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# The Human Papillomavirus E2 Protein

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**Abstract:**

The human papillomavirus (HPV) is the predominant viral infection of the reproductive system, leading to a range of diseases in both males and females, including precancerous lesions that have the potential to develop into cancer. Certain HPV infections can cause the development of genital warts, while others can lead to the formation of abnormal cells that have the potential to progress into cancer. Although preventative vaccines are accessible and have shown encouraging outcomes, the worldwide occurrence and death rates of HPV-related malignancies continue to be elevated, especially in low- and middle-income nations. Currently, there is no known remedy for HPV infection, and innovative therapeutic methods such as immunotherapy have emerged as successful tactics for treating and eradicating malignancies caused by the virus. The oncoproteins E6 and E7 have been widely employed in early immunotherapies, such as HPV therapeutic vaccines, with the goal of treating related illnesses and cancers by inducing a strong cellular immune response to eradicate infected cells.

Moreover, the E2 protein shows great potential in the advancement of HPV therapeutic vaccines because of its crucial function in controlling viral gene expression and replication. This publication provides an overview of the HPV genome and emphasizes the crucial significance of the E2 protein in the life cycle of HPV. We provide an overview of the present state of E2-targeting techniques and examine their potential for use in cancer therapy.

**Keywords:** HPV; E2 protein; HPV-related disease.

## 1. Introduction

Human papillomaviruses (HPVs) are a collection of tiny, non-enveloped viruses made up of double-stranded DNA. The HPV genome is comprised of three separate sections, each responsible for encoding specific functional proteins. The E portion of the papillomavirus genome harbors genes (E1, E2, E4, E5, E6, and E7) that are responsible for viral DNA replication, transcription, and suppression of the immune system. The L region contains the genetic information for two main structural proteins, L1 and L2, that play a crucial role in the creation of the viral capsid. The upstream regulatory region (URR), also known as the Long Control Region (LCR) or non-coding region, contains regulatory elements that control the expression and replication of viral genes [1]. Currently, there are over 200 known variations of HPV, and at least 40 of these variations can infect the genital tract. The genotypes are classified into low-risk and high-risk kinds depending on their correlation with cancer.

There are 15 variants of high-risk HPV (HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) that have been identified as causing invasive cancer. HPV 16 and HPV 18 are the predominant kinds of human papillomavirus (HPV) that cause cervical cancer, making up over 70% of all cases [2]. In recent years, prophylactic HPV vaccines have demonstrated a high level of effectiveness in preventing HPV infections. There are currently three widely available forms of HPV vaccines: the 2-valent vaccination (Cervarix), the 4-valent vaccine (Gardasil), and the 9-valent vaccine (Gardasil 9). These vaccines specifically target different strains of HPV and utilize different immunization dosages and techniques. The efficacy of all three preventive HPV vaccinations has shown good results in avoiding HPV infection. They are highly productive in mitigating the likelihood of contracting HPV-related illnesses, such as cervical cancer, as well as other cancers associated with HPV and genital warts [3]. The global introduction of HPV vaccination programs has greatly contributed to the decrease in HPV-related illness and death on a large scale. Although preventive HPV vaccinations have demonstrated efficacy in preventing new infections, persons who are already infected do not experience the same advantages. As a result, individuals, especially those in disadvantaged groups with restricted access to screening and immunization programs, nevertheless suffer from cervical cancer, genital warts, and other illnesses caused by HPV [4]. Although there have been notable progres-

sions, cervical cancer continues to be a substantial worldwide public health issue, with 662,301 newly diagnosed cases and 348,874 fatalities reported in 2022 [5].

At present, surgical treatments and cytotoxic medications are the main methods used to manage cervical cancer. However, there is currently no viable treatment to eliminate HPV infection and prevent the development of cancer. Nevertheless, due to the crucial roles played by different HPV proteins, multiple treatment medications and techniques are currently being developed [6]. Although most techniques primarily target the oncogenes E6 and E7, a significant challenge that has to be addressed is the immune evasion mechanisms employed by cells infected with HPV. Hence, it is imperative to specifically focus on targeting supplementary proteins or antigens that are unique to the tumor in order to generate more potent and long-lasting immune reactions [4]. The E2 protein, because of its crucial function in the HPV infection process, has great potential. This study seeks to investigate the role of E2 in order to reveal its potential in the treatment of HPV infection.

