Molecular Mechanisms of Human Papillomavirus-Induced Carcinogenesis

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Abstract

Approximately 20% of all cancers are associated with infectious agents. Among them, human papillomaviruses (HPVs) are very common and are now recognized as the etiological agent of cervical cancer, the second most common cancer in women worldwide, and they are increasingly linked with other forms of dysplasia. Carcinogenesis is a complex and multistep process requiring the acquisition of several genetic and/or epigenetic alterations. HPV-induced neoplasia, however, is in part mediated by the intrinsic functions of the viral proteins. In order to replicate its genome, HPV modulates the cell cycle, while deploying mechanisms to escape the host immune response, cellular senescence and apoptosis. As such, HPV infection leads directly and indirectly to genomic instability, further favouring transforming genetic events and progression to malignancy. This review aims to summarize our current understanding of the molecular mechanisms exploited by HPV to induce neoplasia, with an emphasis on the role of the 2 viral oncoproteins E6 and E7. Greater understanding of the role of HPV proteins in these processes will ultimately aid in the development of antiviral therapies, as well as unravel general mechanisms of oncogenesis.

Keywords

Human papillomavirus; Cervical cancer; Genomic instability; Oncogenesis; Viral oncogenes

Introduction

The notion that DNA tumor virus infections can trigger carcinogenesis originated many decades ago while examining malignant progression in domestic rabbits inoculated with wart-inducing viruses [1, 2]. These oncogenic viral particles were later described as cottontail rabbit papillomaviruses and their human counterparts, human papillomaviruses (HPVs), were subsequently identified in human genital warts [3] and cervical biopsies [4]. Since then, infectious agents have been increasingly associated with cancer development, accounting for approximately 20% of cancers worldwide, with HPV responsible for nearly one third of them [5, 6].

The family of papillomaviruses comprises a plethora of different types that infect almost every vertebrate species, while showing a high species specificity [7, 8]. Over 100 HPV types have been identified that infect either cutaneous or mucosal tissues [7], and among them, about 30–40 types infect the genital tract [9] and are presently recognized as the most common sexually transmitted infectious agents [10, 11]. HPVs are commonly found in warts and anogenital dysplasias, although infections frequently remain subclinical. Anogenital HPVs are categorized as ‘low-risk’ or ‘high-risk’ types, according to their association with benign or pre-cancerous and cancerous lesions, respectively. The oncogenic potential of high-risk HPV types was initially demonstrated by Harald zur Hausen, who was recently awarded the 2008 Nobel Prize in Physiology or Medicine for this discovery [12]. High-risk HPVs are now recognized as the etiological agents of cervical cancer [13]. The burden of HPV-associated malignancies worldwide is enormous with 493,000 new cases of cervical cancer diagnosed each year, making it the second most common cancer in women [14, 15]. The incidence in developed countries has drastically declined with the implementation of *Papanicolaou* screening programs, such that approximately 80% of all new cases now occur in developing countries, where cervical cancer prevention programs are not available. Nevertheless, 11,070 new cervical cancer cases were diagnosed in the United States in 2008, with a mortality rate of nearly 35% [16]. HPVs 16 and 18 account for approximately 70% of all these cancers [17]. High-risk HPV types, in particular HPV-16, have also been identified in anal and other genital neoplasia and are associated with a subset of head and neck cancers [18–21]. This review will focus on the molecular events triggered by HPV infections that contribute to carcinogenesis.

