

Modulation of host immune response by human papillomaviruses and role of E6/E7 oncoproteins in development of cervical precursor lesions and cervical cancer.

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Abstract

Human papillomavirus infection is one of the most common sexually transmitted disease globally and etiological cause of cervical precursor lesions, cervical cancer as well as non-genital cancer. Failure of immunocompetent host to timely detect and eradicate HPV-infected cells and malignant altered cells, poses a higher risk for development of HPV-related cervical disease. This review focuses on host innate and adaptive effectors of the immune system and mechanisms used by HPVs to efficiently evade immune response and therefore establish persistent infection with subsequent disease progression.

Keywords: Human papillomavirus, Apoptosis, Cancer, Immune evasion, Antibodies

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Introduction

Papillomaviruses (PVs) which belong to family *Papillomaviridae*, as proposed in 2004 by de Villiers and co-workers are small non-enveloped viruses with closed circular DNA genome [1]. Currently, 198 different Human papillomaviruses are already recognized [2]. They belong to five genera, namely, Alpha, Beta, Gamma, Mu and Nu but based on the carcinogenic potential and biological relevance, the most important species are considered alpha 9 and alpha 7 [3]. According to the International Agency for Research on Cancer (IARC), HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 are human carcinogens [4]. Based on their oncogenic potential and epidemiological relation with cervical cancer they are further classified in 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) assumed hr-types 26, 53, 67, 70, 73, 82 and low-risk types 6, 11, 40, 42, 43, 44 and 71 [5]. Low-risk types (alpha 10 species) are associated with genital condylomata and laryngeal papillomatosis with type 6 being more common in genital condyloma and type 11 frequently found in laryngeal papillomas [6]. Reported worldwide HPV prevalence in cervical cancer is 99.7% thus making this infectious agent, etiological factor for cervical carcinogenesis [7]. Around 40 types infect anogenital region in the both sexes [8] Additionally, HPV infection plays a critical role in head and neck malignancies, with HPV-DNA detected in 25.9% of all head and neck squamous cell carcinomas, predominantly in oropharynx [9]. Albeit, the causal relationship between the high-risk HPV infection, cervical premalignant lesions and squamous cervical cancer is recognized in several epidemiological studies, HPV infection was shown as an obligate but not sufficient factor in cervical carcinogenesis. Cervical precursor lesions identified and properly treated are highly curable and therefore cervical cancer is preventable disease but yet it remains, the third most common cancer in women worldwide and furthermore it presents a major health and public problem particularly in developing and low-resource countries where remains the leading cause of cancer-related death in women [10,11].

HPV infection is one of the most common sexually transmitted diseases in the world with estimated global prevalence up to 10.0% [12]. Nevertheless, published results concerning the HPV prevalence range from 25.60% for the North America up to 43.5% for East European countries [13]. Dependently, on the geographical region, race, ethnicity, and age group, HPV prevalence and genotype distribution varies largely due to several risk factors for HPV acquisition, dissimilar screening feasibilities and variable sensitivity of the detection methods [14].

HPV infection is usually acquired in the beginning of the sexual experience and moreover the majority of HPV infections are of transient nature. Remarkably, the highest prevalence is reported for the age group 25-35 years [15] with declining prevalence for older age probably due to the development of the immunological response and changes in sexual behavior and other cofactors [14]. The majority of the infected subjects are likely to clear the infection within one or two year via cell-mediated immunity and therefore minor cytological abnormalities regress without treatment [16-18]. However, persistent infection with high-risk genotypes carries out an increased risk for development of severe intraepithelial lesions and cervical cancer in HPV infected subjects [19,20].

The term "Cervical Intraepithelial Neoplasia" or CIN date as far back as early 1973 when introduced by Richart. Based on this classification premalignant abnormalities comprise a wide spectrum of premalignant changes from mild dysplasia (CIN1) to moderate dysplasia (CIN2) to severe dysplasia/carcinoma in situ (CIN3/CIS) [21]. Nonetheless, need for uniform unique reporting of cytological findings resulted later on in the terminology known as the Bethesda System, according to which squamous cell abnormalities are divided in categories 1) Atypical squamous cells or ASC which is further divided into ASC of "undetermined significance" (ASC-US) and "cannot exclude high-grade squamous intraepithelial

lesion” or ASC-H 2) Low-grade squamous intraepithelial lesions or LSIL 3) High-grade squamous intraepithelial lesions or HSIL and 4) squamous cell carcinoma or SCC. Glandular abnormalities are classified as atypical glandular cells (AGC), endocervical adenocarcinoma in situ (AIS) and adenocarcinoma [22].

