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The Human Papillomavirus E6 Protein

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Abstract:

The association of human papillomavirus (HPV) infection with various forms of cancer, including oropharyngeal, cervical, and anal cancers, presents a significant global health concern. The HPV E6 oncoprotein plays a crucial role in promoting carcinogenesis by disrupting key cellular pathways involved in cell cycle control, apoptosis, and immune evasion. This review provides a comprehensive analysis of the molecular mechanisms by which HPV E6 induces cancer, with a particular focus on its association with cellular proteins and its role in the progression of malignancies associated with HPV. Additionally, therapeutic approaches targeting HPV E6 are discussed, including small molecule inhibitors, RNA interference strategies, and immunotherapies. These therapeutic modalities show promising potential for selectively targeting HPV-infected cancer cells and improving treatment outcomes for HPV-associated malignancies, particularly cervical cancer. Overall, understanding the molecular mechanisms driven by HPV E6 is essential for the development of targeted therapies and preventive strategies against HPV-induced cancers.

Keywords: E6 protein; Human Papillomavirus; cancer.

1. Introduction

Papillomaviruses constitute a highly diverse family of viruses classified into a hierarchical taxonomic system based on nucleotide sequence similarities in their major capsid protein L1. At the highest level, papillomaviruses are divided into genera when their L1 sequences share less than 60% identity. There are currently 38 established papillomavirus genera, reflecting this degree of genetic divergence. Within genera, papillomavirus species are delineated by sharing 60–70% L1 sequence homology. Finally, virus types represent further sub-classifications within species, exhibiting 71–89% L1 sequence identity, indicative of their early genetic relatedness [1]. This systematic classification allows researchers to study the diversity, evolution, and pathogenicity of different papillomavirus clades. Human papillomavirus (HPV) infects keratinocytes in the basal layer of the stratified epithelium at various anatomical sites, and its replication cycle is intricately linked to the differentiation of infected cells. HPV encompasses numerous types, with mucosal HPV being the most common. Among these, high-risk types like HPV-18 pose a significant threat, being implicated as the primary cause of cervical cancer [2]. At present, there are three prophylactic vaccines on the market. All these vaccines consist of recombinant HPV L1 capsid proteins, which self-assemble into VLPs. These VLPs stimulate a robust B-cell-mediated immune response, which in turn generates type-specific antibodies at high concentrations. Long-lasting immunity can be achieved through vaccination, as the immune response to vaccination is preferable to that of natural infection [3, 4]. All three vaccines are administered as three injections over six months [5]. Its association with numerous varieties of cancer highlights the importance of HPV in the field of oncology. Multiple malignancies, including cervical cancer, anal cancer, oropharyngeal (throat) cancer, vulvar cancer, vaginal cancer, and penile cancer, have been associated with HPV infections. Treatment options for HPV-related cancers are limited and often include surgical procedures, chemotherapy, and radiation therapy, each with its own set of side effects. While it is unlikely that the vaccines will have a significant impact on the incidence of cervical and other HPV-associated malignancies for decades [6], numerous studies have already shown a reduction in the occurrence of HPV infections, precancerous lesions, and genital warts.

The E6 protein is one of the viral oncoproteins that may promote the appearance or development of cancer despite lacking any intrinsic enzymatic activity. E6 exerts its oncogenic effects primarily by deregulating pivotal cellular pathways involved in processes such as cell cycle control, apoptosis, telomerase activation, and genomic instability. E6 participates in the transformation of infected cells,

which will disrupt the normal operation of the cell cycle and lead to uncontrolled cell change. The p53 protein is a critical component in preventing the development of cancer through its ability to initiate cell cycle inhibition, apoptosis, DNA damage, and other forms of cellular stress. Inhibiting the function of the tumor suppressor protein p53 can promote the immortalization of HPV-infected cells. In the development of cancer, the E6 that binds to and targets P53 can play a necessary role. E6 may also help in avoiding the host immune system. By disrupting normal cell cycle regulation and inhibiting p53 operation, E6 can help infected cells avoid detection and elimination from the immune system. E6 is the principal component of the productive life cycle of the virus. It can result in the replication and maintenance of the viral genome within infected cells. E6 commonly promotes connecting with another HPV oncoprotein called E7. The transformation of host cells was catalyzed by E6 and E7 interactions. In the development of preventative and therapeutic strategies for HPV-associated diseases, especially cervical cancer, molecular mechanism research on the E6 protein should take precedence. Vaccines, including the HPV vaccine, which targets particular viral proteins and is crucial in averting HPV infections and related malignancies, have undergone a multitude of advancements [7].