Epidemiology and Clinical Aspects

Mucosal HPV types can infect epithelia of the anogenital and upper aerodigestive tract. Although HPV infections are very common, most remain asymptomatic and are cleared within 6–12 months through an effective immune response [22]. Nonetheless, a small subset of infections ultimately results in neoplasia. The link between HPV infections and cervical cancer is now strongly established, such that HPV is detected in 99.7% of cases [23] and in the vast majority of high-grade neoplasia [24]. HPV infections have also been associated with anal carcinogenesis and HPV is found in 80% of anal cancers, with HPV16 accounting for the majority of cases [25–27]. The occurrence of anal cancers has steadily increased since the 1980s, particularly in the HIV-positive population of men who have sex with men [16, 27, 28]. This has led to the suggestion that screening programs should be implemented within this population and other ones at a higher risk of developing anal dysplasia. Aside from their high susceptibility to anogenital cancers, HIV-positive and other immunosuppressed patients are at high risk of developing non-melanoma skin cancers when infected with HPV, especially at sun-exposed sites [29, 30]. Susceptibility to HPV-induced non-melanoma skin cancers upon UV exposure is also observed in the rare inherited disorder epidermodysplasia verruciformis [31]. Furthermore, HPV has been linked to other types of genital neoplasia, including vulvovaginal and penile cancers [27], and accumulating evidence suggests that HPV is associated with a subset of head and neck cancers, and in particular those of the tonsils. With the use of more sensitive viral DNA detection technologies, recent case-control studies proposed that HPV infection was causally

associated with these cancers and was a risk factor independent from those previously reported, such as cigarette and alcohol consumption [18, 20, 21]. A systematic review of 60 different studies also confirmed a significantly higher prevalence of HPVs in these types of squamous-cell carcinomas [19].

Many factors contribute to the risk of HPV carcinogenesis including pregnancy, high parity, smoking, oral contraceptive use, other sexually transmitted diseases and the number of sexual partners [13]. Increasing evidence suggests that immunosuppressed patients also have a higher risk of carcinoma development and a higher prevalence of multiple HPV infections, as exemplified within the HIV-infected population and in organ transplant recipients [29, 32, 33]. Thus, the medical burden associated with HPV infections is substantial and may become even larger as these viruses are being linked to other malignancies.

Overview of the Viral Life Cycle

The molecular biology of HPV during its normal life cycle has been extensively reviewed [17, 34, 35] and thus is only briefly summarized below. To initially infect dividing cells of the stratified epithelium, HPV virions are believed to reach the basal layer of the skin through microlesions. Mechanisms allowing entry from the extracellular milieu into the cell are poorly understood, but are known to proceed through interaction with cell surface heparan sulphate followed by clathrin- or caveola-mediated endocytosis [36, 37]. Then, translocation of the viral DNA into the nucleus involves the L2 capsid protein and is dependent on its interaction with microtubules [35, 38]. The viral genome consists of approximately 8 kb of circular double-stranded DNA and 8 well-defined open reading frames that are encoded in the early region (E1, E2, E4, E5, E6 and E7) and in the late region (L1 and L2). The genome also contains a third non-coding region, termed the long control region, that is critical for transcription of the viral genes, the initiation of viral DNA replication, and the segregation of the viral genome in mitosis (reviewed in [35]).

HPV relies extensively on host proteins for replication and maintenance of its genome. During a productive infection, the viral genome is maintained at a low copy number as an extrachromosomal element known as episome in the basal undifferentiated cells of the epithelium. Thereafter, the viral life cycle is tightly coupled to the differentiation program of keratinocytes and relies on several cellular factors and viral proteins. Normal epithelial cells undergo terminal differentiation as they migrate towards the upper layers, a condition that would be non-permissive for HPV replication since the virus depends on the host DNA replication machinery for replication of its genome. To maintain the cellular replication machinery active, the viral proteins E6 and E7 are expressed and uncouple cell growth arrest and differentiation primarily through the inactivation of p53 and pRb, respectively. At the core of these events are the facts that inactivation of pRb by E7 forces infected cells to remain in a proliferative state and escape cell cycle exit, while abrogation of p53 by E6 ensures cell survival by preventing apoptosis triggered by this aberrant growth signal. Viral genome replication requires the viral initiator protein E1, which contains a helicase-ATPase activity, and the multifunctional viral protein E2, which helps in the specific recruitment of E1 to the viral DNA. E1 oligomerizes and assembles as a double-hexamer at the viral origin of DNA replication and functionally interacts with several host replication factors, such as

polymerase α -primase, replication protein A, topoisomerase I and cyclin E/Cdk2 [35, 39]. E2 also functions as a transcription factor, capable of trans-activation and repression [40, 41], and as a mediator of genome segregation, which is essential for viral persistence [42, 43]. As the infected cells undergo differentiation, late gene expression and viral genome replication are induced (fig. 1). The amplified genomes are then packaged into infectious virions by the L1 and L2 proteins, which form the subunits of the icosahedral capsid. Finally, viral egress probably occurs by natural tissue desquamation and may be facilitated by the keratin network disrupting ability of E4 [34]. E4 and E5 are both required for viral amplification, although their exact functions in this process are not clearly defined [44–46].

