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Oncogenic Proteins Involved in Persistent HPV Infections, Carcinogenic Potential and Therapeutic Control

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ABSTRACT

Human papillomaviruses (HPV) are a group of small-chain DNA viruses that can cause benign disease or be detected in human malignant lesions. Almost a third of all human cancers caused by infectious agents are due to HPV infections. This review provides a thematic analysis based on data from the literature, focusing on the viral infection spread cycle, focusing on the functional roles of HPV E6 and E7 oncoproteins responsible for abnormal cell proliferation, diagnosis of precancerous status, prevention and therapeutic strategy.

Due to the high carcinogenic activity of HPV16 and HPV18 and onco-E6 and E7, molecular mechanisms and tests are studied that are based on the detection of viral nucleic acids in infected tissues, various modes of therapy, which work by disrupting the activity of E6 and E7. The most well studied cellular targets of the viral oncogenes E6 and E7 are p53 and pRb, targeting a series of cellular elements, including proteins to regulate epigenetic signs, exerting uncontrolled global changes and finally carcinogenesis.

Different treatment modalities for associated HPV carcinogenesis have been shown to be beneficial. Thus, a recent therapeutic strategy based mainly on the CRISPR/Cas9 mechanism of reactivation of TP53 and pRb to induce apoptosis and cell senescence, is an alternative to RNAi interference with E6 and E7.

Despite, HPV research that has led to extraordinary achievements over the past years, further research is needed to characterize the biochemistry of HPV types, the potential risk of persistent infection, and the induction of oncogenesis.

KEYWORDS

E6 E7 oncoproteins; Human papillomavirus; Oncogenic potential risk; Therapeutics against HPVs; Viral-induced cancers

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INTRODUCTION

Human papillomaviruses (HPVs) are part of the Papillomaviridae family and are a large, heterogeneous group of viruses whose genome contains double-stranded circular DNA that infects squamous epithelial cells of the oral cavity and upper respiratory tract, anogenital tract and skin, causing a series of pathological manifestations such as papillomas, dysplasia and cancer [1,2].

Statistics show that HPV is considered to be the cause of about 5% of all human cancers in the world [3], with cervical cancer ranking 4th for women worldwide [4] and 2nd for countries developing [5].

Papillomaviruses found in humans are divided into 5 phylogenetic genes, based on DNA sequence analysis, having different life cycle characteristics [6]. For each gen (alpha, beta, gamma, mu and nu HPV), there are biological and characteristic genomic properties. These viruses were not grown in vitro, being characterized by molecular methods. Currently, of different HPV types described, approximately 40 can infect the epithelial lining of the anogenital tract and other mucosal areas of the human body [7]. Based on their association with benign and malignant anogenital lesions, HPV can be classified as high-risk oncogenic HR-HPV types: E-6,E-7,16,18,31,33,35,39,45,51,52,56,68,73,82 (26,53,66), and low-risk LR-HPV: 11,40,12,43,44,53,54,61,72,73,81 causing particularly benign lesions affecting anogenital, cervical and laryngeal papilloma's [8,9].

Intense research in recent decades on HPV, E6 and E7 oncogenes has highlighted the existence of a dependence between HPV-induced cancers and the expression of major viral oncogenes, E6 and E7 [10]. Targeting E6/E7 viral oncogenes in tumors, or in tumor-derived cell lines for functional inhibition make E6 and E7 potential targets for therapeutic intervention in HPV-induced cancer [11].

HPV INFECTIOUS CYCLE

Human papillomavirus is an epitheliotropic DNA virus, is a 50 nanometer - 60 nanometer diameter, 8 kb - 10 kb, which contains a double-stranded circular DNA genome associated with histone-like proteins and protected by a latex-two capsid L1 protein and L2.

All papillomaviruses encode four conserved base proteins: E1 and E2 as replication factors; L1 and L2 capsid proteins, and oncogene HPVs encode the auxiliary proteins E4, E5, E6 and E7 [12]. These proteins make modulation of the cellular environment necessary for viral replication and are important for immune evasion [13]. The structure of human papillomavirus and the alpha HPV16 genome organization genome is shown in Figure 1 [14,15].