The objective of this exhaustive review is to examine the molecular mechanisms through which the oncogenic effects of the HPV E6 protein are exerted. Our focus will be on the manner in which E6 downregulates critical cellular processes. In addition, we shall evaluate the viability of employing the E6 protein as a target for therapeutic purposes in the context of HPV-associated cancers.

2. The role of the HPV E6 protein in cancer development

The E6 gene, which encodes approximately 150 amino acids, is situated upstream of the E7 open reading frame (ORF). The HPV E6 protein has five functional domains (I~V). The domain I has 1~29 amino acids located at the N terminal; the domain II is located at the zinc finger zone I, which includes the 30~66-position amino acids. The zinc finger structure (Figure 1) is the E6 function component and plays a crucial role in transcription, transformation, immortalization, and binding to cellular proteins. Domain III contains an amino acid at position 67–102 in the protein's central zone; domain IV contains an amino acid at position 103–139 in zinc finger zone II; and domain V contains an amino acid at position 140–151 in terminal C [8]. By inhibiting apoptosis, interfering with intercellular communications, and modifying transcription mechanisms, the HPV E6 protein can establish connections with a variety of proteins within the host cell in order to initiate a cascade of events that culminate in the proliferation of cancerous cells. The cells of the host will undergo a malignant transformation upon interaction with E5, E6, and E7 proteins. The long peptide LXXLL, which is abundant in leucine, mediates the interaction between E6 and the target protein of host cells. LXXLL will be incapable of binding to E6AP and, consequently, unable to interact with p53 in the absence of LXXLL [9]. Cytokine interaction between E6 protein and LXXLL polypeptide disrupts the growth factor dependence of host cells, regulates transcription, impairs apoptosis, damages the immune systems of hosts, and induces damage accumulation in cells; these effects will manifest in the growth cycle of cells.

The E6 protein, despite lacking enzymatic activity, is a key factor in HPV-associated cancer. Through various signaling cascades, it can activate telomerase, degrade and inactivate p53, and induce abnormalities in other tumor suppressor genes. The p53 protein serves as a well-established tumor suppressor, capable of enhancing the expression of p21, which inhibits cyclin-dependent kinase two and reduces the G1/S transition upon activation [10]. Conversely, p53 inactivation leads to the accumulation of abnormal cells and an increased cancer risk. E6's ability to degrade p53 prevents apoptosis in host cells with damaged DNA, thereby facilitating HPV-associated carcinogenesis. Additionally, E6 inhibits extrinsic apoptosis signaling by interacting with TNFR-1, FADD, and Lamin 8, and it can also hinder apoptosis induced by Fas and degrade FADD. The Wnt signaling pathway, which is involved in the development, proliferation, differentiation, adhesion, and cellular polarity, is deregulated in various neoplasms and linked to HPV-related cancers. The PI3K/Akt pathway, crucial for cancer cell survival, has been targeted for cancer treatment. Studies have demonstrated that E6 can activate this pathway by inactivating PTEN through PDZ proteins, resulting in increased pAkt levels and cell proliferation. E6's role in telomerase activation was discovered in 1996, and subsequent research revealed the critical role of E6AP in regulating hTERT. Further investigation identified NFX1-91's protein-protein interaction with the HPV E6/E6AP complex.