Viral Genome Integration and Oncoprotein Expression

The preceding section described the molecular events involved in the normal life cycle of HPV, during which the viral genome is always maintained in episomal form. However, in the majority of high-grade cervical lesions and cancers, the viral DNA is not found as an extrachromosomal element but rather is integrated into the host genomic DNA [34, 35, 47]. This rare and aberrant integration event greatly facilitates tumorigenesis and appears to seldom involve disruption of cellular transcribed sequences [48, 49], although it has been suggested to occasionally affect certain tumor suppressor genes [50–53]. HPV integration contributes to oncogenesis mainly when it involves disruption of the E2 open reading frame [54]. Aside from its essential role in viral replication, E2 also functions as a transcriptional repressor of the viral oncogenes, E6 and E7. As such, HPV integration events that disrupt E2 expression result in increased expression of the viral oncoproteins which, in turn, promotes cellular immortalization and transformation [55, 56]. The expression of E6 and E7 alone has been shown to immortalize cultured human foreskin keratinocytes in a cooperative manner [57, 58]. Moreover, continued expression of E6 and E7 is essential to maintain cervical carcinoma cells in a transformed state, as illustrated by the fact that restoring E2 expression in cervical cancer cell lines represses E6 and E7 expression and induces cell cycle arrest and eventually senescence [59, 60]. Both E6 and E7 are tumorigenic when expressed separately or together in transgenic mice, but induce distinct malignancies [61, 62]. This can be explained by their different mechanism of transformation emphasizing their cooperative function in tumor development.

The oncogenic potential of HPV E7 was first demonstrated by its ability to immortalize rodent fibroblasts [63, 64]. Subsequently, development of high-grade dysplasias was observed in transgenic mice expressing E7 [61, 65]. E7 consists of approximately 100 amino acids and contains regions of high-sequence homology to adenovirus E1A and SV40 large T antigen [66]. These domains are known to bind to the family of cellular ‘pocket proteins’ including pRb, p107 and p130 (fig. 2a) [35, 67]. Through these interactions, the viral oncogene E7 functions primarily to modulate the proliferation status of infected cells. Normal cells use the pRb family members to regulate the G1/S transition by constitutively sequestering the E2F family of transcription factors. For normal S-phase entry, pRb hyperphosphorylation is induced by the cell cycle regulators cyclin-dependent kinases (Cdk). This leads to the activation of E2F and the subsequent trans-activation of its target genes, which is essential for the expression of many proteins involved in S-phase, such as those needed for DNA replication. In HPV-infected cells, activation of the E2F transcription

factors is brought about by E7, which directly binds to pRb (reviewed in [35]). In high-risk HPVs, this interaction results in the proteasome-dependent degradation of pRb, possibly through the recruitment of an E3-ubiquitin ligase that has yet to be identified [68, 69]. Some studies reported that the cullin 2 ubiquitin ligase complex is implicated in HPV-16 E7-mediated pRb destabilization, but the conservation of this mechanism among HPV types is uncertain [70]. E7 induction of cellular proliferation is a critical step for tumorigenesis and depends on its capacity to bind and destabilize pRb [71, 72].