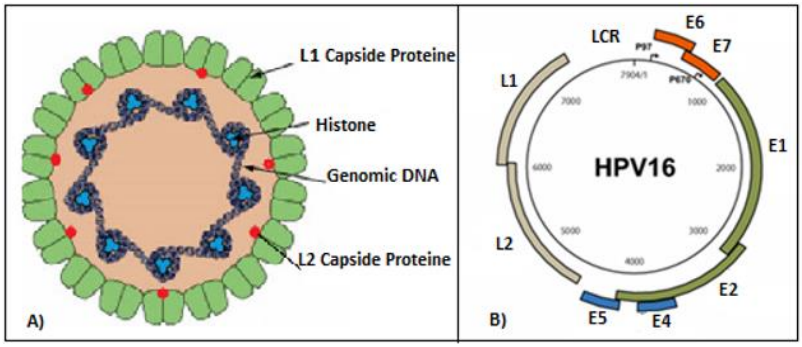


Figure 1: (A) Structure of human papillomavirus; **(B)** Genome organization of the high-risk Alpha HPV 16 types.

Human papillomavirus performs a single propagation pathway, limited to the layering of the flattened epithelial tissue of the mucosa and skin depending on their tropism [16].

The HPV life cycle is closely related to the host cell biology and begins by infecting stem cells in the basal layer of the epithelium by micro-abrasion. This mechanism allows access to self-renewing cells and promotes cell proliferation as part of the wound healing process, which could help establish viral infection [17].

The mechanism of propagation of the HPV viral infection cycle according to the studies conducted and published in the literature cited by Fernandes et al. (2012) and comprises the following propagation steps: i) after entering the cells, the virus require the expression of the E1 and E2 genes to maintain a small number of copies of the genome. These proteins bind to the viral origin of replication and recruit cellular DNA polymerases and other proteins required for DNA replication. ii) In the suprabasal layer, the expression of the E1, E2, E5, E6 and E7 genes contributes to the maintenance of the viral genome and induces cell proliferation, increasing the number of HPV-infected cells in the epithelium, resulting in a larger number of cells that will ultimately produce infectious virions. iii) In the highly differentiated cells of the same epithelial layer, activation of the differentiation-dependent promoter occurs and the gene expression E1, E2, E6 and E7 is maintained. In parallel, there will be an activation of E4 gene expression, the product of which will induce the amplification of viral genome replication, significantly increasing the number of virus copies per cell at the same time as L1 and L2 gene expression. iv) In the granular layer, the late genetic products, the major and minor proteins of the viral capsid, L1 and L2, respectively, are assembled to assemble viral capsids and virion formation that reach the cornified layer of the epithelium and are released [18] (Figure 2).

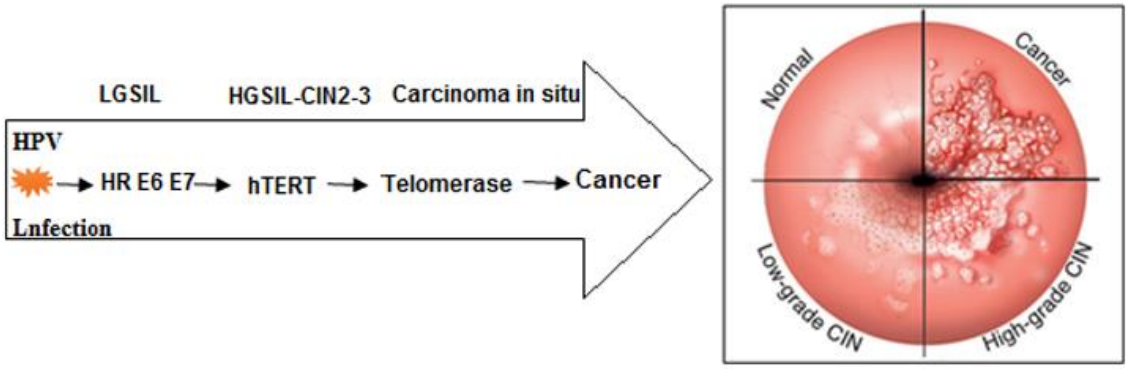


Figure 2: L1 and L2, respectively, are assembled to assemble viral capsids and virion formation that reach the cornified layer of the epithelium and are released.

The HPV life cycle has two stages: One maintenance phase and one differentiation-dependent phase [19, 20]. In the maintenance phase, viral proteins are expressed at very low levels in undifferentiated cells, which contribute to immunity evasion and persistence. During the differentiation phase, HPV-infected cells leave the basal layer inducing high levels of viral protein synthesis, delaying the expression of antigens viral infections in locations less susceptible to host immune response.

This mechanism determines different duration of infection for HL or HR-HPV types: HR-HPV, especially HPV16, has a longer clearance time (> 6 months - 12 months) and is more likely to develop a persistent infection [21].