Even though, several risk factors should be considered in the multistep complex process of cervical carcinogenesis including early sexual debut, younger age at first full-time pregnancy five or more full-term pregnancy, long-term hormonal contraception, previous exposure to other sexually transmitted disease predominantly, *Chlamydia trachomatis* and Herpes Simplex virus-2, HIV-related immunosuppression, tobacco smoking, lack of the antioxidants and genetic alterations, mainly persistent infections with high-risk HPVs, pose a higher risk for disease progression with cervical cancer as endpoint.

Persistence of infection is undoubtedly linked with numerous mechanisms by which HPVs avoid host immune response and this review focuses on the different evasion mechanisms of HPVs and role of E6/E7 in cervical disease.

HPV genome organization

The small viral genome of these non-lytic viruses contains around 8,000 base-pairs (bp) and is simply organized in three regions, the long control region (LCR) or upstream regulatory region (URR) which presents the non-coding region, early transcription region that comprise of the E1, E2, E4, E5, E6, E7 open reading frames (ORF) and late transcription region with L1/L2 genes which encode for L1 and L2 capsid proteins [23].

Despite the fact that, LCR in non-coding region it contains several silencer and enhancer sequences for gene transcription. The promoter for the early genes is located in the long control region, while the late viral transcription is regulated by late promoter p670, which is positioned in E7 region [23].

HPV genome replication begins with the E1/E2 gene expression so that E2 binds to the LCR and recruits E1-replication factor, a specific-viral DNA helicase [24] which in the meantime binds to its particular binding site in the viral origin of replication [25]. Therefore, E2 participates in replication, transcription and genome segregation and it can act as pro-apoptotic factor in normal and HPV-infected cells [24-27]. Since it is able to modulate viral expression through interacting with LCR it can also capably suppress E6/E7 expression, so loss or disruption of the E1/E2 gene function will lead to E6/E7 over expression [25].

In human papillomaviruses, no E3 open reading frame was identified while E4 can facilitate genome amplification and expression of the late viral genes [23].

E5 is a short transmembrane protein [28] with anti-apoptotic activity and important in viral immunoevasion [29] By increasing EGFR (epidermal growth-factor receptor) signaling [30] as well as by increasing mitogenic activity of the growth factor ET-1(endothelin-1) supports viral replication and cell proliferation in basal keratinocytes during the early phases of viral infection [31].

E5 mRNA is highly expressed in upper epithelial layers since in differentiating cells promotes proliferation thereby acting as a modulator of late viral functions [32].

E6 and E7 non-structural proteins demonstrate their oncogenic potential primarily through interfering with the cell cycle by inactivating the tumor suppressor genes and by transcriptional up-regulation of the catalytic subunit of the TERT gene [33,34].

Major capsid protein, L1 mediates viral entry by interacting with heparin sulphate proteoglycan on the basal membrane, to ensure a precondition for *in vivo* infection while during the late phase of the viral life cycle L1 with L2, which is a minor protein part of viral capsid are expressed in superficial epithelial layers in mature keratinocytes and contribute to the virus infectivity by participating in the assembly of the viral particles and virion release [35-37].

HPV life cycle and role of E6/E7 oncoproteins in cervical carcinogenesis

Infection of the basal epithelial cells by Human papillomaviruses is realized through micro-abrasions and micro traumas of the epithelium. Their viral cycle is entirely dependent on the differentiation of the host epithelial cells. After infection, viral life cycle in the host cells goes through three phases a) establishment or latent phase b) maintenance phase and c) amplification or productive phase [23]. In establishment phase, low copy number of episome, usually up to 200 copies per cell is maintained due to E1/E2 gene expression in the basal cells [23] while the productive infection is characterised by amplification of the viral genome to a high copy number and virion release from mature, differentiating keratinocytes from the upper layers of epithelium to guarantee dissemination of the infection to other cells [25,38]. In low-grade lesions viral DNA is usually episomal whereas in high-grade lesions and cervical cancer where viral expression deregulation is present, typically integrated sequences are found into the host chromosome with consequent E6/E7 overexpression [25].

Early oncoproteins, E6 and E7 have a decisive role in the progression of cervical precursor lesions and cervical carcinogenesis through a number of molecular pathways. One of the mechanisms by which E6 disrupt cell cycle circuit consist of p53 gene product inactivation through E6/E6 associated protein (E6AP) complex which catalyzes p53 ubiquitination and hence targets it to the proteasome degradation [39]. The p53 gene product is an important transcriptional regulator and is activated by DNA damage or stress. It inhibits cell proliferation by activating the p21 cyclin-dependent kinase (CDK) inhibitor gene and also induces apoptosis. Therefore, without this defense working properly, cell is driven in the direction of unrestricted proliferation and malignancy [39]. Besides p53 protein, E6 targets other apoptotic proteins including Bak, c-Myc and caspase-8 [40] which is degraded in a similar way to p53 protein, through ubiquitin-proteasome process. This way E6 interferes with extrinsic and intrinsic apoptotic pathways [40].