Deregulated and sustained cellular proliferation signals often trigger cell growth arrest and apoptosis. This is also observed in E7-expressing cells, which undergo apoptosis in a p53-dependent manner [72]. This is overcome by the expression of E6, the second viral oncoprotein, which specifically binds to and inactivates p53, preventing activation of its anti-proliferative and pro-apoptotic target genes. High-risk E6 associates with the cellular E3 ubiquitin ligase E6-associated protein (E6-AP), the prototypical member of the HECT family (homologous to E6-AP C-terminus), to form a ternary complex with p53 [73]. E6-AP does not regulate p53 stability in non-infected cells [74], but, when in complex with E6, it strongly induces p53 poly-ubiquitination and its subsequent proteasomal degradation [75]. In vitro, E6 overexpression alone is sufficient to immortalize mammary epithelial cells [76], a process that requires E6-AP association and down-regulation of p53 [77, 78]. Nevertheless, even when the proteasome is inhibited, E6 still retains some p53-inhibitory functions, possibly by preventing its nuclear translocation [79] and/or by inhibiting its transcriptional coactivator p300/CBP [80, 81]. Of interest is that the immortalizing potential of E6 is exclusive to high-risk HPV types, suggesting that intrinsic differences between proteins of low and high risk, as well as distinct features of high-risk E6, explain its oncogenic properties. It is known that E6 from different HPV types bind and inhibit p53 with variable potencies, and that only high-risk E6 is capable of mediating p53 degradation [82]. Interestingly, low-risk E6 proteins, such as the one from HPV-11, can associate with E6-AP, although they do not induce degradation of p53 [83]. This raises the possibility that low-risk E6 can promote the degradation of cellular substrates not yet identified. Another feature unique to the high-risk HPV types is a PDZ-binding domain at the C-terminal end [x(T/S)x(V/L)], which enables E6 to interact with a subset of PDZ domain-containing proteins including hDlg, hScribb, MUPP-1, PATJ, TIP-2/GIPC, PTPN13 and MAGIs 1, 2 and 3, to promote their proteasomal degradation (fig. 2b) [84–89]. E6 activities have also been investigated in transgenic mice models expressing wild-type or mutant forms of E6 [90]. These studies demonstrated that the E6 region implicated in p53 inactivation and the C-terminal PDZ-binding domain independently contribute to oncogenesis. Interestingly, splicing variants of E6 that retain the ability to interact with E6-AP but lack the PDZ-binding domain are also expressed in vivo. These variants, globally referred to as E6*, inhibit p53 degradation induced by full-length E6 and thus may serve to negatively regulate the immortalizing activities of E6 [91].

To date, numerous interacting partners of E6 and E7 have been identified, several of which will be discussed in the subsequent sections. These proteins have not all been characterized to the same extent, such that the relevance of some of them to HPV infections and carcinogenesis still remains to be established. Nonetheless, it is clear that E6 and E7 overexpression alone is not sufficient to induce malignancy. For instance, E6 and E7

immortalized human foreskin keratinocytes are not fully transformed and are unable to induce tumors when grafted into nude mice unless the cells are passaged several times to acquire additional genetic changes [92]. The same likely occurs in humans, such that most infections remain benign and transient, with regression occurring in the first 2 years, and persistent infection required for development of neoplasia [11]. Tumorigenesis is a multistep process necessitating the acquisition of several genetic alterations [93]. For example, mutations in the Ras oncogene have been detected in many high-grade and cancerous cervical lesions in humans [94, 95], suggesting that activating mutations in RAS may be one of these genetic modifications cooperating with HPV to promote carcinogenesis. In support of this, activated Ras was shown to cooperate with the 2 HPV oncogenes E6 and E7 in the transformation of rodent primary cells [96, 97]. In normal cells, spontaneous mutations rarely occur, but HPV-infected cells exhibit a high genomic instability which is, in part, directly induced by viral proteins.

Genomic Instability

The low frequency of HPV-induced carcinogenesis and the fact that persistent HPV infection is required for neoplasia to occur underlines the need for additional cellular events for progression to malignancy. Cancer has often been described as a disease of genomic instability, particularly in epithelial cells [98]. Expression of E6 and E7 alone is sufficient to induce genomic instability and the accumulation of secondary mutations [99–101]. By doing so, E6 and E7 may facilitate viral genome integration [49] which in turn would result in their increased expression and further promote instability [102, 103]. This order of events is not perfectly understood. As such, an unintended consequence of integration is that it facilitates progression to malignancy (fig. 3). Through this phenomenon and in response to their environment, transforming cells are clonally selected in a Darwinian manner [93, 104]. Genomic instability is simultaneously caused by the E7-mediated activation of Cdk2 [105] and by the disruption of key cell cycle regulatory events such as the G1/S and mitotic checkpoints [106, 107].

