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Review Article

The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review

Sheila V. Graham[1,2]

[1]MRC-University of Glasgow Centre for Virus Research; Institute of Infection, Immunity and Inflammation; College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Glasgow G61 1QH, Scotland, U.K.; [2]Rm 254, Jarrett Building, Garscube Estate, University of Glasgow, Glasgow G61 1QH, Scotland, U.K.

Correspondence: Sheila V. Graham (Sheila.Graham@gla.ac.uk)

HPVs (human papillomaviruses) infect epithelial cells and their replication cycle is intimately linked to epithelial differentiation. There are over 200 different HPV genotypes identified to date and each displays a strict tissue specificity for infection. HPV infection can result in a range of benign lesions, for example verrucas on the feet, common warts on the hands, or genital warts. HPV infects dividing basal epithelial cells where its dsDNA episomal genome enters the nuclei. Upon basal cell division, an infected daughter cell begins the process of keratinocyte differentiation that triggers a tightly orchestrated pattern of viral gene expression to accomplish a productive infection. A subset of mucosal-infective HPVs, the so-called 'high risk' (HR) HPVs, cause cervical disease, categorized as low or high grade. Most individuals will experience transient HR-HPV infection during their lifetime but these infections will not progress to clinically significant cervical disease or cancer because the immune system eventually recognizes and clears the virus. Cancer progression is due to persistent infection with an HR-HPV. HR-HPV infection is the cause of >99.7% cervical cancers in women, and a subset of oropharyngeal cancers, predominantly in men. HPV16 (HR-HPV genotype 16) is the most prevalent worldwide and the major cause of HPV-associated cancers. At the molecular level, cancer progression is due to increased expression of the viral oncoproteins E6 and E7, which activate the cell cycle, inhibit apoptosis, and allow accumulation of DNA damage. This review aims to describe the productive life cycle of HPV and discuss the roles of the viral proteins in HPV replication. Routes to viral persistence and cancer progression are also discussed.

Introduction

HPVs (human papillomaviruses) are non-enveloped icosahedral, circular, dsDNA viruses of approximately 55 nm in diameter. They infect cutaneous and mucosal epithelia. There are over 200 different HPV genotypes that mainly cause benign lesions or warts [1,2]. For example, HPV genotypes 1 and 2 (HPV1, HPV2) cause verrucas on the feet (plantar lesions), while HPV2 and 4 cause common warts on the hands. However, approximately 40 HPVs are sexually transmitted infections of the anogenital region where infection manifests either as genital warts (caused by HPV6 and 11) or other anogenital lesions (condyloma accuminata; caused by the other anogenital-infective HPVs) [3] . Such lesions have been identified on the cervix, the vulva, vagina, and anus in women [3,4]. There evidence for HPV-associated lesions of the anus and penis in men but these are less well characterized [5-7]. Lack of knowledge of HPV-associated diseases of these sites is due to low rates of presentation in the clinic [8]. For a specific subset of anogenital HPVs, the HR (high risk) HPVs, persistent infection, over a period of several years, together with associated changes in the infected host cell, can cause anogenital cancers. It is estimated that HPVs are the cause of more than 5% of all the human cancers [9]. The most prevalent HR-HPV is HPV16 (HR-HPV genotype 16). It is the most prevalent sexually transmitted viral infection worldwide [10]. There are approximately 14 other HPVs classified as HR but other types may also be associated with anogenital cancers (Table

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Table 1 Sites of infection of HPVs

Site	HPV-associated diseases	HPV types
Skin	Wart	1, 2, 3, 4, 7, 10, 26, 27, 28, 29, 41, 48, 50, 57, 60, 63, 65, 75, 76, 77, 88, 95
	EV wart	5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 46, 47, 49, 75, 76, 80, 92, 93, 96
Oropharynx, larynx, oral cavity	Head and neck cancers	16 mainly (also other HR types at low frequency)
Oral cavity	FEH	13,32
Larynx	Laryngeal papillomatosis	6,11
Anogenital tract	Genital warts (low risk)	6, 11
	Intraepithelial neoplasia (low risk)	40, 42, 43, 44, 53, 54, 61, 72, 73, 81
	Intraepithelial neoplasia and cervical cancer (high risk)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73, 82 (26, 53, 66)

This is not an exhaustive list.
HPV types in brackets may be high risk (HR). Abbreviations: EV wart, epidermodysplasia verruciformis; FEH, focal epithelial hyperplasia.

1) [3]. HPVs are associated with cancers at anatomical sites other than the anogenital region. Over the last 20 years, there has been a highly significant increase in incidence of HPV-associated tumors of the oropharynx, particularly in young men in developed countries [11]. Although vaccines against HR-HPV infection have been widely available since 2006–2008, they are prophylactic and normally delivered only to girls [12]. Vaccine acceptability has been low in many countries and recently, it has dropped drastically in some countries where vaccination rates were originally high [12]. Moreover, these vaccines are the most expensive ever produced, and so are difficult to roll out in the developing world where over 80% of significant HPV-associated diseases occur. Thus, new therapies require to be developed to aid the worldwide effort to treat HPV-associated diseases. Knowledge of the HPV infectious life cycle and HPV-associated cancer progression has increased substantially over the last decades leading to several potential new avenues for diagnosis and treatment of HPV-associated cancers [13]. This review will focus on the HPV infectious life cycle and how this is linked to HPV-associated diseases.

HPV clasification

There are over 207 HPVs classified to date [1,2]. Characterization of new HPVs involves sequencing of the viral genome and comparison with known HPV genomes. Therefore, HPVs are categorized into genotypes. Each genotype differs from another by at least 10% sequence difference in the highly conserved *L1* gene region [14]. Currently, five evolutionary HPV genotype groups (α, β, γ, μ, and ν) have been defined [1,2]. Of these, the largest group is the α group [1,2]. This group contains 64 HPVs that mainly infect mucosal epithelia. Approximately 40 of these HPVs can infect the anogenital tract and include the approximately 15 so-called 'HR' types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 51, 56, 58, 59, 68, 73, 82) that have been classified as oncogenic and are found to cause anogenital cancers (Table 1) [3]. Over 80% of the population will experience an anogenital α-HPV infection during their lifetime [15]. Indeed, the majority of adults become infected with HR-HPV upon sexual debut and the highest incidence age range of infection is 20–25 years of age in the developed world [16].

The next largest group is the β-group HPVs that mainly infect cutaneous epithelia (Table 1) [17]. More than 50 types have been identified and characterized, but it is likely that many more exist because fragments of uncharacterized HPVs continue to be isolated from cutaneous lesions [14,18]. The β group, together with UV irradiation, can be associated with human tumors, especially non-melanoma squamous cell carcinomas, the most common human cancer [19]. HPVs of the remaining three groups (γ, μ, and ν) normally cause only benign disease.

HPV-associated diseases

The vast majority of HPV research has focussed on HPV16, its replicative life cycle, and its role in cervical cancer. In 1995, WHO (World Health Organization) defined HPV16 as a viral tumor-promoting agent. HPV16 causes approximately 55% of cervical cancers, HPV18 causes approximately 15% of cases and the remainder are caused by the other HR-HPVs [3]. Nowadays, over 270000 women die from cervical cancer per annum worldwide with over 500000 cases diagnosed per annum [15]. It is the fourth most common cancer in women but is the most common cause of cancer-related deaths in women under the age of 35. The peak age of incidence of cervical cancer is 49 years