THE ROLE OF E6 AND E7 ONCOPROTEINS IN CARCINOGENESIS

It has been shown that alpha-HPV types infect the mucosal epithelium, the virus enters through the microinjection and infects the basal epithelial cells [22] and that the virus does not have its own replicative equipment being dependent on the cell division and stratification of the epithelium occurs from basal to suprabasal layers. In this process, HPV, E6 and E7 oncoproteins play a crucial role. Their common action on different cellular pathways implicated in the regulation of cell cycle control and apoptosis allows the virus to maintain the cellular proliferation largely of the differentiated suprabasal region that allows the amplification of the viral genome [23,24]. Although the normal productive viral life cycle of HR-HPV alpha types is a well-regulated and coordinated process, in some cases generally, during persistent infection, viral DNA is randomly integrated into the host genome, resulting in immortalization cellular and ultimately malignant progression.

E6 and E7 are oncoproteins of the alpha-HPVs type, characterized by a low conservation degree and a high degree of specialization compared to the main proteins of the virus. Based on multiple interactions with cellular proteins, E6 and E7 activate cell proliferation and inactivate cell cycle control points with the goal of promoting viral replication in differentiated cells [25]. Conclusions of studies over the years highlight the underlying functions of the HPV oncogene mechanism: E7 mediated degradation of hypo phosphorylated retinoblastoma protein (pRb) family members; E6 mediated degradation of tumor-suppressor protein 53 (p53) and PDZ domain proteins, and E6 mediated telomerase enzyme regulation [13,26,27]. Major properties of HPV E6 and E7 oncogenes and the oncogenic pathways of E6 and E7 are summarized in Table 1 [13,28]. High-risk HPV types produce oncoproteins E6 and E7, which bind normal p53 and pRb tumor suppressor proteins and induce mutations over time E6 proteins bind and degrade p53, which is tumor suppressor, and E7 binds and inactivates the retinoblastoma protein family [29].

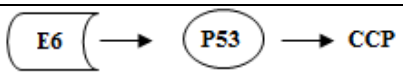
Major Properties	Oncogenic Pathways of E6 and E7
E6 • Proteasome-mediated degradation of p53 • Induction of telomerase expression • Degradation of PDZ domain proteins involved in cell polarity • Inhibition of innate immune response	
E7 • Epigenetic reprogramming of cells by Upregulation of KDM6A and KDM6B • Abrogation of pRb/E2F pathway by pRb degradation • Induction of DDR in differentiated cells to promote viral DNA amplification • Inhibition of innate immune response	

Table 1: Major properties and oncogenic pathways of HPV E6 and E7Legend: CCP: Cell cycle progression.

Normally, pRb binds to the E2F transcription factor, which plays a role in the progression of the cell cycle from phase G1 to phase S following cyclin interaction and cyclin-dependent kinases, thus p21 protein as regulator of cells cycle progression at G1 S phase is controlled by the tumor protein p53 [30]. In the presence of E7, there is an inactive E7-pRb complex that disrupts the attachment of E2F to pRb and allows E2F to bind to DNA and to induce cell growth and proliferation. The E6 and E7 proteins of the

different HPV types exert a variable effect during the malignant transformation process: HPV 16 and HPV 18 have a high oncogenic potential, whereas those produced by HPV 6 and HPV 11 are incapable of binding and inactive p53 and pRb [31].

Some studies have suggested that the disruption of E6/E7 expression, even in the absence of genome integration, is a critical event in determining neoplastic degree [32], which is classified according to the extent to which basal-like cells expand in the sub-base layers [33]. E6 and E7 proteins produced by high-risk HPV types play an essential role in carcinogenesis, being expressed in both premalignant and advanced cervical lesions.

THERAPEUTIC OPPORTUNITIES

Prevention

Some studies state that in most healthy people, about 70% of HPV infections spontaneously resolve within one year and 90% within two years; the rest of the infections are persistent [34]. Elimination of the infection requires an effective cellular immune response, while persistent infections with oncogenic HPV types are associated with an immune response failure and an increased risk of progression to cancer [34]. Hence, host immunity, including innate and adaptive immune responses, plays an important role in the elimination of infected cells, and there is a greater incidence of HPV-related and cancer-related diseases in immunocompromised individuals [35]. It takes an average of 12 years - 15 years before a persistent HPV infection progresses stepwise, premalignal (intraepithelial squamous lesions) and becomes clinically meaningful cervical carcinoma [36].