Abrogation of the mitotic checkpoint promotes aneuploidy, a common feature of cancer cells which is characterized by an abnormal number of chromosomes (gain or loss) resulting from a non-symmetrical segregation of chromosomes during mitosis. Many studies reported this phenomenon in cells expressing E7 alone and have suggested that HPV-infected cells induce chromosomal aberrations, even in p53 unaltered cells [101, 108]. Aneuploidy can also be observed in early premalignant lesions and several recent studies have revealed that E7 induces centrosome abnormalities prior to genomic instability [109]. The implication of aneuploidy in tumorigenesis is highly controversial for it is currently unknown whether it is an inducer of transformation or the result of cellular deregulation. While this debate is beyond the scope of this paper, it is clear that HPV induces mitotic defects. Globally, E6 and E7 seem to possess intrinsic functions to induce genomic instability that are both independent and synergistic.

Like many other viral infections, HPV interacts with some DNA repair pathways [110]. It is suspected that DNA damage, as well as DNA virus infection and integration, lead to the activation of common cellular repair mechanisms. Consequently, a virus could benefit from

interacting with the DNA repair machinery, both to inhibit its antiproliferative properties and to exploit some of its intrinsic abilities to mediate specific DNA transactions. This is exemplified by the recently identified relationship between HPV and the Fanconi anemia (FA) pathway. FA is a genetic disease characterized by a high genomic instability, which results in an increased susceptibility to bone marrow failure and cancers, such as squamous cell carcinomas (SCC) [111]. These pathological features are linked to defects in one of the 13 currently known FA genes ensuring genomic integrity [112]. Some studies suggested that squamous cell carcinoma in FA patients is tightly associated with HPV infection, making FA potentially the second inherited disease that increases susceptibility to HPV-induced carcinogenesis, in addition to epidermodysplasia verruciformis [113]. The link between the FA pathway and HPV infections also appear to hold true in non-FA patients, since some FA genes become epigenetically inactivated during cervical carcinogenesis [114]. Epigenetic silencing of tumor suppressor genes by promoter hypermethylation is also a common phenomenon in cervical carcinogenesis, along with mutations, deletions and chromosome translocations [115–117]. During early infection, the FA pathway may provide an early response mechanism to DNA perturbations induced by E6 and E7. Studies have shown that expression of E7 alone is sufficient to activate the FA pathway [118] and to induce expression of the downstream effector FANCD2 in a E2F-dependent manner [119]. Of interest, the FA pathway may regulate genomic integrity at common fragile sites, which are often the sites of HPV integration [118, 120, 121]. Moreover, inactivation of FA genes represents an important molecular step for cancer progression. Many FA genes have been linked to chemotherapy resistance in multiple cancers [122], although whether this occurs in cervical cancer remains to be investigated. Although HPV infection exploits some functions of the DNA repair machinery, it also, by its association with genetic and epigenetic events, represses these pathways, thereby promoting the increased genomic plasticity required for cancer progression.

Limitless and Sustained Growth

Cancerous cells lose their potential to regulate the cell cycle and undergo uncontrolled growth. It is not surprising that HPV contributes directly to cellular transformation by stimulating unrestrained cell division and removing the requirement for mitogenic growth signals. The oncoprotein E7 is the major protein contributing to cell proliferation in HPV infection. Aside from its central role in inactivating pRb and related pocket proteins to promote transcription of E2F target genes, recent research has unravelled some new functions of E7 in cellular transformation. E7 not only indirectly induces Cdk2 expression by inhibiting pRb, but also binds and activates Cdk2 directly while in complex with either cyclin A or E [123]. E7 also counteracts the effect of the Cdk inhibitors p21 [124, 125] and p27 [126] by directly binding to them and inhibiting their function. In addition, since p21 and p27 are target genes of p53, their expression is reduced by E6 in HPV-infected cells. Furthermore, E7 binds and inhibits class I histone deacetylases, whose activity is important for E2F2-mediated transcription and progression through S-phase [127, 128]. In addition to E7, several groups have also implicated E5 as an activator of cellular proliferation. Of particular importance to this process is the ability of E5 to interfere with the degradation and/or trafficking of the epidermal growth factor receptor and which leads to the sustained

activation of epidermal growth factor signalling [129]. However, since E5 expression is commonly lost after integration, its role may be important only in the early stages of carcinogenesis [130, 131].

