

# Regulation of human oncogenes' expression by human papillomavirus (HPV) 16 E6 protein

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Human papillomavirus (HPV) is a DNA virus from the Papillomaviridae family that is capable of infecting humans. Infection with HPV causes anogenital neoplastic lesions in men and in women. HPV is transmitted by sexual contact generally by asymptomatic carriers. These viruses establish productive infections only in keratinocytes of the skin or mucous membranes. Most HPV infections are subclinical and will cause no physical symptoms; however, in some peoples subclinical infections will become clinical and may cause benign papilloma or cancers, which mainly involve genital regions, and the head and neck areas. In this manuscript, we discuss HPV virology, the molecular mechanisms of carcinogenesis and the regulation of human oncogenes' expression by E6 HPV 16 protein.

**Keywords:** cancer; genome expression; human papillomavirus; protein p53, protein pRb,

## 1. Introduction

The human papillomaviruses (HPVs) are a diverse group of DNA viruses that establishes productive infections only in the laminated epithelium of the skin and in mucous membranes. More than 100 HPV types are known and the standard classification in types of high and low risk of these viruses was performed based on their cancer pathogenesis[1].

Most HPVs cause no symptoms, currently; the available studies show that the prevalence of asymptomatic genital HPV infections in the population is very high [2]. Some types of HPVs can cause warts, while others can cause subclinical infections, which can lead to cervical cancer, in women or cancer of the anus and penis in men [3]. The most often transmission of HPVs occurs through skin to skin contact, so viral particles can penetrate first into mucosa and then, into epithelial cells [4]. Other HPV types affect non-anogenital localizations, such

as the head and neck areas. The term head and neck cancer includes malignancy in an area that comprises the skin, oral cavity, salivary glands, lip, pharynx, larynx, nasal cavity, paranasal sinuses and soft tissues of the neck and ear [5,6].

The molecular mechanism of HPV carcinogenesis can be explained by the regulation and function of the two viral oncogenes E6 and E7. These oncogenes, increase the rate of cell division or inhibit programmed cell death, thereby they will increase the risk of malignant transformation [7,8]. The great advances for fight against these viruses have been developed, such as prophylactic vaccination and high sensitivity-specificity methodologies of detection [9].

## 2. HPV Genome

HPVs have an icosahedral capsid and their genome is double-stranded circular DNA, with 8,000 nucleotide base pairs long. The HPV genome is divided into an early region (E), encoding

six genes (E1, E2, E4, E5, E6, and E7) that are expressed immediately after initial infection of a host cell, and a late region (L) encoding a major capsid protein L1 and also a minor capsid protein L2 and a non-coding regulatory region (NCR) of approximately 1 kb [6,10].

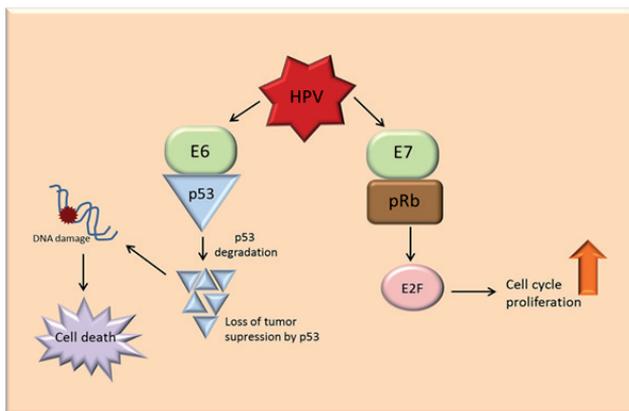
The L1 and L2 late proteins form capsomers of the virus that encapsidates the viral DNA. The L1 is the major capsid protein. L1 self-assembles into pentameric capsomers. L1 capsids assembled *in vitro* are the basis for prophylactic vaccines against several HPV types, because the amino acid sequences of L1 are well-conserved. The L2 protein is a minor component of the viral capsid. In addition to cooperation of L2 with L1 by the packaging of the viral DNA into the virion, L2 interacts with a number of cellular proteins during the infectious entry process. After the initial binding of the virion to the cell, L2 must be cleaved by the cellular protease [11].

The E1 encodes a protein that binds to the viral origin of replication. It has a helicase activity for separation of DNA strands. E2 is a transcriptional activator of virus gene expression. The E2 protein binds to E1 and stimulates viral DNA replication. E2 regulates a distribution of viral genomes to each daughter cell after cell division. The genetic changes, which inactivate E2 expression, tend to increase the expression of the E6 and E7 oncogenes, resulting in further genetic destabilization [9].

The E4 protein is expressed in a later phase of viral infection with a role in the assembly and release of the viral particle protein, facilitating virion release into the squamous epithelial tissue. Viral mutants incapable of expressing E4 do not support the replication of the viral DNA. E4 has also participated in arresting cells in the G2 phase of the cell cycle. The E5 are small and hydrophobic proteins that destabilize the function of many membrane proteins. E5 stimulates the transforming activity of the epidermal growth factor receptor resulting in the increased cell proliferation. These pro-

teins associated with cancer activate the signal cascade initiated by epidermal growth factor and could prevent the infected cell from being eliminated by killer T cells [12].

The E6 and E7 HPV proteins are implied in tumorigenesis and are known to induce degradation of the tumor suppressor genes p53 and pRb, respectively (Fig. 1) [13]. They can suppress apoptosis and alter the function of factors



**Figure 1:** E6 and E7 oncoproteins of human papillomavirus (HPV) prevent tumor suppressor protein (p53) and retinoblastoma protein (pRb) from stopping the growth of damaged cells

involved in cell-cycle regulation, thereby facilitating prolongation of the proliferative stage of keratinocyte differentiation [14]. The major role of E6 is the degradation of p53, reducing the cell's ability to respond to DNA damage.

