, very little is known about the natural history of infections of the vulva, vagina, penis, or anus — although studies have been conducted on the HPV-infected anus of HIV-positive men and women have been published [21]. Tumors are much rarer in these sites than in the cervix, and vaginal intraepithelial neoplasia or cancers are among the rarest of all gynecologic malignant lesions.

The prevalence of carcinogenic HPV in all these anatomical locations is similar, and ranges between 9% and 50%. The rarity of cancers at these locations points to the cervical — and possibly the anal — squamocolumnar metaplastic epithelium as the place of choice for the development of cancerous lesions. Concerning the penis, results should be interpreted with caution because they may be influenced by the site (shaft, urethra, or foreskin) and collection procedure. The distribution of HPV types is interesting, as HPV-84 is often found in penile smears but is rare in cervical cancer.

Recent reports have explored new possibilities for the detection of HPV in men, including self-collected samples [15,16]. However, prospective studies of HPV infection and risk of penile intraepithelial neoplasia are starting to generate data that will increase our knowledge of the natural history of these infections. In a cohort of young Danish men, HPV persistence was associated with having a high-risk type at enrollment; having multiple HPV types at enrollment; and being a current smoker [17,18]. Persistence of HPV in both men and women could be further affected by the regular use of condoms, as indicated in a recent study [19]. The sexually transmitted nature of HPV infections underscores the need to involve men in effective cervical cancer prevention strategies, including HPV vaccine programs.

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