Normal proliferating cells can only divide a limited number of times, since their lifespan is restricted by telomere shortening that occurs at each cell division. This erosion of telomeres ultimately leads to senescence or cell death and it is thought to be a mechanism used in prevention of unlimited cell growth [132]. During tumorigenesis, the activation of the telomerase enzymatic component hTERT usually occurs to thwart this natural proliferation barrier and promote cellular immortalization. Not surprisingly, telomerase activity is detected in many cancer cells, as well as in numerous cervical lesions and neoplasia [133, 134]. Reactivation of hTERT activity, when combined with pRb inactivation, has been suggested to be an essential step in the process of immortalization [135]. Although reactivation of telomerase activity in cancer cells commonly arises from genetic alterations, HPV has been shown to possess intrinsic mechanisms for that purpose. Activation of hTERT has been observed in HPV16 E6-expressing cells and it is thought to be, at least in part, due to an increase in hTERT transcription brought about by the cooperative binding of E6 and Myc at the hTERT promoter [136]. It has also been shown that hTERT induction is not simply caused by Myc activation, but rather results from a combinatorial effect with other transcription factors such as Sp1 [137]. E6 activation of hTERT was reported to depend on its association with E6-AP, possibly implicating the degradation of a hTERT regulator or transcriptional repressor, with NFX1-91 postulated to be one of these repressors [138–140]. However, not all studies have found a need for E6-AP in hTERT induction [141]. Collectively, these findings indicate that telomerase activation is directly induced by HPV, primarily through a stimulatory effect of E6 on transcription from the hTERT promoter.

Inhibition of p53-Independent Cell Death

Sustained uncontrolled cellular proliferation and accumulation of genomic alterations often trigger apoptosis. As described above, a major function of E6 is to antagonize p53 in order to prevent the p53-dependent apoptosis that results from the action of E7. However, experiments in p53-null mice showed that E6 is also able to inhibit other p53-independent apoptotic pathways, supporting other functions for this protein in viral carcinogenesis (fig. 4) [142]. Specifically, E6 was shown to inhibit Bax [143] and to mediate the E6-AP-dependent degradation of Bak [144] by the proteasome, a mechanism conserved among high- and low-risk HPV types [145]. Furthermore, since Bax and Bak expression is regulated by p53, their levels are lower in E6-expressing cells. Interestingly, Bak is highly expressed in the upper layers of the stratified epithelium, making it a potentially important barrier for HPVs [146]. In the context of viral infection, apoptosis is commonly triggered by extrinsic signalling from the family of death receptors [147]. HPV ensures cell survival by interacting with these signalling pathways, such as the one activated by TNF- α , and possibly TRAIL and Fas [148–151]. Many other potential pro-apoptotic proteins reported to interact with HPV have been described and are reviewed elsewhere [152]. Prevention of apoptosis is also suspected to allow UV-damaged HPV-positive keratinocytes to progress to non-melanoma skin cancer [153]. Overall, HPVs possess numerous mechanisms to circumvent apoptosis, which is essential to allow productive infection that is also persistent.

Tissue Integrity and Invasion

Cellular anchorage to ECM is essential for most normal cells to progress through the cell cycle. Loss of this interaction not only restricts proliferation, but also induces an apoptotic pathway, referred to as anoikis [154]. This cellular mechanism prevents dysplastic growth and is one of the natural barriers to tumorigenesis [155]. Connection between ECM and the cytoskeleton is mediated through numerous proteins, forming complex macromolecular structures called focal adhesions. These protein complexes, which are important to achieve proper tissue architecture and cohesion, possess intrinsic signalling properties to control cell growth and survival. It is not surprising that HPV proteins can interfere with these molecules through several means in order to prevent anoikis and promote tissue invasion by infected cells [84]. Specifically, E6 was found to interact with many focal adhesion molecules, including Paxillin, Zyxin and Fibulin-1, disrupting interaction between the cytoskeleton and the ECM [156–159]. It was also shown recently that E7 interacts directly with the retinoblastoma protein associated factor p600, and hence may facilitate anchorage-independent cell proliferation [160].