Moreover, when the E6 protein cooperates with E7 protein 7, it will enhance cell proliferation. E6 and E7 interact with each other, which can cause cells to split up without control [8]. This can make cells multiply faster and look abnormal, and quickly splitting up cell masses is a clear sign of cancerous growth. Interacting with cellular proteins involved in apoptosis regulation can disturb the apoptotic pathways. This suppressor of apoptosis can enable HPV-infected cells to survive and accumulate additional changes, contributing to the progression towards

malignancy [11]. E6 may also help avoid the host immune system. By disrupting normal cell cycle regulation and inhibiting p53 function, E6 helps infected cells avoid detection and elimination by the immune system [12]. In particular, HPV DNA, including the E6 gene, can be integrated into the host cell genome. This integration is associated with a higher risk of cancer development. Integrated E6 genes may undergo changes that enhance their oncogenic potential, which may lead to the durable expression of E6 and the progression of infected cells toward a cancerous state. Understanding the molecular mechanisms driven by E6 will play a necessary role in developing targeted therapies and preventive strategies against HPV-associated cancers.

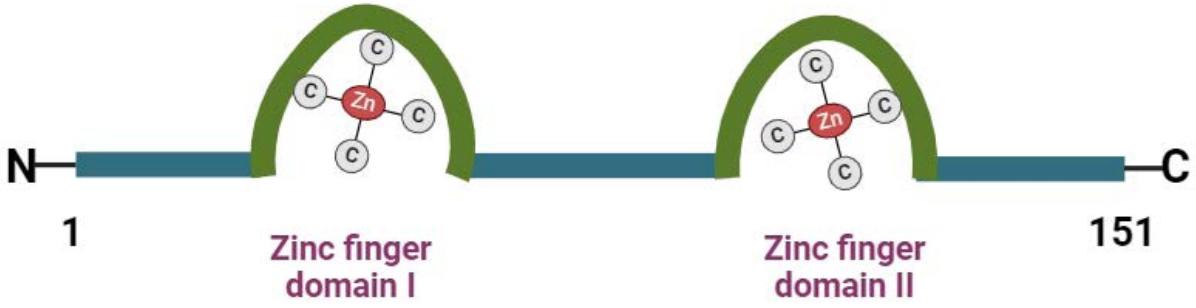

Figure 1. Schematic of the HPV E6 zinc finger structure

3. Therapeutic Approaches Targeting HPV E6

Given the pivotal role of the HPV E6 oncoprotein in driving HPV-associated carcinogenesis, it represents an attractive therapeutic target. Several approaches to inhibit or counteract the oncogenic functions of E6 are under active investigation. Treatment strategies can intervene at various levels, including reducing HPV and oncogene expression using short hairpin RNAs (shRNAs) or small molecule inhibitors, targeting cellular signaling pathways with specific inhibitors or phytochemicals, and inducing cancer cell death through chemotherapy, radiotherapy, or immunotherapy. HPV oncogenes E5, E6, and E7 activate cellular signaling pathways to promote cancer cell survival. When E6 dysregulates signaling pathways in cancer, modulation of these pathways holds therapeutic implications for treatment. Small molecules can be utilized to reverse these altered pathways, particularly targeting the main signaling pathways involved in HPV-associated cancers. Small molecule compounds that can disrupt the interactions between E6 and its key cellular binding partners, such as p53 and E6AP, are being developed. Preventing E6 from binding and triggering the degradation of p53 could restore this critical tumor suppressor pathway. Likewise, inhibiting the E6-E6AP interaction may impede E6's ability to promote telomerase activation and immortalization. Additionally, small molecules targeting signaling nodes dysregulated by E6, including the PI3K/Akt, EGFR, ERK, VEGF, and mTOR pathways, are being explored as therapeutic strategies. RNA interference (RNAi) strategies using short-hairpin RNAs (shRNAs) or siRNAs to deplete E6 expression represent another therapeutic modality. Reducing E6 levels could resensitize HPV-positive cancer cells to chemotherapy and radiation by reactivating p53 and other tumor suppressive pathways subverted by E6. Clinical trials evaluating intratumoral injections of E6/E7-targeting shRNAs in HPV-associated cancers are underway [13]. Since the sustained expression of E6 and E7 oncoproteins is a hallmark of HPV-driven cancers, they serve as attractive tumor-specific antigens for immunotherapeutic targeting. Therapeutic cancer vaccines delivering E6 and E7 antigens aim to elicit robust anti-tumor immunity. These can take the form of peptide or protein-based vaccines, virus-like particles, or naked DNA vectors encoding E6/E7. Innate immune stimulants like the toll-like receptor agonist imiquimod may be synergistic when combined with these vaccines. In summary, the HPV E6 oncoprotein is a compelling target for molecular therapeutic intervention, given its multifaceted role in HPV oncogenesis. Continued research into E6 inhibitors, RNAi, and immunotherapies could yield more effective treatments for HPV-associated malignancies.