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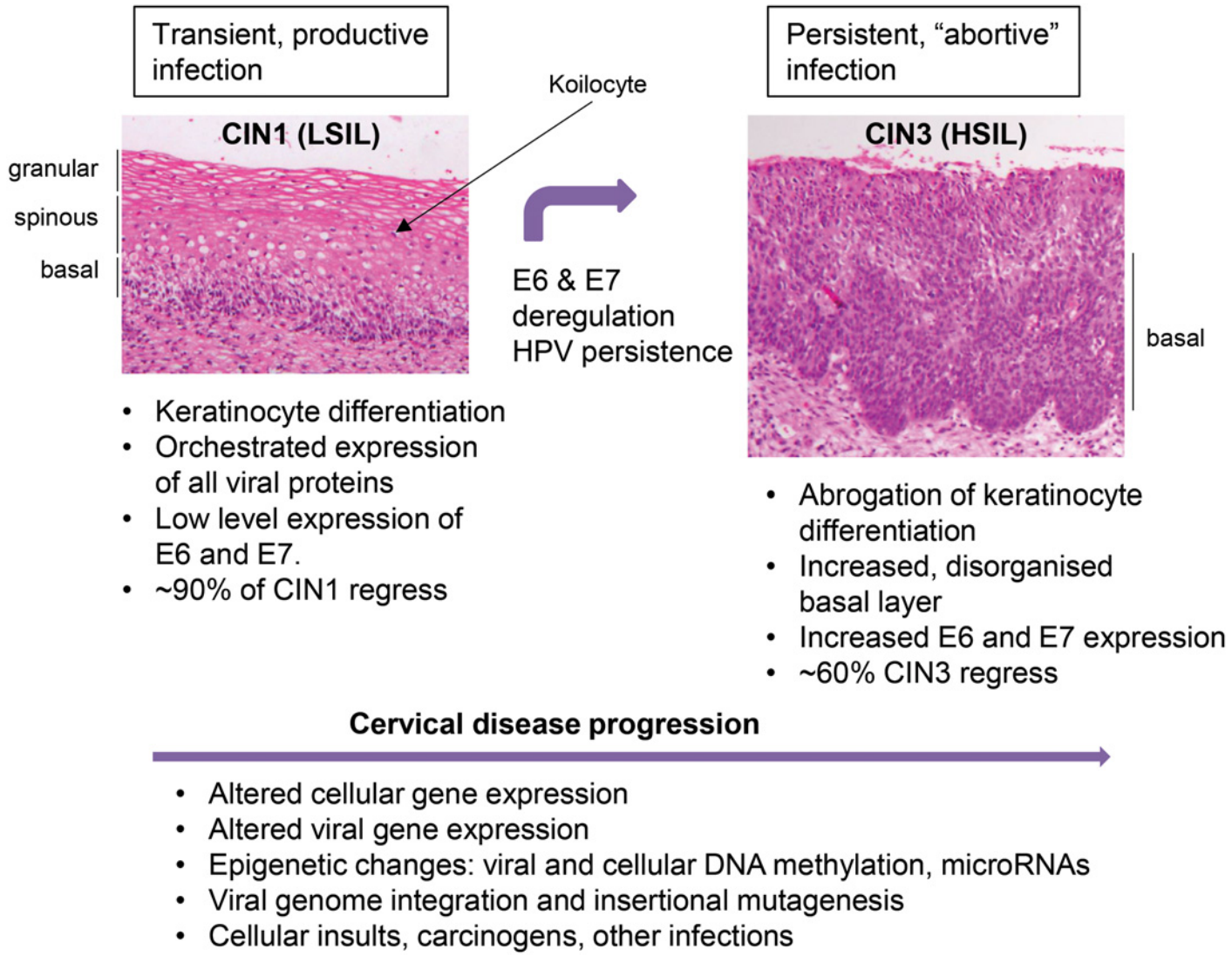

Figure 1. Cervical-disease progression and lists of contributing factors

The images show Hematoxylin and Eosin-stained cervical lesions. On the left hand side is shown a tissue section of low-grade disease (CIN1 (cervical intraepithelial neoplasia 1) (LSIL; low-grade squamous intraepithelial lesion)). On the right hand side is shown a tissue section of high-grade disease (CIN3 (HSIL; high-grade SIL)). Nuclei are stained purple, cytoplasms are stained pink. The granular, spinous, and basal layers are indicated for CIN1. A typical koilocyte is indicated with an arrow. The approximate extend of the significantly expanded basal compartment in CIN3 is indicated. Progression from CIN1 to CIN3 is due to persistent infection and up-regulation of E6 and E7 oncoprotein expression. A bullet point list of features of each stage of disease is beneath each image. A bullet point list of factors contributing to cervical-disease progression is shown beneath the purple block arrow.

and the majority of diagnoses are in women aged 30–55 [16]. Normally, HR-HPVs cause transient infections that are eventually cleared over a period of several months by the immune system. Cancer formation does not result from such infections [3]. However, over time, if changes occur to the viral genome, and/or to the infected host cell, transient infection can become persistent. If persistent infection is not detected and cleared by the immune system, there is a possibility of progression to cancer (Figure 1) [20]. Cancer progression is not a good outcome for the virus because the associated changes to the infected cell abrogate the viral replicative life cycle and progeny virions cannot be produced. It is important to point out that cancer progression due to persistent HR-HPV infection is a rare event.

Cervical cancer is preceded by cervical disease, which is a direct result of HPV infection [9]. Cervical disease is common. It is currently detected by Pap smear testing wherein cells are scraped from the surface of the cervix and examined under the microscope for abnormalities: koilocytes (cells containing a compressed nucleus with a

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perinuclear vacuole), and abnormally dividing cells that may indicate HPV infection (Figure 1). Following a positive cytological test, women are referred to colposcopy clinics for further diagnosis and treatment. In the U.K., over 100000 women are referred to colposcopy (detailed examination of the cervix) per annum. Cervical disease is classified either into a three-stage system, termed as 'cervical intraepithelial neoplasia' (CIN) or a two-stage system, termed as SIL (squamous intraepithelial lesion) [21]. CIN is categorized into CIN1, CIN2, and CIN3, and there is good evidence for a progression from CIN1 to 3 (or LSIL to HSIL) underlying cervical cancer formation (Figure 1) [22]. CIN1 is thought to represent transient HPV infection that has a low probability of progression to cervical cancer [23]. This corresponds to low-grade (L) SIL. Infection with multiple HPV types is very common in CIN1 [24] but infection is eventually cleared over a period of several months by the immune system. Approximately 80–90% of cases of CIN1 regress [23]. CIN2 or CIN3 (high grade (H) SIL)) in most cases represent a persistent, unproductive infection (Figure 1). This equates to precancerous disease, but even for CIN3, up to 60% of lesions will regress spontaneously [23]. High-grade CIN lesions harbor a similar range of anogenital-infective HPVs to that found in cervical cancer [25].

As noted above, HR-HPV can cause other anogenital preneoplastic diseases (VIN; vulvar intraepithelial neoplasia, AIN; anal intraepithelial neoplasia etc.) and cancers such as vulvar, vaginal, and anal cancers in women, and anal and penile cancers in men. The HPV genotype distribution in these cancers is still being worked out. However, at least for female anogenital cancers, they seem to harbor the same HR-HPV set as observed in cervical cancers, and multifocal HR-HPV-associated lesions are frequently observed [26]. This makes sense because of the sexually transmitted nature of the disease. Unlike cervical disease and cervical cancer, a smaller proportion of cancers at other sites are attributable to HR-HPV infection. For example, HR-HPV was detected in 84% of vulvar intraepithelial neoplasia [27] and 74% of vaginal cancers [28]. The one exception to the rule is that penile cancers can sometimes be associated with the low risk anogenital-infective HPV6 and 11 [5]. Information on the etiology and pathogenesis associated with the activities of HPV6 and 11 in these lesions is still lacking and it remains to be determined how viruses that normally do not cause cancer in other sites can do so on the penis. Anal infections are common in women, and men who have sex with men. These usually regress in immunologically competent individuals [6] but anal precancerous disease and anal cancers are a significant problem in HIV-positive patients [29]. Women who experience high-grade cervical disease (CIN3) or vulvar cancer are more likely to develop anal cancer due to persistent HPV infection across the anogenital region [30]. Finally, many people, especially young adults (up to 2% of the population), can suffer from genital warts caused by the two well-defined low risk anogenital infective HPVs, HPV6 and 11 [31]. In some cases, multiple visible lesions, and the stigma of a sexually transmitted infection, can lead to sexual dysfunction in affected individuals. Although benign, treatment of these lesions is costly in terms of clinical time [31].