## 2. HPV life cycle and E2 protein functions

HPV invades developing epithelial cells via small traumas. The virus initially gains entry into the cell through endocytosis, which is made possible by the contact between the viral capsid proteins L1 and L2 and receptors on the epithelial cells [7]. Afterwards, the virus enters the nucleus of the infected cell in order to carry out transcription and reproduction. The proteins E1 and E2 are expressed early and have important functions in controlling the life cycle of the host cell and gene replication. During the process of cellular development, infected cells continue to replicate the virus, reaching a later stage of viral replication. In this stage, L1 proteins spontaneously form a five-part shell in the cytoplasm, which is then moved to the nucleus. Viral particles are formed when L1 proteins and L2 proteins come together within the nucleus [8]. Ultimately, these viral particles are expelled from the cell by shedding and breakdown, mainly happening in deceased superficial epithelial cells. This step facilitates the transmission of the virus and the establishment of infection in adjacent cells.

The E2 protein has a crucial function in the life cycle of HPV, specifically in the process of viral replication. The process of viral replication is dependent on the interaction between proteins produced by the host cell and proteins encoded by the virus, such as the E1 and E2 proteins. The E2 protein is composed of a transactivation domain, which is made up of 201 amino acids at the N-terminus, and a DNA-binding domain, which consists of 84 amino acids at the C-terminus. The linkage between these two sections is facilitated by a hinge region that is abundant in serine and arginine [9]. Usually, E2 acts as a dimer and attaches to particular E2 binding sites (BSs) in the long control region (LCR) of the virus using its DNA-binding domain. Afterward, E2 enlists the DNA helicase E1 by means of its transactivation domain, thus commencing viral replication. In addition, E2 assists in the recruitment of additional cellular replication components, supporting both the initiation and elongation stages of DNA replication [10]. E2 proteins play a vital function in preserving the episomal state of HPV by attaching viral DNA to the chromatin of the host. The anchoring mechanism in question relies on the transactivation domain of E2, which forms interactions with specific cellular proteins located on the host chromosome, including human bromodomain protein 4 (Brd4) [11].

Furthermore, E2 acts as a transcriptional repressor for the viral E6 and E7 oncoproteins. It is also involved in apoptosis, which is controlled by many mechanisms, including p53-dependent and p53-independent pathways, receptor signaling pathways, and mitochondria-dependent processes. Research has shown that E2 can trigger apoptosis indirectly via altering the expression of E6 and E7 and directly by interacting with p53 [12]. Within high-risk HPV strains, E2 controls the process of transcribing E6 and E7 by inhibiting the function of certain transcription factors. Specifically, the promoter region of the E6/E7 oncogenes harbors binding sites for the E2 protein. When E2 dimers attach to these locations, they replace transcription factors such as Sp1 and TFID, leading to the suppression of the promoter and consequent hindrance of oncogene transcription [13]. Furthermore, studies indicate that E2 can control RNA processing, which in turn affects the regulation of translation. The several activities of the E2 protein in the HPV life cycle are emphasized, demonstrating its crucial importance and promise as a target for therapeutic therapies [14].

## 3. E2 Protein in HPV-related Disease

HPV is mainly spread by direct contact between the skin of infected individuals and the skin of uninfected individuals, particularly targeting specialized layers of flat cells called squamous epithelium. It can infect almost any region of the human skin. Low-risk HPV infections commonly present as benign genital warts. Nevertheless, these warts have been linked to malignancies of the vulva, vagina, oral cavity/pharynx, penis, and anus [15]. Fortunately, the majority of HPV infections, approximately 70%, resolve spontaneously within one year, and over 90% resolve within two years. Only a minority of persistent

infections have the potential to give rise to aberrant cells that can potentially grow into cancer [16]. The immune system plays a vital role in regulating HPV infections. T lymphocytes and innate lymphoid cells (ILCs) identify contaminated cells and secrete cytotoxic agents to eradicate them.