Primary and secondary cervical cancer prevention strategies by vaccination and screening are currently contributing to reducing the incidence of cervical cancer [37]. Cervical screening improves stratification of the risk of women with HPV infection and indicates which HPV positive woman has the highest risk of cancer. Prevention of HPV is essential to reduce the risk of infection or further spread of the virus. Safer sex practices can significantly reduce the risk of transmission. There are also three different vaccines to prevent many of the high-risk HPV strains: Cervarix, Gardasil and Gardasil 9. The new non-valent HPV vaccine (9vHPV, Gardasil 9) was approved for use in the US in 2014 Canada, Australia and the European Union in 2015. This vaccine includes high-risk HPV types 16,18,31,33,45,52 and 58 and, in addition, low risk HPV 6 and 11 HPV 9-valent (V503) [38] Van Damme 2016], similar to current vaccines, uses viral particle-like (VLP). The V503 contains 5 additional types of HPV (types 31/33/45/52/28 to HPV). Generally, the 9vHPV vaccine offers the potential to prevent approximately 90% of cases of cervical cancer and HPV, vaginal, anal, as well as nearly 90% of genital warts worldwide [39-41]. For infected people, the Pap smear test was introduced in 1941, which was successful in screening cervical lesions associated with surgically treatable HPV, but there is no equivalent test for oropharyngeal cancer (OPC) because of the inaccessibility of the infection site.

Diagnosis

High-risk HPV testing is done through multiple methods. Histological tests are also performed by highlighting a concomitant histological abnormality, providing the reference standard for cervical infections, based on morphological characteristics and not considering HPV biomarkers. Thus, three degrees of intraepithelial cervical neoplasia (CIN) are highlighted by the amount of vertical extension of abnormal cells in the cervix epithelium [42].

The mechanism of HPV infection propagation with worsening cytological changes and parallel greater histological involvement, with multiple layers of the stratified squamous epithelium, CIN2/3 (cervical intraepithelial neoplasia 2 or 3) histologic changes due to an active HPV infection [27], is summarized in Figure 2.

Carcinoma in situ is the full thickness involvement of stratified squamous epithelium without breakdown of the basement membrane [27]. At present, a histological confirmed CIN3 lesion is a clear indication for surgical treatment [43].

DNA methylation analysis as a high-risk HPV triage test for women may be an attractive alternative to cytology as this is possible for direct brush autosamplers and has shown good correlation with physician readings [44]. Molecular detection methods, such as multiple HPV polymerase chain reaction tests, have been shown to detect more positive specimens than both s-LA and m-LA when clinical specimen DNA was isolated [45]. Fast assays using immunoassay method are used to detect E6/E7 on HP6 16/18/45 oncoproteins with high specificity for E6 in particular [46].

Since HPV cannot be grown, tests have been developed that are based on the detection of viral nucleic acids in infected tissues. Most tests detect viral DNA by various amplification techniques, thus confirming the presence of infection in cervical cells [31]. However, DNA-based tests (such as the HPV genotype detection assay) have an important limitation in that most HPV infections are transient and the positive predictive value of a DNA result -HPV for the development of high-risk cervical lesions is quite low.

In order to highlight HPV oncogenic activity, tests based on the detection of messenger RNA for E6/E7 proteins originated from five high-risk HPV types (16,18,31,33,45) and then from 14 HPV oncogene types: 16,18,31,33,35,39,45,51,52,56,58,59,66 and 68. E6/E7 mRNA translates direct viral activity and corresponds to the initiation and maintenance of pre-cancerous lesions, so RNA based assays have a predictive value superior to DNA testing for the risk of developing cervical cancer. E6/E7 mRNA levels were found to increase proportionally to the severity of the lesions, so that the detection of the transcription product would have a higher prognostic value and could improve the specificity and predictive value of HPV DNA in screening, as confirmed by studies [47].

Therapeutics strategy

Despite recent advances and different treatment modalities that have been shown to be beneficial to some extent, there is currently no effective treatment for associated HPV carcinogenesis. Even though precise molecular targets (pathways) have been characterized and some approaches to their inhibition have been demonstrated, which do not currently have an effective treatment approach; it is just as important as finding new methods for the known goals and mechanisms [48]. The HPV therapeutic vaccine aims to induce in-vivo virus-specific T cell responses against already established HPV infections and lesions. Thus, T cells reach the site of the tumor acting without restrictions [49].