Over the last 20 years, there has been an exponential increase in HPV-associated oropharyngeal cancers (tongue base, soft palate, and pharyngeal walls), particularly in young males in high-income countries [11]. For example, in the U.S.A., rates of HPV detection in oropharyngeal cancers have increased over three-fold over the last two decades [32]. HPV infection of the oropharynx is thought to be due to changes in oral sexual practices [33]. In the U.S.A., the numbers of these HPV-associated cancers has already exceeded cervical cancers (CDC: http://www.cdc.gov/cancer/hpv/statistics/index.html.) and this trend is predicted to be observed in other high-income countries by 2020. HPV has been detected at highest levels in cancers of the tonsil, tongue base, and pharynx (Table 1) [34]. Of all the HPV-positive cases, between 80 and 90% are positive for HPV16, depending on the anatomical site [11]. Surprisingly, although other HPVs can be present, they are usually not detected at equivalent levels to anogenital types [34] suggesting that, although all anogenital HPVs could be sexually transmitted to the oral cavity, HPV16 replicates or persists better, or more readily causes cancer progression in certain sites in the head and neck. Interestingly, HPV positivity appears to indicate better response to radio- and chemotherapy in patients, although this may be related to the younger age distribution of these patients compared with the relatively older age group of HPV-negative patients [35].

HPV has been found to be associated with a number of other cancers, including prostate, colon, bladder, esophageal, and breast cancers [7]. The evidence includes detection of viral nucleic acid, and/or EM observation of viral particles, but in most cases a causative association has not been demonstrated. Breast cancer studies have been the most frequently reported [36]. HPV DNA and RNA detection and observation of HPV-associated morphological changes in breast epithelial cells, including koilocytes, a morphological feature of cervical disease, comprise the evidence [37]. However, there are many studies where HPV has not been detected in breast cancer casting doubt on the causative role of HPV infection [38]. HPV could be present, but not active, in HPV-positive breast tumors.

Many HPVs of each of the different genotypic groups cause transient infection of the cutaneous epithelium at a wide range of anatomical sites (Table 1) [39,40]. Skin lesions, such as common warts on the hands or verrucas on the feet, are benign but they can become medically important if the lesions are unsightly or cause embarrassment, spread rapidly or persist, or are prone to injury. The β-HPVs all infect the cutaneous epithelium and are a part of

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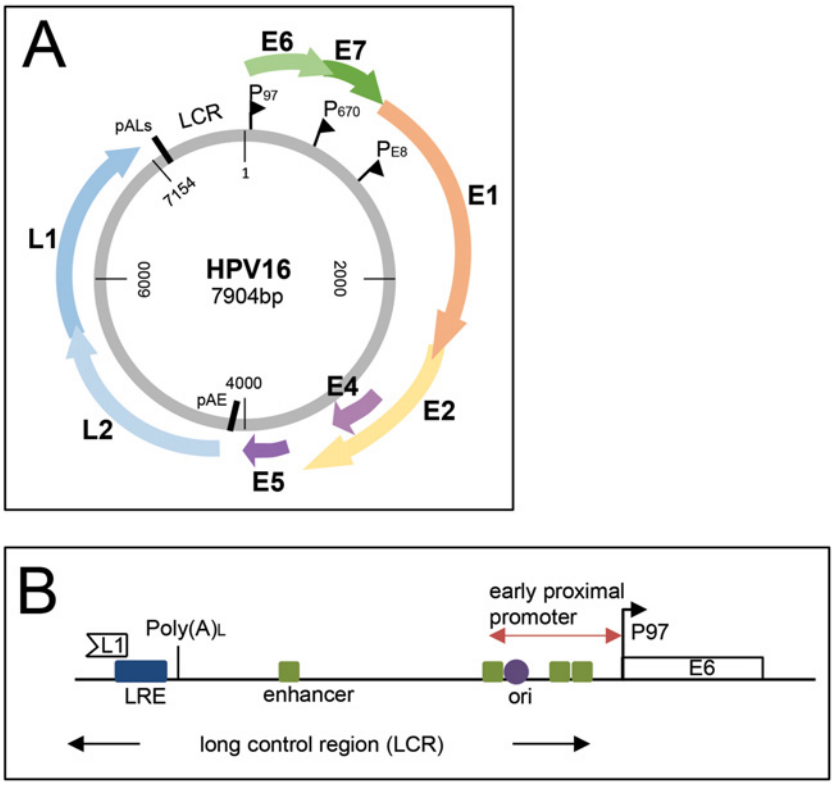

Figure 2. The HPV16 genome

(**A**) Diagram of the circular dsHPV16 genome (gray circle). Other HPV genomes are very similar in organization. Viral ORFs are indicated with colored arcs above the genome. Promoters are indicated with P and chevrons (P97, P670, PE8). Early (pAE) and late (pALs) polyadenylation sites are indicated with short straight lines above the circular genome. The LCR is indicated with a blue line. (**B**) Details of the LCR sometimes called the URR. The E6 ORF is shown as an open rectangle. The end of the L1 ORF is shown as a partial open rectangle. The early promoter (P97) is indicated with and arrowhead. The proximal promoter region is indicated with a double arrowhead red line. Four E2 binding sites in the LCR are indicated with green squares. The origin of replication (ori) to which E1 binds is shown as a purple circle. The late polyadenylation site is indicated by Poly(A)$_L$ and a straight line. The late regulatory element that controls late gene expression is indicated with a blue box. Locations of the different features are not to scale.

the normal human virome from infancy [39,40]. However, in some cases β-HPVs can cause cancer. The majority of such cancers occur in immunosuppressed individuals and in patients with the inherited recessive genetic disorder EV (epidermodysplasia verruciformis) [41,42]. Key β-HPV types associated with increased risk of non-melanoma squamous cell carcinomas include HPVs, 5, 8, 17, 20, 24, 36, and 38 (Table 1) [43]. β-HPV types 5 and 8 were first isolated and identified as carcinogenic in EV patients. EV patients accumulate increased cutaneous HPV infections from birth, and in infancy, can develop flat warts on sun-exposed areas of the skin that can eventually progress to squamous cell carcinomas [44]. Individuals with EV harbor mutations in two genes, *EVER1* (TMC6) or *EVER2* (TMC8). These mutations alter the way in which HPV is detected and cleared by the immune system [45]. This results in the possibility of massive persistent infection of the cutaneous epithelium, which, if not cleared, can lead to cancer.

The HPV infectious life cycle

Virion structure

All HPVs possess an episomal DNA genome of approximately 8 kb in size (Figure 2A) [1,2]. The genome can be divided into three functional sections, the early (E) region encoding at least seven viral proteins that have regulatory functions in the infected epithelial cell (E1, E2, E4, E5, E6, E7, E8), the late (L) region that encodes the two viral structural proteins L1 and L2, that form the viral capsid, and the LCR (long control region) otherwise called the upstream regulatory region (URR) (Figure 2B) [1,2]. The LCR contains the viral *cis*-acting regulatory sequences that

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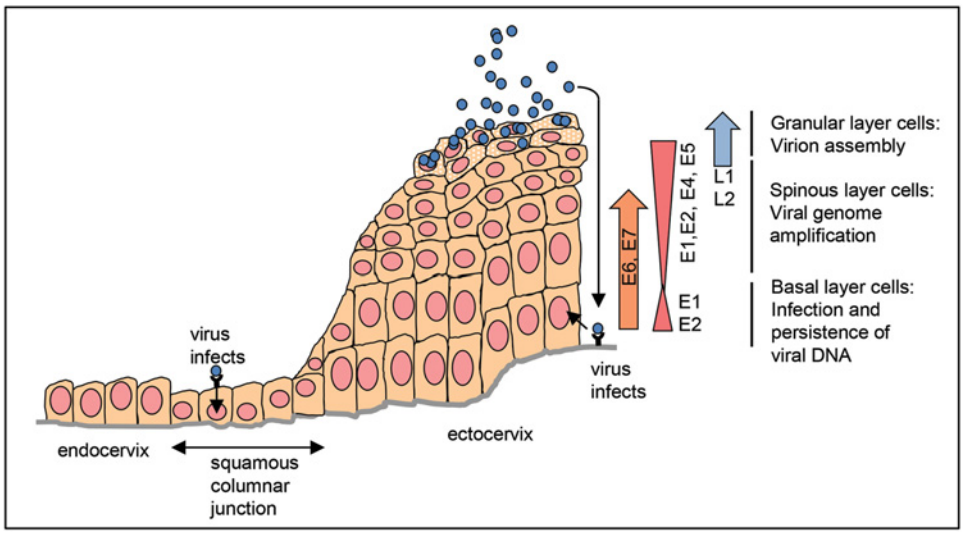