The E6 protein also activates telomerase, an enzyme that maintains the telomeric DNA at the ends of linear chromosomes. Without telomerase, telomeres shorten upon each cell division, and causes cell senescence. Almost all human cancers and immortalized cell lines have highly active telomerase. Additionally, E6 can act as a transcriptional cofactor and also bind to signaling proteins and impede normal protein activity. The E6 protein is the target of therapeutic HPV vaccines designed to eradicate established cancer tumors.

The E7 protein is a small polypeptide of 100 amino acids. The carboxyl-terminus of E7 contains a similar zinc-binding domain as E6 [15].

The primary function of the E7 protein is to inactivate the members of the pRb family of tumor suppressor proteins. Together with E6, E7 serves to prevent apoptosis and promote cell cycle progression, for replication of the viral DNA. E7 also interacts with various other proteins, like important regulators of the cell growth. The E7 protein induces abnormal centrosome duplication, abnormal mitoses, aneuploidy and genomic instability [14-16]. E6-E7 oncogene expression is considered necessary for carcinogenesis and maintenance of the malignant phenotype of these cancers [6,17].

### 3. Regulation of the genome expression by E6 protein

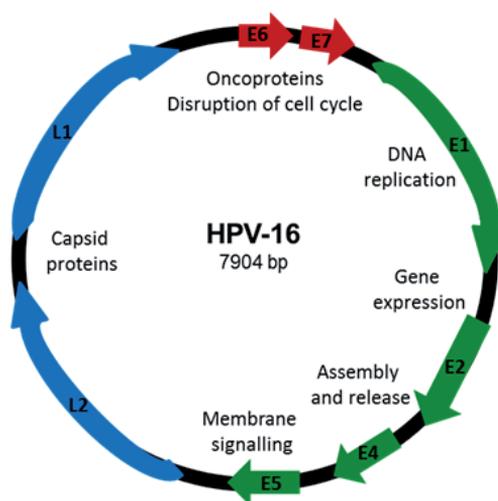
HPV-16 was included into the high-risk HPV types, and is the most common one in all cases of cervical cancer; also raise the risk of developing oropharyngeal cancer [5,18]. HPV-16 genome is about 7900 bp long (Fig. 2). High-risk HPV types act as primary transforming viral proteins to inactivate the p53 and pRb pathways that result in cell proliferation and resistance to apoptosis [13,15]. This leads to the accumulation of DNA damage and mutations that give rise to cell transformation and carcinoma development [19].

Yu et al investigated the relation between high-risk HPV-16 infection and p53 mutation in lung carcinomas and its association with tumor behavior. The study indicated that mutation in the p53 and HPV-16 infection might coordinate the development of lung squamous cell carcinomas, and their coexistence was associated with a poor prognosis [20].

Similarly, Fujita et al investigated HPV infection and the expression of p53 in verrucous carcinoma (VC). The presence of HPV-16 DNA and the E6 protein was inversely associated with the expression of p53. Oral VC tumorigenesis may involve the inactivation of p53, which is associated with HPV infection [21].

The E6 protein of HPV-16 is a small polypeptide (150 amino acids) that contains two zinc-binding domains [22]. This protein has a transcription-modulatory activity for a wide variety of viral promoters, mainly adenovirus or herpes simplex virus [23].

The mechanism in the regulation of the E6 HPV-16 gene expression was induced by post-transcriptional RNA splicing. Kezhi Lin et al established a method to specifically amplify E6 HPV-16 associated transcripts. Six groups of E6 transcription patterns were identified from HPV-16 positive cervical tumor tissue. The transcription pattern of the E6 HPV-16 gene was closely associated with the stages of cervical carcinogenesis, and may also be involved in the development of cervical cancer [24].



**Figure 2:** HPV-16 genome, early genes are designated E1 to E7 and late genes, L1, and L2 and description of their functions are shown in this figure. E6 and E7, oncoproteins, are indicated in red boxes, E1-E5 in green boxes and capsid proteins in blue boxes

Other studies about regulation of the E6 HPV-16 gene expression showed that mouse fibronectin (FN) gene as a putative cellular target whose expression is up-regulated by E6 oncoprotein. E6 HPV-16 transactivates the FN promoters in a p53-independent manner [25]. Notch1 gene is a determinant of keratinocyte differentiation and functions as a tumor suppressor in mammalian epidermis. This gene can be down-regulated by E6 through p53 degradation in normal human epithelial cells [26]. On the other hand, the involvement of HPV-16 infections in oral cavity of squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC) remains elusive [27,28].

A protein known as, Foxhead box M1

(FOXM1), regulates cell cycle so as to promote tumor progression. Also this protein is upregulated by E2F1, which is released by pRb phosphorylation through p53 inactivation [29]. FOXM1 interacts with E7 HPV-16 to promote the transformation of primary embryo fibroblasts and FOXM1 also interacts with E6 HPV-16 to increase the expression of mediated NKX2-1 (also known as thyroid transcription factor 1). Consequently, FOXM1 induced by the E6/NKX2-1 axis is responsible for HPV-mediated tumor progression and metastasis [29,30].

#### 4. Conclusions

Nowadays, there is a lot of analytical and clinical information about HPVs available that can be used for detection, development the vaccines against these viruses, and in the prognostic assessment of the patients who have developed cancer.

But further insights into the mechanistic actions of the E6 and E7 oncoproteins, on tumor progression at a level of regulation expression of these genes are needed for to support the fight against different types of cancer that are caused by HPVs.

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#### Conflicts of Interest

The authors declare they have no potential conflicts of interests concerning drugs, products, services or another research outputs in this study. The Editorial Board declares that the manuscript met the ICMJE „uniform requirements“ for biomedical papers.

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