Although, it is demonstrated that HPV-16 E6 oncoprotein can activate telomerase in human keratinocytes and mammary cells [41] and that E6 increases *TERT* gene transcription levels with consequent telomerase activity and immortalized phenotype of

the cells, mechanism by which E6 activates catalytic subunit of telomerase (hTERT) is mainly obscure [34,42]. However, their mutual action is substantial to mediate cell immortalization and malignant transformation [43,44]. E7 oncoprotein binds retinoblastoma protein (*Rb*) with subsequent activation of the E2F transcription, which in turn increases cyclin E gene expression and hence promotes unrestricted cell proliferation [45]. It also inhibits activity of the p21 and p27 which are important cyclin-kinase inhibitors [46,47] while depending on the cell type and viral type, E7 can act as pro-apoptotic or anti-apoptotic factor [40].

Importantly, it was demonstrated that E7 oncoprotein from low and high-risk types directly associate with the cyclin A/CDK2 and cyclin E/CDK2 and that this association is independent from the pRb family member [48]. Thus, by disrupting CDK2 activity and by inactivating Rb protein, HPVs are capable to sustain G1 to S-phase entry and progression in mature cells [49]. E7 associates with histone deacetylases class-I and thereby, plays a role in epigenetic programming [50]. Finally, E6 and E7 induce chromosomal/genomic instability and therefore malignant conversion is due to the accumulation of the genetic mutations [51].

Innate and adaptive immune host response and HPV infection

Innate and adaptive components of the immune response are important in HPV clearance and elimination of the infected cells. The innate immune system comprises complement, lysozyme, natural killer cells, macrophages and dendritic cells [52] whereas T and B cells and their products comprise adaptive immunity. Progressive lesions due to prolonged viral persistence which lead to cervical cancer occur in a small fraction of HPV infected subjects, if they fail to clear or control infection mainly mediated by cellular immune response.

There is a strong evidence that integrated viral genome with E6/E7 overexpression is regular event in severe intraepithelial lesions and cancer [53]. However, in cervical cancer caused by HPV 16, integrated viral sequences were found in 72% of cases while, HPV DNA was in episomal state in 27% of these cancers, implying that integration of viral genome into the host genome, is not always prerequisite for malignant transformation [54]. Therefore, the crucial step in understanding the complex relation between the virus and the host immune competence is how the HPVs evade innate and adaptive response in order to prolong infection and ensure progression of HPV- related disease. Another central issue is certainly, which mechanisms are used by malignant cells to evade the appropriate immune response [55].

HPV low viral expression and replication is confined to the basal epithelial cells. Dendritic cells are matured antigen presenting cells (APC) and by expressing antigen/MHC class complexes they activate B lymphocytes and T -cell immunity [52]. Evidence suggest that E7 and E5 oncoproteins via MHC class I and II complex downregulation, actively inhibit presentation of viral antigens to immune patrol, thereby efficiently prevent CD8+ and CD4+ activity. E5 causes retention of the HLA class I complexes in the golgi apparatus [56] and also via inhibition of acidification of endosomes in human foreskin keratinocytes as well as by inhibiting invariant chain (Ii) degradation, can block

formation of mature MHC class II dimer [57] while E7 was shown to repress MHC class I heavy chain promoter [58]. Above mentioned E5/E7 activities will result in no recognition and no eradication of infected and transformed cells by immune system, hence enable viral survival and persistence. Additionally, after HPV infection, DC activation and migration to ensure local pro-inflammatory milieu is not likely to occur due to non-lytic nature of these viruses and as described above because of low levels of viral peptides exposed to immune surveillance. Locally, at the infected sites number of Langerhans cells (LC) is reduced due to E6 activity in E-cadherin mediated adhesion between the keratinocytes and LC [59]. Transcriptional repression of E-cadherin by E6 [60] and low levels of this transmembrane protein in HPV infected cells [61] restrict viral exposure to immune system and disable T-cell priming [61]. Furthermore, viral assembly, DNA packaging and virion release conditioned and accompanied with L1 expression are realized in terminally differentiating cells which normally undergo apoptosis, thus evading direct communication with vascular and lymphatic milieu [62]. L1 is highly immunogenic albeit without potential to induce immune response under those circumstances since L1 expression is suppressed in basal cells [63]. Humoral response is also impaired due to absent viraemia since there is no blood-born phase [62].