Figure 3. Virus infection of the cervix, keratinocyte differentiation, and HPV protein expression

HPV can bind receptors on the basement membrane (gray line) and go on to infect basal layer cells of the epithelium. Alternatively, HPV can enter cells in the squamous columnar junction. Most cervical cancers are thought to arise from this zone [48]. Division of an infected basal epithelial cells can give rise to a transit amplifying cell that is capable of differentiation. Viral genomes are segregated into daughter cells upon basal cell division and can be carried into upper epithelial layers. The keratinocyte differentiation process allows an orchestrated pattern of viral gene expression as indicated on the right hand side. Cells are shown in beige, nuclei in pink, and virus particles in pale blue. The basement membrane is shown as a gray line. Entry receptors are shown as a line with an arc above.

control viral replication and transcription, and post-transcriptional control via the LRE (late regulatory element) [13]. The genome is encapsidated in an icosahedral shell that comprises 360 copies of the L1 protein organized into 72 pentameric capsomeres that probably have one copy of L2 at the center [46].

HPV cell entry

HPV can gain entry into the epithelium through microabrasions [47] or for the HR-HPVs infecting the cervical epithelium, by entering cells of the single layered squamous cellular junction between the endo- and ectocervix, (Figure 3) [48]. At least for the HR-HPVs, it seems necessary for efficient establishment of infection that they infect actively dividing basal, or stem, epithelial cells [49,50]. HPV L1 capsid protein binds cellular receptors located on the basement membrane or on the surface of basal layer cells [51]. Heparin sulphate proteoglycans (HSPGs) seem to be the primary receptor for initial binding [52]. When HPV binds HSPGs, there is a cyclophilin B-mediated conformational change in the viral capsid such that the N-terminus of the L2 component is exposed on the surface of the virion [53]. The N-terminus is then cleaved by furin and/or PC5/6 and this allows binding to a secondary receptor on the plasma membrane of the target cell [54,55]. Recent studies have revealed the involvement of epidermal growth factor (EGF) receptors (EGFRs) [56], integrins (α6 integrin) [56-58], tetraspanin-enriched membrane microdomains [59], laminins [60], syndecan-1 [56,61], the annexin-A2 heterotetramer [62,63], and vimentin [64] as entry receptors of HR-HPV. The receptor strategy used may be dependent upon the HPV genotype, the cell type to be infected, or several different receptor strategies may be applicable in a single infection. HPV enters cells by an endocytosis mechanism that has most similarities with micropinocytosis [65]. The virus travels through membrane-bound cytoplasmic components and the *trans*-Golgi network [66-68], although there may be some involvement of the ER (endoplasmic reticulum) in trafficking [68,69]. Finally, the viral episomal genome is transported through a tubulin-mediated pathway [70] to the nucleus where it may enter via nuclear pores or following breakdown of the nuclear membrane during mitosis [49,71]. HPV reaches the nucleus approximately 24 h following cellular attachment where the capsid disassembles and incoming L2 and the viral genome associated with PML (promyelocytic leukaemia) nuclear bodies [72]. This nuclear location is commonly used by DNA viruses to initiate viral transcription [73] but while many viruses disrupt PMLs upon nuclear infection, HPV seems to require PML integrity to establish nuclear infection [72].

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The early phase of the viral replication cycle: E1 and E2

Upon nuclear entry into the dividing cells of the basal layer or ectocervix viral early transcription is initiated. One study on HPV31 revealed that RNA encoding the viral replication/transcription factors E1 and E2 was the first RNA species to be detected upon nuclear infection [74]. This makes sense because the first goal of the incoming virus is to carry out initial replication of its genome. Moreover, early expression of the viral transcription factor E2 would allow correct regulation of the viral early promoter to direct expression of the E6 and E7 regulatory proteins that ensure continued survival of HPV-infected cells [75]. E2 possesses one DNA-binding and one protein-binding domain linked by a flexible hinge region [76]. It forms a homodimer that can bind to four palindromic sites in the LCR (Figure 2B). Three of these sites are located adjacent to the origin of replication and are required for E1-activated viral replication [77] . E2 binds E1, which then binds as a dimer of hexamers to the viral origin of replication and recruits the cellular DNA replication machinery [78,79]. Initial replication of an incoming HPV genome generates approximately 50–100 episomal copies per nucleus [80]. Limited viral genome amplification has been shown to be controlled by the E8^ E2 protein via the cellular NCoR/SMRT complex [81]. This constitutes the first phase of viral DNA replication and genome maintenance. In infected basal cells, circular viral genomes are replicated in concert with replication of cellular DNA and equally partitioned into daughter cells through tethering of virus genomes to host cell chromosomes via E2 bound to the viral LCR and chromatin-binding proteins [77]. Brd4, via E2, is the most studied anchor for HPV genomes to cellular chromosomes [82]. However, there may be alternatives other than Brd4 for attachment of HPV genomes, including MKlp2 [83], ChlR1 [84], and TopBP1 [85]. On the other hand, the E1–E2 complex itself may be sufficient for some HPVs to locate to cellular chromosomes [77].

Viral proteins are probably expressed at low levels in infected basal cells [86] to avoid activating the local immune response [87]. This is achieved by E2 transcriptionally repressing the P97 promoter by inhibiting access of transcription factors to the promoter and by altering chromatin conformation [88-90]. In this way, HPV is capable of maintaining infection of epithelial cells over a significant period of time. Division of an infected basal epithelial cell can produce a transit amplifying cell that is capable of differentiating and moving into the upper epithelial layers [91]. These cells carry the viral genomes with them as they move through the upper epithelial layers. HPV has evolved to carry out its replication cycle in exquisite concert with epithelial differentiation and an orchestrated program of viral gene expression is carried out during epithelial differentiation (Figure 3) [20].

HPV E6 and E7 protein activities during the replication cycle

HPVs possess an early promoter (for HPV16: P97; for HPV18: P105) that is responsible for expression of proteins at early stages of the replication cycle (Figure 2B) [13]. These include the E6 and E7 viral oncoproteins [92,93]. Despite their designation as oncoproteins, their expression is essential for the normal replicative HPV life cycle. Early *in situ* hybridization studies indicated increased levels of the mRNA encoding these proteins in the lower to mid-upper epithelial layers [94-96]. However, expression of biomarkers that respond to E6 and E7 was found to decrease in the upper epithelial layers [97]. Therefore, biological activity of these proteins may be most important in the early phase of viral replication in basal epithelial cells. For example, E6 has been shown to be required for episomal genome maintenance [98-100]. E7 expression, early in infection, activates the G_1 to S-phase checkpoint in keratinocytes that would normally undergo terminal differentiation, thus expanding the compartment of epithelial cells active in DNA replication [93]. This is an important step to accomplish the second, productive, phase of viral genome replication in cells of the mid to upper epithelial layers that would normally exit the cell cycle. E7 activates the cell cycle of infected, differentiating cells by binding and releasing, or degrading, pRb and other pocket proteins, p107 and p130, from a transcriptional repression complex containing the E2F transcription factor [101-105]. E2F becomes free to activate transcription of a number of cell cycle-related genes such as cyclins A and E, thus stimulating G_1 to S-phase transition [75]. E7 can also interact with and abrogate E2F inhibitory transcription complex activity leading to stimulation of promoter activity of growth control genes [106]. Added to this is a complex series of possible stimulatory and inhibitory activities of E7 on cellular transcription factors such as STAT1, NF-κB, IRF1, SMAD2/3, TBP, Miz 1, B-Myb, c-Myc, c-Jun, c-Fos, E2F1, and E2F6 [107]. This suggests the potential of E7 to make very significant transcriptional changes in infected cells. However, the expression level of the protein, and where it is expressed in the different layers of the infected epithelium, will have some impact on these activities. Moreover, during the viral life cycle, E7 is always present with E6 in the infected cells due to the bicistronic nature of the E6/E7 coding region of the genome [1,2]. Therefore, activities of either protein will be affected by the other and it is clear that they often act co-operatively, for example in avoiding immune detection [87].