The oncoproteins E6 and E7 considered to be almost ideal targets for cervical cancer immunotherapy [50] have been included in most therapeutic vaccines. In recent years, a new CRISPR/Cas9 therapeutic strategy has been developed that has entered clinical trials. The CRISPR/Cas9-mediated silencing mechanism of E6 and E7 depend mainly on the reactivation of TP53 and pRb to induce apoptosis and cell senescence. The characteristics of this CRISPR/Cas9 strategy compared to RNAi are presented in Table 2 [48].

A series of studies support the features and opportunity of applying this CRISPR-centric technology: a CRISPR/Cas9 sequence targeting E6 mRNA, reduces full-length mRNA levels and increases TP53 protein level [51]; CRISPR/Cas9 inhibition of E7 as a potential therapeutic intervention for the treatment of cervical cancer [52]; CRISPR/Cas9 targeting the promoter and ORF of the E6/E7 transcripts reduces E6 and E7 mRNA level, increases TP53 protein

level, lowers RB protein level, promotes apoptosis and inhibits SiHa cell growth [28], Cas9 exhibits attenuated growth in vivo; Kennedy et al. 2014 designed CRISPR/Cas9 specifically targeting E6/E7 HPV16 or HPV18 mRNAs [53]. The results of intratumoral administration resulted in inhibition of tumor growth and induction of apoptosis in vivo, CRISPR/Cas9 being appropriate as potential adjuvant therapy for cervical cancer. Furthermore, the CRISPR/Cas9-based tools have been successfully applied in various organisms and in a wide range of research areas such as high-performance genetic screens, knockout genes in multiple species, and targeting pathogens to eradicate infections such as HPV, HIV and HBV.

Targets	CRISPR/Cas9	RNAi
Loss function mechanism	Frame shift DNA mutation	Post-transcriptional RNA degradation
Result	Permanent knockout	Reversible knockdown
Transgenes	Cas9 nuclease gRNA	si/shRNA
Guiding sequence	gRNA	si/shRNA
Required sequence information	Transcriptome	Transcriptome
Off-target space	Cuts as monomer	Transcriptome genome
Transcript variants region	All variants	All variants

Table 2: CRISPR/Cas9 strategy compared to RNAi.

People infected with HPV who develop cancer generally receive the same treatment as patients whose tumors do not contain HPV infections, depending on the type and stage of the tumors. Depending on the stage and extent of the disease, treatment options include surgery, radiotherapy, chemotherapy and various combinations thereof. Methods commonly used to treat precancerous changes in the cervix include: Cryosurgery (freeze-freezing); Loop electrosurgical excision procedure (LEEP) (Cremer); Electrosurgical excision procedure at the loop or removal of cervical tissue using a hot wire loop; Surgical consultation (scalpel surgery, laser or both to remove a piece of cone-shaped tissue from the cervix and the cervical canal) and the laser vaporization console (the use of a laser to destroy the cervical tissue) [54].

A number of drugs targeting epigenetic mechanisms have been developed in recent years for the treatment of cervical cancer: Histone deacetylase inhibitors (HDAC and DNA methyltransferase 1 (DNMT1). In addition, new drugs based on E6/E7-binding liposomal peptides could be developed. This blocks their interaction with other cellular structures, including epigenetics through a cascading effect on cellular pathways that are modulated by E6/E7 [55].

CONCLUSION

Some types of human papillomavirus (HPV) are highly associated with the development of cervical cancer in women, penile cancer in men, and anal and oropharyngeal cancers in both sexes. Over 15% of human cancers can be attributed to agent infections and nearly one-third of them are due to HPV infections [56] Plumer 2016. Low-risk oncogenic HPV causes genital warts (condylomas) and low dysplasia, while high-risk oncogenic HPV causes high-grade lesions (cervical intraepithelial neoplasia 2+), which are precursors to cervical cancer. Large-scale epidemiological studies have helped to identify the oncogenic risk of most of these HPVs.

Generally, HPV oncoproteins E6 and E7 are the primary viral factors responsible for the initiation and progression of HPV-related cancers through the inactivation of p53 and pRb. RNA interference with E6 and E7, as well as their functions in inhibiting the actions of various molecules, is a promising approach for the treatment of cervical cancers.

Because prophylactic vaccination is still in its infancy, focusing on such therapeutic measures is still warranted.

Despite HPV research that has led to extraordinary achievements over the past four decades, significantly improving the screening and prophylaxis of HPV-induced lesions, further research is needed to characterize the biochemistry and epidemiology of large numbers of HPV types in order to assesses the potential for risk of persistent infection and progression to oncogenesis.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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