4. Summary

HPV-induced cancers are expected to remain a significant global health challenge for the foreseeable future. The sustained expression of E6 oncogenes is critical for the growth and survival of HPV-positive cancer cells, often in conjunction with other cellular alterations. E6 presents promising therapeutic targets, as its inhibition rapidly induces senescence in HPV-positive cancer cells, primarily by restoring the anti-proliferative p53 signaling pathways. Cervical cancer, predominantly caused by HPV infection,

is a major health concern, particularly affecting women in underserved regions. Due to challenges in early diagnosis and poor prognosis, cervical cancer has a high mortality rate. Current therapeutic strategies focus on modulating E6 activity, with approaches targeting E6 demonstrating significant efficacy in selectively targeting malignant cells. These approaches include various forms of vaccines, advanced genome editing techniques, RNA interference, and small-molecule compounds designed to inhibit E6 activity, leading to reduced populations of HPV-infected cervical cancer cells. Cell-mediated immunotherapy has emerged as another successful treatment modality aimed at eradicating HPV-infected tumor cells. These therapeutic strategies not only effectively address E6-associated symptoms but also contribute significantly to ongoing research efforts in this field, offering promising avenues for combating HPV-induced cancers.

References

[1]de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004 Jun 20;324(1):17-27.

[2]Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020 Jul;40(5):602-608. doi: 10.1080/01443615.2019.1634030. Epub 2019 Sep 10. Erratum in: J Obstet Gynaecol. 2020 May;40(4):590.

[3]Naud PS, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, Zahaf T, Catteau G, Geeraerts B, Descamps D. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother. 2014; 10:2147–2162.

[4]Nygard M, Saah A, Munk C, Tryggvadottir L, Enerly E, Hortlund M, Sigurdardottir LG, Vuocolo S, Kjaer SK, Dillner J. Evaluation of the Long-Term Anti-Human Papillomavirus 6 (HPV6), 11, 16, and 18 Immune Responses Generated by the Quadrivalent HPV Vaccine. Clin Vaccine Immunol. 2015; 22:943–948.

[5]Schiller J, Lowy D. Explanations for the high potency of HPV prophylactic vaccines. Vaccine. 2018 Aug 6;36(32 Pt A):4768-4773.

[6]Barr E, Tamms G. Quadrivalent human papillomavirus vaccine. Clin Infect Dis. 2007 Sep 1;45(5):609-7.

[7]Cheng L, Wang Y, Du J. Human Papillomavirus Vaccines: An Updated Review. Vaccines (Basel). 2020 Jul 16;8(3):391.

[8]Tomaić V. Functional Roles of E6 and E7 Oncoproteins in HPV-Induced Malignancies at Diverse Anatomical Sites. Cancers (Basel). 2016 Oct 19;8(10):95.

[9]Ansari T, Brimer N, Vande Pol SB. Peptide interactions stabilize and restructure human papillomavirus type 16 E6 to interact with p53. J Virol. 2012 Oct;86(20):11386-91.

[10]He G, Siddik ZH, Huang Z, Wang R, Koomen J, Kobayashi R, Khokhar AR, Kuang J. Induction of p21 by p53 following DNA damage inhibits both Cdk4 and Cdk2 activities. Oncogene. 2005 Apr 21;24(18):2929-43.

[11]Pal A, Kundu R. Human Papillomavirus E6, and E7: The Cervical Cancer Hallmarks and Targets for Therapy. Front Microbiol. 2020 Jan 21;10:3116.

[12]Pal A, Kundu R. Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy. Front Microbiol. 2020 Jan 21;10:3116.

[13]Jung HS, Rajasekaran N, Ju W, Shin YK. Human Papillomavirus: Current and Future RNAi Therapeutic Strategies for Cervical Cancer. J Clin Med. 2015 May 21;4(5):1126-55.