Normally, cells respond to any unscheduled cell proliferation event by inducing apoptosis. Therefore, HPV E7 activity might be expected to induce cell death. To avoid this, HR-HPVs express the E6 protein, which binds the

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ubiquitin ligase, E6AP (E6-associated protein) and p53, a key regulator of apoptosis and targets it for degradation [75,108]. Further, HR E6 proteins can abrogate p53 function by binding the histone acetyltransferase CBP/p300, and inhibiting p53 transcriptional activity [109,110]. E6 proteins can also cause a conformation change in p53 through binding. This inhibits its transcriptional transactivation properties [111,112]. Finally, E6 can sequester p53 in the cytoplasm, meaning that it cannot carry out its nuclear transcription activation functions [113]. Some β-papillomavirus E6 proteins can bind p53 but cannot target it for degradation [114]. β-HPV E6 proteins can bind CBP/p300, but the mechanism of inactivation is different from that of the α HR-HPVs and involves blocking phosphorylation of p300 leading to destabilization of the protein and reduced levels in infected cells [115]. β-E6 proteins also bind MAML, and SMAD2 and 3, to inhibit Notch signaling [116,117]. The Notch pathway is responsible for promoting keratinocyte differentiation [118]. Thus, β-HPV E6 can directly inhibit differentiation, as can α-HR-HPV [119]. E6 also promotes proteasomal degradation of tuberin, part of the TSC2 (tuberous sclerosis complex 2), to interfere with insulin signaling and maintain mTOR activity, protein translation, and cellular proliferation [120]. HPV16 E6 has been shown to bind to, but not degrade, paxillin, a protein that plays essential roles in the structural organization of the cell [121]. It is possible that this interaction could result in restructuring the infected cells to facilitate viral egress.

High and low risk, and β HPV E6 proteins can bind the proapototic Bak protein and target it for degradation [122-125]. Bak has been found to be most active in the upper layers of the skin where HPV replication occurs [122]. It is thought that in uninfected cells, E6AP regulates the levels of Bak. However HPV18 E6 can decrease Bak levels through proteasomal degradation [122]. This prevents Bak-mediated permeabilization of the mitochondrial and ER membranes, therefore avoiding the activation of the caspase cascade and apoptosis. Therefore, this is another route to inhibition of apoptosis and it has been proposed that this pathway specifically links to the DNA damage response [123,124]. These various roles of high and low risk, α and β E6 proteins are clearly important for viral replication.

E6 interaction with, and degradation of PDZ (PSD95/hDlg/ZO-1) domain proteins that control cell shape, cell signaling, and polarity is also important for the viral life cycle [126]. The E6 proteins of HR-HPVs contain a C-terminal PBM (PDZ-binding motif) allowing binding to PDZ proteins such as human homolog of *Drosophila* Discs Large (hDlg), Scribble, and MAGI 1, 2, and 3 [126]. The E6 PBM is required for episomal maintenance and genome amplification of HR-HPVs, indicating that PDZ proteins are important for the HPV life cycle [127-129]. PDZ domain-containing proteins can bind to and stabilize low levels of E6 expressed during an infection leading to enhanced episome maintenance [129]. This PBM motif may also be required for cell cycle activation of HPV-infected cells and inhibition of apoptosis via NF-κB [130]. The fact that this E6 motif is missing in low risk E6 proteins suggests that it may be associated with tumor-promoting properties of the HR-HPVs [126]. However, a recent study revealed that PDZ-binding predates acquisition of oncogenic properties by the α papillomaviruses and it has been suggested that E6 regulation of PDZ proteins allowed ancient HPVs to gain access to new sites of infection such as the cervical transformation zone [131]. The E6 PBM also allows a regulatory interaction with SNX27 (sorting nexin 27), a protein that controls endosomal transport, nutrient acquisition, and cell proliferation [132]. E6 control of these pathways via SNX27 could have importance in keratinocyte metabolism during viral infection.

HPV16 E6 has been shown to bind to IRF-3 (interferon (IFN) regulatory factor-3) and abrogate transcriptional activity on the IFN-β promoter [133]. E6 also interferes with IFN α-mediated signaling via the STAT/Tyk2 pathway by inhibiting autophosphorylation of Tyk2 and ISG3α, a component of the ISGF3 transcription complex that activates IFN responsive genes [134]. HPV16 E6, together with E7, also prevents expression of TLR9 (toll-like receptor 9) that recognizes dsDNA in an infected cell to activate the innate immune response [135]. Recently the β-HPV type 38 E6 and E7 proteins were also shown to down-regulate TLR9 and importantly, this could be linked to cell cycle activation and increased keratinocyte proliferation [136]. Moreover, both E6 and E7 inhibit cytokines such as TNFα (tumor necrosis factor α), TGF β (transforming growth factor β), and the IFNs, thus altering cytokine signaling and innate immunity [87]. HR-HPV E6 represses IFNκ by DNA methylation to abrogate cellular responses to viral infection [137,138]. Finally, HPV E6 controls IL1-β via E6AP and p53-mediated degradation [139].

Recently, there has been an explosion of interest in the roles of non-coding RNAs during HPV infection. The majority of studies have examined miRNAs, 22–24 nt RNAs that control the stability and translation of mRNAs. Although other tumor viruses encode miRNAs, HR-HPVs do not [140]. However, E6 controls expression of cellular miRNAs, *miR-23b*, *miR-218*, and *miR-34a*. *miR-34* is controlled via p53 and loss of this factor via E6-mediated degradation results in release of inhibition of a wide range of cell cycle and apoptosis-related gene expression [141]. E7 control of *miR-203* has been shown to be important for viral genome amplification. *miR-203* controls expression of ΔNp63 and an increase in levels of this transcription factor by E7 suppression of *miR-203* leads to prolonged cell cycle activation in differentiating keratinocytes [142]. In fact, E6 and E7 control of many cellular transcription factors has the potential to alter expression of many RNA polymerase II-transcribed miRNAs that could be involved in the viral replication cycle. Importantly, miRNAs can modulate viral gene expression. *miR-145* targets sequences in the

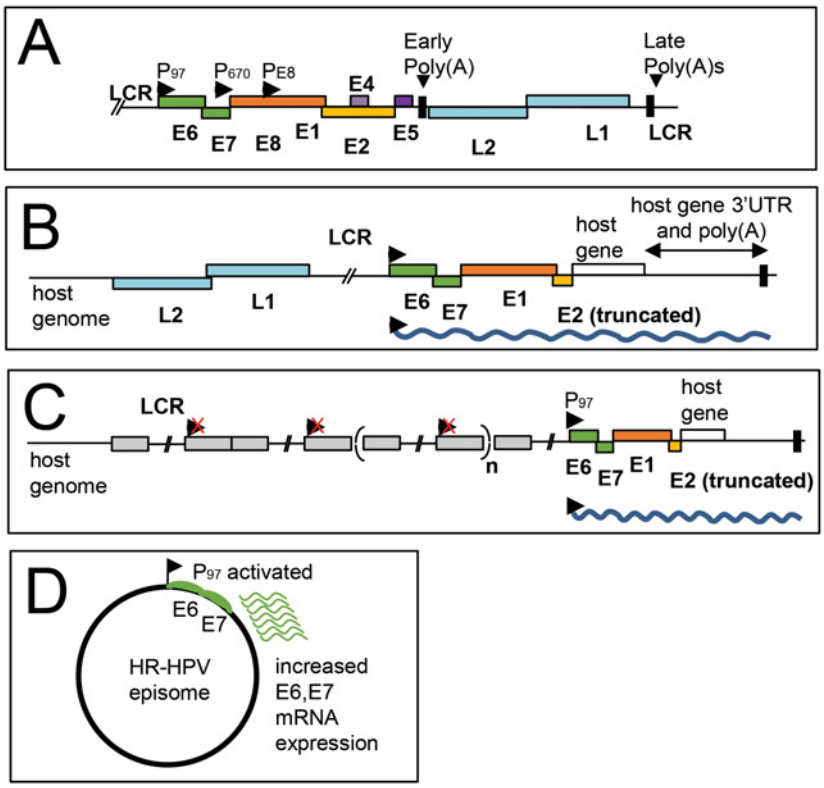

Figure 4. Mechanisms of activation of E6 and E7 expression during cervical tumor progression