Natural killer cells (NK) or cytolytic lymphocytes are innate components, involved in anti-viral and anti-tumoral activity [64]. NKc expresses inhibitory MHC class I receptors which ensure NK self- tolerance and initiate NK-mediated cytotoxicity in MHC class I deficient cells [65]. In patients with high grade lesions and cervical cancer, their activating receptors NKp30, NKp46 was shown to be down-regulated hence highlighting role of the NKc in HPV infection and cancer [66]. NF- κ B is transcription factor activated after nuclear translocation and by binding to specific DNA sites can stimulate expression of genes involved in inflammatory response, anti-apoptosis and innate/adaptive immunity [67]. E6/E7 expressing cells can alter expression of the NF- κ B inducible genes and hence have essential role in immunoevasion strategies and HPV-driven carcinogenesis [68]. Disruption of NF- κ B/I κ B complexes by overexpression of antisense RNA resulted in cellular malignant transformation [69] and activation of this nuclear transcription factor in HPV immortalized human keratinocytes contributes to malignant progression [70] whereas AP-1 and NF- κ B inhibitors can block malignant alteration [71].

Innate and adaptive immune response is bridged not only by the actions of APCs but also by the functions of interferons which are cytokine mediators capable to activate macrophages, induce B and T cells and stimulate antiviral state in uninfected cells [72] E6 interferes with IFN pathway by inhibiting INF- α and IFN- β expression and IFN-responsive genes as well as by decreasing nuclear STAT-1 proteins which in turns inhibits IFN production [68].

Furthermore, HPV 16 E6 specifically interact with transcriptional activator IRF-3 which is important factor for expression of IFN- α and IFN- β genes [73]. Interaction between HPV 18 E6 and Tyk-2 with decreased IFN- α signaling was also observed in HT1080 cells [74]. This way HPVs block antiviral, antiangiogenic and antiproliferative functions of IFNs [75].

IFN alpha/beta is produced after recognition of viral peptides by cellular pattern-recognition receptors (PRRs) like toll-like receptors (TLRs). Increased expression of TLR 2, 3, 7, 8 and 9 was associated with HPV 16 viral clearance unlike viral persistence [76] and therefore treatment of genital warts with TLR7 agonist Imiquimod was shown moderately efficient [77]. In HPV infected subject's viral persistence is also due to altered mucosal immunity since high levels of pro-inflammatory cytokines IL-1 α/β and IL-8 and low levels of anti-inflammatory cytokines were found in women diagnosed with CIN1/CIN3 [78].

Second line of immune defense is adaptive immunity which acts by antigen-specific effectors and is characterized by immunologic memory [52]. CD8+ T cells can inhibit pathogen spreading and viral replication while CD4+ T cell or T helper cells are initially activated during the adaptive response acting by cytokine production. Th1 cells which produce TNF α and IFN γ and Th2 cells which produce IL-4, IL-5 and IL-13 are both subsets of T helper cells [52]. Higher levels of Th1 and Th 2 were found in low-grade lesions and high grade lesions respectively, while cervical cancer was associated with expression of Treg cells [79].

Regulatory T-cells are CD4+T cells with fundamental role in immunological tolerance to prevent autoimmune diseases and can suppress immune activity in antigen dependent manner [80]. Cervical Treg cells were low in CIN regressors compared with non-regressors hence by promoting immunological tolerance these cells have a role in viral immune evasion of neoplastic cells [81].

PD-1 expressed in T cells and PD-L1 expressed in APC and T cells are immunomodulatory mediators that inhibit T cell response [82]. Up-regulation of PD-1 in T cells and PD-L1 in DCs suppress T cell-mediated immunity and thus interfere with viral clearance and contribute in persistence and disease progression [83].

Using cDNA microarrays to analyze gene expression in HPV E6/E7 infected cervical keratinocytes, was demonstrated that HPV16 E6 alters expression of interferon responsive genes, NF- κ B stimulated genes and genes that regulate cell cycle progression [68].

Apoptosis or programmed cell death is physiological process essential for maintaining cell homeostasis, morphogenesis, and development and has a central role in the etiopathogenesis of viral infections, autoimmune disorders, neurodegenerative diseases and immunodeficiencies [84]. Apoptotic response is triggered by specific signals from extra and intracellular environment and is realized through extrinsic and intrinsic path [84]. Extrinsic apoptotic pathway is initiated with activation of the receptor by its respective death ligand [85] which mainly belong to tumor necrosis factor family or TNF and the central event is activation of the executioner caspase 3 which further leads to programmed cell death [40]. Apoptotic stimuli for the intrinsic or mitochondrial pathway are DNA damage, radiation and osmotic stress [86] and chemotherapeutic drugs [87]. In this regard, different viruses have developed different strategies to modulate or to interfere with apoptotic mechanism in order to maintain virus latency within the host cell or to improve survival of the infected cell [84].