E6 also interacts with several cellular PDZ domain-containing proteins involved in cell adhesion, proliferation, and apicobasal polarity, to promote their proteasomal degradation through E6-AP-dependent and -independent mechanisms. The effect of E6 on these proteins, many of which have tumor suppressor properties [161], disrupts normal cell adhesion, contributes to tissue invasion and metastasis, and favours proliferation of supra-basal epithelial cells. Inhibition of PDZ domain-containing proteins is a mechanism highly conserved among high-risk HPV types to induce hyperplasia [88, 89]. An unusual example of this conservation is the finding that Rhesus papillomavirus targets PDZ domain-containing proteins using its E7 protein rather than through E6 [162].

Immune Evasion

Like many viruses, HPV has developed mechanisms to escape immune surveillance. This is of particular significance when considering that cervical carcinogenesis is linked to HPV persistence. One means by which HPV escapes immune detection is built into its natural life cycle. Primary infection occurs in the basal cells of the stratified epithelium where viral genomes are maintained only at very low levels. Viral proteins are also very weakly expressed and confined mainly to the nucleus of basal cells. Increased protein expression only occurs as keratinocytes migrate through the upper layers of the epithelium where the adaptive immune system has limited access. Finally, newly assembled viral particles are released by natural shedding, a process that does not involve cell lysis, thereby preventing dendritic cell activation, pro-inflammatory cytokine liberation, and antigen presentation by Langerhans cells in the proximal layers of the epithelium [163, 164]. HPV has also evolved mechanisms to counteract intracellular antiviral defense pathways. An interferon (IFN) response is usually triggered upon viral infection, which proceeds through IFN- α and IFN- β secretion, both of which harbour anti-proliferative and pro-apoptotic abilities. Interferon immunoregulatory effects were shown to be directly inhibited by HPV, both by reducing interferon expression and by interfering with its signalling pathways (reviewed in [165]). Gene expression profiling studies have shown that HPV-31 downregulates IFN-responsive

genes [166]. Furthermore, E6 is known to physically interact with IRF-3 and inhibit its transactivation ability, and by doing so blocks IFN- β expression [167]. HPV-18 E6 has also been proposed to interact with and impair activation of Tyk2, an important mediator of IFN-receptor signalling [168]. Simultaneously, E7 can interfere with the IFN response by inhibiting IRF-1 and ISGF-3 [169–171]. Another innate intracellular antiviral response is mediated by the double-stranded RNA protein kinase (PKR), an IFN-inducible protein, which is also targeted by HPV [172]. This protein kinase has been shown to be mis-localized in HPV-infected cells and its phosphorylation level reduced in cells expressing E6 and E7, suggesting a modulation of this pathway by HPV. Moreover, E5 has been proposed to interfere with MHC1 mediated antigen presentation by deregulating endosomal acidification and trafficking, and thus, prevents immune recognition [173]. Altogether, these immune evasion mechanisms probably function in concert to facilitate viral persistence, a known risk factor for cancer progression.

Conclusion

The role of HPV infection in carcinogenesis is now well established and the molecular events triggered by viral proteins are becoming better characterized. It is important to realize, however, that the implication of HPV infection in oncogenesis may be an indirect consequence of keeping infected cells in a proliferative state. To ensure replication and maintenance of their genome, HPVs have acquired several mechanisms to usurp the host DNA synthesis machinery, while escaping the host intracellular and immune defenses. Moreover, viral proteins of high-risk HPV types contain distinct features that promote cell growth, despite cellular attempts to initiate apoptosis in response to uncontrolled proliferation and/or disruption of cellular adhesion, which represent natural mechanisms to prevent dysplasia. In some cases, infection becomes suboptimal and results in viral genome integration. This in turn amplifies genomic instability and the acquisition of additional genetic and epigenetic changes needed for cancer progression. Prophylactic vaccines have recently been developed and approved for preventing infection by the 2 most common oncogenic types (HPVs 16 and 18), and those causing most cases of benign genital warts (HPVs 6 and 11). Unfortunately, and despite substantial efforts made towards the discovery of small molecule inhibitors of HPV (reviewed in [39]), antiviral drugs to treat already infected patients are still lacking. It is hoped that a greater understanding of the molecular events involved in viral pathogenesis will ultimately contribute to the development of novel antiviral therapies, as well as serve as a model to understand carcinogenesis in general.