(**A**) Linear diagram of the HPV16 genome showing the eight ORFs (colored boxes) the three characterized promoters (forward facing arrows) and the early and late polyadenylation sites (thick black vertical lines). Green colored boxes indicate the E6 and E7 coding regions. Orange/yellow colored boxes indicate E1 and E2 coding regions. Lilac/purple colored boxes indicate the E4 and E5 coding regions. Blue colored boxes represent the L1 and L2 capsid encoding genes. (**B**) This diagram illustrates insertion of a single copy of the HPV genome with disruption in the E2 ORF. This leads to overexpression from the P97 promoter of the viral oncoproteins. Integration of the HPV16 genome can disrupt a host gene leading to transcription from the P97 promoter of a truncated viral early gene region fused to the 3′-UTR and polyadenylation site of the host gene (open box). This can cause increased stability in the E6 E7 bicistronic mRNA leading to increased expression levels of the oncoproteins. Integration may also cause insertional mutagenesis of the host gene, which could have oncogenic effects. The gene color scheme is the same as that used in (A). The wavy blue line indicates a viral-host gene fusion transcript. (**C**). Multiple integration can also occur but expression of the majority are epigenetically silenced by methylation of the P97 promoter region. The gene color scheme is the same as that used in (A). Gray boxes, silenced viral genome copies (not to scale). Arrows with a red cross, epigenetically silenced p97 promoters. (**D**) Episomal genomes can be found in some cervical cancer cells. In this case, the P97 promoter is activated epigenetically leading to increased levels of viral oncogene mRNAs (green wavy lines). Other viral proteins are also expressed (not indicated) [200].

HPV31 E1 and E2 gene regions. The subsequent loss of these viral replication factors results in a reduction in viral genome amplification and late gene expression [143].

The late phase of the viral replication cycle

The late phase of the viral life cycle involves vegetative viral DNA replication and subsequently, virion formation. Increased expression of the viral E1 and E2 proteins is required to accomplish this phase. The late stage of the life cycle is marked by activation (due to changes in cell signaling) of the viral major late promoter (HPV16: P670; HPV18: P811) that is situated in the *E7* gene region (Figures 2A and 4A) [144,145]. This results in increased expression, not only of E1 and E2, but also of E4 and E5 [13]. The E8 promoter remains active at this stage and E8^ E2 can still repress viral genome amplification [81]. Late stage DNA replication, probably using a rolling circle mechanism, yields many thousands of progeny viral genomes [146]. HR-HPV E4 proteins are the most abundant viral regulatory factors and play essential roles in the differentiated keratinocytes which support viral genome amplification [97] and late events

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in the life cycle [147-149]. In contrast, the low risk HPV11 E4 has been found not to be essential for viral genome amplification [150].

The roles of the E4 and E5 proteins

HPV16 and 18 E4 proteins can associate with and stabilize, E2 suggesting the possibility of a regulatory loop centering on viral replication and transcription [151]. E4 can also enhance genome amplification directly by regulating cell cycle arrest in G_2-phase [152,153] and activating cellular kinases [154]. E5 is a transmembrane ER-resident protein [155] that can stabilize EGFR and stimulate MAPK (mitogen-activated protein kinase) activity, suggesting that it can control cell division pathways [156]. The fact that HPV genomes containing inactivating mutations in the E5 ORF have lower levels of viral genome amplification than wild-type may also be related to E5 interaction with cellular signaling [157,158]. E4's ability to arrest cells in G_2-phase could counteract the effect of E7 stimulation of G_1 to S-phase transition [147,152,159].

E4 and E5 have additional roles in the HPV life cycle. HPV1 E4 may control RNA processing in the infected cell through the SRPK1 (SR protein kinase 1) [160]; while HPV16 E4 can bind a DEAD box RNA helicase that could contribute to post-transcriptional control of viral gene expression [161]. In the context of natural levels of expression from intact HPV genomes, the most important role for E4 late in the virus replication cycle may be to restructure cytokeratin filaments to render cells fragile and more liable to release progeny virions [162-164]. E5 helps the virus evade the immune response by repression of MHC presentation of viral peptides [165]. Apart from EGFR and MAPK signaling, other signaling affected by E5 includes ERK (extracellular signal regulated kinase) 1/2, AKT, and p38 pathways and through these E5 can regulate apoptosis [155]. Finally, HPV16 E5 is capable of forming a viroporin that could have ion channel activity in infected cells and this may be linked to E5's enhancement of EGFR activity [166]. However, further work is required to elucidate this potentially very important function of E5.

A major cellular pathway crucial for completion of genome amplification and viral late gene expression is the DNA damage response pathway. ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related) kinases are the key sensors of DNA damage and regulators of damage repair [167]. Both kinases play a key role in HR HPV replication by helping to recruit cellular DNA replication and repair factors [168]. In the early phase of the life cycle, ATM may be necessary for establishment and maintenance of HPV genomes in basal epithelial cell via E1-mediated activation [169]. In addition, E1 and E2 together create viral replication factories that contain a wide range of DNA damage repair proteins and this is likely to facilitate viral genome replication [170]. In the late phase, E7 has been shown to activate the ATM DNA damage pathway required for vegetative viral genome amplification in differentiated keratinocytes [171]. E7 seems to control stability of DNA repair proteins through its Rb-binding domain, but further work is required to elucidate the mechanism [172]. In addition, E1 can activate the DNA damage response that results in recruitment of ATM/ATR to viral replication centers [173-175]. Less is known about HPV control of ATR however, E7, in stimulating cell cycle activation in differentiating keratinocytes, may indirectly activate ATR through replication stress [169].

Following viral genome amplification and capsid protein synthesis, virion formation takes place in the nucleus. At least for some HPVs (e.g. HPV16 and 31), capsid protein expression is delayed until infected cells reach the granular layer [97]. This delay in expression of the highly immunogenic capsid proteins the uppermost epithelial layer may allow the virus to evade the host immune response [176]. However, the ability of other HPVs to yield a productive infection despite expression of their capsid proteins closer to the basal epithelial layer [86] suggests that other means to avoid the host immune response must exist. The capsid proteins are expressed from mRNAs that initiate at the late promoter (P670 for HPV16, P811 for HPV18) that becomes activated in cells supporting viral genome amplification [13]. Therefore, restriction of expression of the capsid proteins to differentiated granular layer keratinocytes is probably not controlled at the transcriptional level. Various post-transcriptional mechanisms have been described that may contribute to delayed capsid protein expression, including alternative splicing and polyadenylation, mRNA stability, and control of translation [13,177]. There are a number of cellular RNA-binding proteins that could control capsid protein expression through binding a regulatory element in the late 3' untranslated region, including the mRNA stability regulator HuR [178], hnRNP A1 [179], splicing factor 1 (SRSF1 (SF2/ASF)) [180], and U1snRNP (U1 small nuclear ribonucleoprotein) [181], all of which show increased expression upon keratinocyte differentiation [182]. Understanding the link between cellular differentiation and capsid protein expression could indicate strategies to disrupt it because unscheduled expression of the capsid proteins in the lower epithelial layers might allow activation of a robust antiviral immune response.