All non-structural HPV-oncoproteins interfere with apoptosis. E2 was shown to efficiently activate p53 transcription and suppress E6 expression in E2-transfected HeLa cells which resulted in cell death and arrest of the G1 phase [88].

The p53-independent apoptotic activity of the E2 which is carried out through caspase 8 with E2 cleaved during apoptosis was also previously demonstrated [89] thus highlighting the vital role of E2 in progression of precancerous lesions to cervical cancer, since E2 disruption or deletion enables unrestricted expression of E6/E7 oncoproteins [45].

E5 prevents apoptosis in infected keratinocytes during the early stages of infection, predominantly by hindering ligand-mediated apoptosis (Fas ligand and TRAIL or tumor necrosis factor-related apoptosis-inducing ligand) [90] and also by using mitochondrial apoptotic way via proteasome degradation of the Bax-apoptotic protein [91].

In general, HPV E6 uses p53-dependent and p53 -independent pathways in order to evade host immune surveillance. Inhibition of intrinsic apoptosis throughout E6-mediated degradation of p53 or p53-dependent pathway is extensively studied [92]. However, E6 antiapoptotic activity is carried out also through association with other pro-apoptotic proteins. One of the p53-independent pathways of E6-mediated apoptosis is by Bak apoptotic protein degradation via a similar ubiquitinylation process described for p53 [93]. Through the same proteasome degradation process, E6 prevents myc-induced apoptosis [94].

Adaptor molecule FADD contain death effectors domains (DED) which enables recruitment of procaspase-8 to the death-inducing signaling complex (DISC) Executioner caspases 3, 6 and 7 are then activated by caspase-8 and apoptosis is initiated [95]. In this regard, it was demonstrated that cells expressing E6, through degradation of the procaspase-8 and Fas-associated death domain (FADD) inhibits TRAIL-triggered apoptosis [96].

Therefore, the interaction between the initiator caspase-8 and FADD with E6 protein enables HPVs to interfere with extrinsic apoptosis and thus prevent removal of the infected cells. This is however only one of the mechanisms used by E6 to interfere with extrinsic signaling pathways.

E7, apart from its fundamental function in E2F transcription with subsequent progression from G1 to S phase of the cell cycle [45] E7 shows also pro and anti-apoptotic activity in different cell types [40]. It was demonstrated that E7 increased spontaneous and induced cell demise in primary human keratinocytes while E6/E7 co-expression showed anti-apoptotic effect mediated by tumor necrosis factor alpha [97].

Humoral response and HPV infection

Evident that L1 is highly immunogenic and that has ability to self-assemble into VLPs while inducing genotype-specific serological response through neutralizing antibodies opened a possibility for vaccine development to prevent HPV-related disease [98,99].

Although, in natural infections serological response was weak with low concentration of neutralizing antibodies, seroconversion observed after vaccination was shown highly protective with detectable antibodies up to 5 years after vaccination [62,100].

In general, there is sufficient evidence that vaccination provides protection against non-vaccine HPV types or so called cross-protection [101].

Yet, if these antibodies can protect the individuals with established humoral response against re-infection with the same HPV type, remains largely unclear since evidence suggest that virus remains in the latent state even after the regression of benign lesions occurs [102] confirming our poor understanding of the latent HPV infection.

Conclusion

Human papillomaviruses have evolved with humans and assure their replication, survival and propagation in a hostile host environment which is equipped with complicated defense machinery. They interfere successfully and competently avoid local mucosal microenvironment patrolling, downregulate adherence transmembrane mediators, inhibit MHC class I/II complex activity, impede apoptotic pathways, inhibit interferon functions and alter expression of the genes involved in the control of the cell cycle and immunity. Despite the efficient viral immune-evasion, majority of the infected subjects are able to clear infection insinuating that nor infection neither persistence are sufficient for cancer development and that our knowledge regarding the multi-mechanisms involved in viral hidden, endurance and resistance are far from being completely understood.

Clarifying the complex multi-factorial relationship between the host immunity and HPV infection as well as molecular pathways used by HPVs to interfere with host immunity presents an enthralling future objective for development of new therapeutic approach in management of the HPV-driven disease and neoplasia.

Conflicts of Interest

The author declares that no conflicts of interest exist.

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Ethical Approval

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