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Fig. 1.

Schematic representation of the HPV life cycle in the context of a differentiating epithelium. Sections of normal uninfected (left) and HPV-infected epithelia (right) are represented in this illustration. The HPV life cycle is dependent on the differentiation program that keratinocytes undergo within a stratified epithelium. HPV virions infect dividing keratinocytes of the basal cell layer where they establish and maintain their genome as a low copy number episome in the nucleus of these cells. Although un-infected cells ultimately differentiate and lose their nuclei, expression of the viral oncogenes E6 and E7 prevents terminal differentiation of infected keratinocytes and keeps them in a proliferative state needed for viral DNA synthesis. As the cells migrate towards the upper layers of the epithelium, the viral genome is amplified and late gene expression is induced. Synthesis of the 2 capsid proteins allows for the viral DNA to be packaged into mature virions, which are then released from the top portion of the epithelium through natural shedding. The different cell layers of the epithelium and the viral events occurring within them are indicated on the left and right side of the figure, respectively.

Fig. 2.

Schematic representation of the HPV oncoproteins E6 and E7. **a** E7 harbours 1 zinc finger (Zn) in its C-terminal domain and 2 amino-terminal conserved regions. Conserved region 2 contains the consensus sequence LxCxE required for pRb binding, and is highly conserved among HPV types, as well as in adenovirus type 5 E1A protein (Ad5 E1A) and simian virus 40 large T antigen (SV40 large T). **b** E6 harbours 2 zinc finger domains. A C-terminal PDZ-binding domain of the consensus sequence x-(T/S)-x-(V/L) is also present in the E6 proteins from high-risk HPV types (HPVs 16 and 18), but is absent in their low-risk counterparts (HPVs 6 and 11).

Fig. 3.

Molecular mechanisms of HPV-induced cellular proliferation and genomic instability. HPV E7 induces cellular proliferation by 4 main mechanisms that culminate in the activation of E2F and re-entry of cells into S-phase. These mechanisms involve: (1) inhibition and degradation of pRb and related pocket proteins p107 and p130; (2) stimulation of cyclinA/E-Cdk2 synthesis and activity; (3) inhibition of the Cdk2 inhibitors p21 and p27; (4) inhibition of specific histone deacetylases (HDAC) involved in E2F repression. E6 also contributes to cellular proliferation through its inhibition of p53, which results in decreased transcription of p21 and p27 and prevention of E7-induced apoptosis. These synergistic effects of E6 and E7 result in uncontrolled cell division and high genomic instability, possibly facilitating viral integration into the host genome. Integration events that disrupt the E2 open reading frame lead to an increase in E6 and E7 expression and associated genomic instability, a hallmark of cancerous cells.

Fig. 4.

Cellular proteins and processes affected by the HPV E6 oncoprotein. E6 interacts with several cellular proteins involved in apoptosis, tissue integrity, telomerase regulation and IFN antiviral response. The primary anti-apoptotic function of E6 is to inhibit p53. E6 also interferes with other cellular proteins involved in intrinsic and extrinsic apoptotic pathways, including Bax, Bak and downstream effectors of death receptor signalling. Additionally, E6 contributes to the disruption of tissue integrity by binding to focal adhesion molecules and PDZ domain-containing proteins. E6 also helps HPV-induced cellular immortalization by its induction of the telomerase hTERT proceeding through transcriptional activation by Myc and Sp1, as well as by inhibition of the transcriptional repressor NFX1-91. Finally, E6 also inhibits the host IFN antiviral response.