L2 is synthesized prior to L1 and is imported into the nucleus [183]. L1 proteins self-assemble into pentameric capsomeres in the cytoplasm and these structures are transported into the nucleus [184]. Post-synthesis, viral genomes

locate to PML through E2 bound to its cognate sites in the LCR [185]. Thus viral genomes are located adjacent to viral capsid proteins ready for assembly. L1 and L2 interact and L2 is probably incorporated into assembling virus particles at the centers of the capsomeres, although the L1/L2 interface is poorly defined as yet [186,187]. L2 seems to be required for efficient DNA packaging and virion assembly [188]. Fully formed virions are released inside dead squames that are shed from the epithelial surface. Free virions can survive for some time in the environment and normally reinfect cells at sites adjacent to where they are shed [163].

Apart from the proteins mentioned above, there are potentially other viral proteins expressed during the life cycle. For example, the *E6E7* gene region of the HPV16 genome gives rise to at least four alternatively spliced mRNAs (E6 full length (E6fl), E6*I E6*II, E6*III (also called E6*X)) that have been detected in HPV-positive patient samples [189]. E6*I protein, which represents the N-terminal portion of E6, is significantly more abundant than E6fl, at least at the mRNA level. E6*I has antiapoptotic properties and may regulate E6 itself [190]. In addition, E6*I may cause oxidative stress and DNA damage, processes that could be important for the infectious viral life cycle but also for HPV-associated tumor progression [191]. Proteins encoded by the other spliced isoforms have not yet been investigated.

Viral proteins interact with each other

Elucidating the role of the various viral proteins in viral replication is complicated by several facts: (i) protein multifunctionality, (ii) most viral proteins have been shown to bind to one another, (iii) changes in expression profiles of these proteins during epithelial differentiation, and (iv) subcellular compartmentalization. As discussed above, E1 and E2 interact at the viral origin of replication to initiate viral replication [77]. However, E2 has also been shown to bind L1, L2, E6, E6*I, E7, and E4. E2 binding to L2 appears to have a role during establishment of infection in basal epithelial cells [185] where it localizes with L2 to ND10 domains in infected cell nuclei. Moreover, L2 can inhibit E2 transcriptional transactivation but not E2-mediated viral DNA replication [192]. Thus, it is tempting to speculate that L2 could act as an early switch between activation of viral early transcription and the limited viral genome replication that occurs in newly infected cells. E2 is capable of recruiting E7 to mitotic chromosomes in late mitosis [193]. It has been suggested that E2–E7 oligomers might result in inhibition of E2 repression of E6 and E7 expression to contribute to HR-HPV-associated tumor progression [194]. The interaction between E2 and E6 isoforms changes the subnuclear localization of the proteins, which has potential to alter their cellular functions [195]. Similarly, upon binding to E4, E2 undergoes cytoplasmic relocation and stabilization [151] but any role for cytoplasmic E2 has yet to be explored. E2 and L1 associate at PML nuclear bodies during virion assembly [185]. It is likely that there are more functions to be discovered for each protein and co-operative complexes of viral proteins may have additional functions to those of the individual proteins.

HPVs can induce tumorigenesis

Although there is clear evidence that HR-HPVs have a causative association with a number of cancers, the progression of an HPV infection to an HPV-associated cancer is a rare event [3]. Most importantly, in terms of viral replication, cancer progression is an unproductive or 'dead-end' event for the virus. The primary cause of progression to cancer seems to be persistent infection, over a period of several years, of basal and stem epithelial cells with at least one of the HR-HPVs [75]. However, although persistent infection is a necessary event, it may not be sufficient to drive full tumorigenesis. For cervical cancer, HR-HPV infection of the region between the ecto- and endocervix may be more prone to result in a persistent infection because HPV productive replication cannot be properly controlled in this single cell basal layer [48]. Given that most people become infected with HPV as young adults, the normal decades-long time for progression to cancer, and the rarity of this event, indicates that other secondary events can co-operate with persistent HR-HPV infection to lead to cancer formation. The main risk factors for cervical cancer have been identified as immune status (immunocompromised, immunosuppressed), smoking, oral combined contraceptive use, high parity, and other sexually transmitted infections, particularly *Chlamydia* [87,196,197]. HIV patients have a five-fold greater risk of developing HPV-associated cervical cancer [198] probably due to increased likelihood of acquiring a persistent HPV infection. More recently, there is evidence that the vaginal microbiome can have either a protective or stimulatory effect on cervical disease progression [199]. This could be due to the specific composition of any bacterial population that might exert different effects on anogenital HPV persistence. Persistent infection may equal an elongated maintenance phase of viral genome replication in the undifferentiated epithelial cell(s) initially targetted for infection [200]. Whatever anatomical site is infected, the key change required for progression to malignancy is increased expression of the viral oncoproteins E6 and E7 in dividing, infected cells.

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Increased E6 and E7 activity can stimulate cell growth, inhibit differentiation, and induce chromosomal instability that will result in tumorigenesis. In most cases (estimated to be between 70 and 85% of cases), changes in oncoprotein expression results from integration of the HR-HPV genome into the host genome (Figure 4) [201]. Integration is not a part of the normal HPV replication cycle. This is an 'abortive' infection where integration of a single viral genome copy results in concomitant deletion of parts of the viral genome but retention of the early P97 promoter and E6 and E7 coding regions (Figure 4 B), or integration of multiple viral genomes most of which become epigenetically silenced (Figure 4C) [75]. A common theme of either event is that the viral E2 transcription factor is no longer expressed and thus cannot repress the P97 promoter to restrict E6 and E7 expression [75]. The primary role of loss of E2-mediated repression of HR-HPV oncogene expression from integrated viral genomes was demonstrated by experiments where E2 overexpression in cervical tumor cells such as HeLa cells resulted in reversion of the tumor phenotype [202,203]. Recently, it was demonstrated that integration could result in formation of a Brd4-controlled 'super enhancer' that could cause increased viral oncoprotein expression [204]. However, E6/E7 overexpression may also be achieved by increased oncoprotein mRNA stability due to integrant genomes being transcribed as a part of a cellular ORF where the resulting mRNA is more stable, and therefore, more efficiently translated [205]. Moreover, acquisition of resistance to cytokine signaling can lead to cellular transcription factors such as the AP1 complex further activating viral transcription [206].

The pathway to viral integration during persistence is still unclear [200]. Moreover, because approximately 15% of cervical cancers harbor episomal HPV genomes, it is considered possible that tumor progression can result from misregulation of episomal HPV genomes [200]. In this case, E2 expression is retained but E6 and E7 expression are increased, possibly through epigenetic and/or chromatin conformation changes around the P97 promoter. On the other hand, experiments in the HPV16-positive W12 tumorigenesis model have indicated that although latent integrants may coexist with the normal complement of episomal genomes in the initially infected cell, selection of cells containing transcriptionally active integrants must be accompanied by loss of episomes that express the E2 transcriptional repressor [207]. Integration could be an indirect result of tethering viral episomes adjacent to fragile site regions of chromosomes that accumulate the DNA break repair factors essential for viral DNA amplification [208]. Alternatively, the E6*I protein has been shown to induce oxidative stress and DNA damage. Therefore, its expression in persistently infected cells could predispose to genome damage and integration [191]. However, a timeline for these events during persistence could be different for every tumor and difficult to work out.

Although continued expression of the E6 and E7 oncoproteins is required to maintain the transformed phenotype, it is not sufficient for cellular transformation. Expression of E7, but not E6, can immortalize keratinocytes. However, expression of both oncoproteins provide co-operative activity that can readily immortalize cells [75]. HR-HPV E6 proteins activate telomerase reverse transcriptase (TERT) and telomerase. These enzymes are essential for maintaining the lengths of telomeres at the end of chromosomes that is necessary for immortalization of cells [75,209]. Activation of telomerase and maintenance of the lengths of telomeres is a hallmark of cancer cells. Therefore, it has been proposed that this function of E6 has a key role in development of high-grade lesions and progression to cancer. HPV16 E6-mediated loss of p53 allows mutations to accumulate in cells in which apoptosis is inhibited [92]. In addition, HPV16 E6, HPV8 E6, and HPV1 E6 can all bind and inhibit the DNA repair protein XRCC1 to prevent repair of single-stranded breaks allowing accumulation of mutations in the host genome. This function of E6 may also release cellular DNA polymerase β and promote DNA replication [210].

There is a strong evidence that E7 can cause errors in centrosome duplication, perhaps through misregulation of cyclin E/cyclin-dependent kinase 2 (CDK2) complexes, leading to overduplication and genomic instability [211]. High and low risk E7 proteins interact with NuMA (nuclear mitotic apparatus protein 1) causing its delocalization and resulting in mitotic errors [212]. HPV16 E7 can bind and affect γ-tubulin recruitment to centrosomes [213], a process regulated by dynein, also relocalized by E7 [214]. Experimental evidence indicates that even prior to cancer progression E6 and E7 can co-operatively induce centrosome abnormalities as an early event in tumor progression [215]. E6 and E7 may also be able to bypass G_2-M checkpoints that would otherwise signal cells with accumulated mutated chromosomes for destruction [216].

The increased levels of viral oncoproteins found in HPV-positive tumor cells may cause sustained activation or inhibition of growth control pathways. For example, the E6-mediated stimulation of growth-related (for example EGF and MAPK) signaling pathways [127] would abrogate cellular growth control allowing cell transformation and tumor invasion to occur [217]. Moreover, E6 control of PDZ proteins, tumor suppressors that control cell shape and polarity and integrate cellular signaling [218], has been shown to play a major role in tumor formation [126]. For example, the staining pattern of hDlg, the human homolog of the *Drosophila* Dlg tumor suppressor in transformed cells is very different from that of non-transformed cells [219,220]. In normal epithelial cells, hDlg is found predominantly at tight

junction plaques between the neighboring cells, however in HR-HPV E6-transformed cells hDlg staining is predominantly cytoplasmic suggesting a loss of cell–cell communication between neighboring transformed cells [219,220]. As E6 has been shown to bind to PDZ domain containing proteins, it is thought that this protein is responsible for redistribution of hDlg during transformation [221,222]. However, E6 may misregulate many others such PDZ proteins to contribute to tumorigenesis [126]

E7 interacts with a wide range of transcription regulators and can repress or activate expression of cell cycle control proteins such as cyclin kinase inhibitors p21 and p27 and cyclins A and E [75]. Furthermore, E7 binds E2F6, a member of the polycomb repressor complex that normally associates with chromatin, and prevents its normal repressive functions on E2F-activated S-phase genes [223]. E7 also can allow anchorage-independent growth and cellular transformation by binding p600, a retinoblastoma-associated protein [224]. All these interactions and regulatory pathways are important for the normal infectious life cycle of HR-HPVs. However, when misregulated due to increased E6/E7 expression these signaling and cellular regulation pathways can contribute to tumor progression. E7 can also promote telomere maintenance, thus supporting the function of E6 in this regard [225]. Both oncoproteins can regulate the cellular miRNA profile and increased oncogenic, and in particular, decreased tumor suppressive miRNAs have been found in cervical tumors [226]

Finally, the role of HR-HPV E5 in the transformation process should be taken into account. Although there is convincing evidence of the role of E5 in the transformation process during infection with bovine papillomaviruses [227], HR-HPV E5 has only weak transforming activity and therefore its role during the transformation process is more obscure [155]. Following viral genome integration into host chromosomes, E5 is usually no longer expressed. However, E5 has many documented activities that could contribute to the transformation process and it appears to augment the functions of E6 and E7 in tumor progression [228-230]. E5 controls cellular signaling in keratinocytes. It binds to the 16K subunit c of the proton pump vacuolar ATPase and reduces endosomal acidification [231,232]. This leads to the activation of the EGFR which stimulates proliferation of basal layer epithelial cells [233]. At the same time, E5 can interact with keratinocyte growth factor receptor signaling to inhibit autophagy [234] and cause a decrease in proliferation and differentiation of suprabasal keratinocytes [235]. Overall this would result in a significant increase in the proliferative compartment of the epithelium, which may be an early event in tumor progression. Indeed, E5 expression in transgenic mice leads to changes in growth and differentiation of keratinocytes and induces epithelial tumors in an EGFR-dependent fashion [236] and evidence suggests that E5 could contribute to the promotion and progression stages of tumorigenesis [237]. Other signaling pathways such as MAPK and PI3 kinase are also activated [156,238].

Conclusion

HPV infection and its relationship to HPV-associated tumor progression has been the subject of intense research activity for over 50 years. There are still significant gaps in our understanding of both strands of HPV pathogenesis. E6 and E7 clearly make a very significant contribution to tumor progression. Tight regulation by E2 of expression levels of these oncoproteins in the dividing cells of an infected epithelium is the key to avoid tumor formation. Dampening down amplification levels of viral genomes during a normal infectious cycle via the E8^ E2 protein may also help to reduce the chances of elevated levels of E6 and E7 in differentiated keratinocytes. The fine balance between the activities of E6, E7, E2, E4, and E5 on the cell cycle and apoptosis to allow viral genome amplification, while avoiding viral genome integration into the host genome, is crucial for the virus to complete its infectious life cycle. There are still many fascinating aspects of the HPV life cycle to be elucidated, and our knowledge of the life cycle of HPVs other than the more common HR types is still limited. Understanding HPV-associated cancer progression in sites other than the cervix will be key to future development of interventions to alleviate HPV-associated diseases.

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Competing interests

The author declares that there are no competing interests associated with the manuscript.

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Abbreviations

Akt, Protein kinase B; AP1, Activator protein 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; Brd4, Bromodomain-containing protein 4; B-Myb, Myeloblastosis oncoprotein B; CBP, CREB binding protein; CIN, cervical intraepithelial neoplasia; C-Jun, Cellular JNK transcription factor; C-Fos, Cellular Finkel-Biskis-Jinkins murine osteosarcoma virus oncogene; ChlR1, Chromatin loss related 1; C-Myc, Cellular myelocytomatosis oncogene; DEAD, amino acids D-E-A-D-containing; △p63, Delta phosphorylated 63 protein; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EM, Electron microscopy; ER, endoplasmic reticulum; ERK, extracellular signal regulated kinase; EV, epidermodysplasia verruciformis; E6AP, E6-associated protein; hDlg, homolog of *Drosophila* Discs Large; hnRNP, heterongenous nuclear ribonucleoprotein; HPV, human papillomavirus; HPV16, HR-HPV genotype 16; HR, high risk; HR-HPV, high risk HPV; HSIL, high-grade squamous intraepithelial lesion; HSPG, heparin sulphate proteoglycan; HuR, Human antigen R; IFN, interferon; LCR, long control region; LSIL, low-grade squamous intraepithelial lesion; NCoR, Nuclear receptor corepressor 2; MAGI 1, Membrane-associated guanylate kinase 1; MAML, Mastermind-like proteins 1; MAPK, mitogen-activated protein kinase; Miz 1, Mizu 1; MKlp2, Mitotic kinesin-like proteins 2; mTOR, Mechanistic target of rapamycin; ND10, Nuclear domain 1; NF-kB, Nuclear factor kB; Pap, Papanikolaou; PBM, PDZ-binding motif; PC5/6, Proprotein convertase 5/6; PDZ, PSD95/hDlg/ZO-1; PI3, Phosphoinositide 3; PML, promyelocytic leukaemia; pRb, Phosphorylated retinoblastoma protein; SIL, squamous intraepithelial lesion; SMAD2/3, Similar to mothers against decapentaplegic 2/3; SMRT, Silencing mediator of retinoic acid and thyroid hormone receptor; SNX27, sorting nexin 27; SRSF1 (SF2/ASF), Serine arginine-rich splicing factor; SR, Serine arginine-rich; STAT1, Signal transducer and activator of transcription 1; TBP, TAT binding protein; TLR9, toll-like receptor 9; TopBP1, Topoisomerase binding protein 1; URR, upstream regulatory region.

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