Furthermore, the immune system is activated and produces antibodies to provide defense against future infections caused by the same strain of HPV [17]. Nevertheless, the capacity of HPV to sequester itself within epithelial cells can impede the initiation of the immune system, resulting in ineffective immunological responses. Immunosuppressed populations and individuals with impaired immune systems, such as those with HIV, have a greater likelihood of developing HPV-associated malignancies. This highlights the significance of preserving a robust immune system in the prevention of illnesses and malignancies associated with HPV [18]. Persistent HPV infections result in the disruption of normal cellular functions and the disturbance of late events in the viral life cycle. This is caused by the integration of viral DNA with host DNA and the continuous production of the viral proto-oncogenes E6 and E7 [19]. The development of a disease is associated with the disturbance or absence of transcriptional repression produced by the viral tumor suppressor protein E2. Genital warts are formed when the E2 protein's function is blocked, which results in higher levels of HPV gene transcription and replication. This also stimulates cell proliferation, ultimately causing the development of genital warts.

Moreover, the lack of the E2 protein has been detected in numerous cervical cancer cell lines that exhibit viral oncogenes. Reintroducing E2 into cervical cancer cell lines has been discovered to have harmful consequences on cell growth. E2's capacity to suppress the production of natural E6/E7 and trigger cell cycle arrest in the G1 phase, as well as apoptosis, is responsible for this phenomenon [20]. Therefore, the absence or interruption of E2 function is crucial in the advancement of HPV-related illnesses, specifically cervical cancer.

## 4. Therapeutic Targeting of HPV E2

Currently, the main approaches for treating cervical cancer are surgical interventions and cytotoxic drugs. Unfortunately, there is presently no efficacious medication accessible to eliminate HPV infection and avert the progression of malignancy. However, because multiple HPV proteins play a key role, continuing research is being conducted to develop therapeutic drugs and procedures that precisely target the E2 protein [6]. Based on the replication mechanism of HPV DNA, the development of inhibitors that disrupt the HPV E1-E2 interaction and E1 helicase activity is a promising therapeutic approach. By the high-throughput screening, the first series of inhibitors of the HPV11 indanones to block the E1-E2 complex and the viral DNA replication initiation assembly were discovered, and more active analogs - indandione inhibitors - have been synthesized [21]. These inhibitors were found to act by binding to the E2 transactivation domain, blocking the binding of the E1 protein. Although indandione inhibitors demonstrated strong efficacy against the E2 proteins of low-risk HPV6 and HPV11, they exhibited no efficacy against high-risk HPV16 and HPV18 [22].

Furthermore, the suppression of E1 helicase activity can also efficiently impede the replication of viral DNA.

Consequently, certain pharmaceutical companies have allocated resources towards the discovery of inhibitors that target the E1 ATPase/helicase. Regrettably, despite the discovery of tiny compounds that impede the function of this enzyme, none of these endeavors have yielded a feasible remedy [6]. The E2 protein has substantial potential in the advancement of HPV therapeutic vaccines because of its critical function in controlling viral gene expression and replication. Therapeutic vaccine formulations that specifically target the E2 protein have the potential to interfere with the expression of E6 and E7, resulting in the inhibition of cells infected or transformed by HPV. Furthermore, vaccinations based on E2 could provide benefits in terms of safety, as they can specifically target viral regulatory proteins, potentially reducing the likelihood of autoimmune reactions against the body's antigens.

Many studies have shown the effect of E2 on the development of vaccines for precancerous lesions. The therapeutic HPV vaccine MVA E2 uses the Modified Vaccinia Ankara (MVA) vector encoding the E2 protein of HPV-16 and HPV-18. The MVA virus is a very weakened strain of the poxvirus that has been derived from the smallpox vaccine. Its effectiveness as a vaccine carrier has been thoroughly tested and verified, ensuring its safety. Furthermore, the vaccinia virus is a type of virus that contains two strands of DNA and has a substantial and unchanging genetic material that can produce a wide range of antigens. This virus has been extensively utilized as an immunogen [23]. Studies have demonstrated that MVA E2 effectively halts the proliferation of human tumors in mice and triggers the regression of tumors in rabbits with existing tumors. A phase III trial showed that MVA E2 exhibited a 90% effectiveness rate in treating anogenital intraepithelial lesions generated by HPV.

Furthermore, it has been seen that patients have generated a targeted and destructive immune response to cells that have been changed by papillomavirus. This finding indicates that the MVA E2 therapeutic vaccination can

stimulate long-lasting immunity and effectively prevent recurrence in the majority of patients [24]. Nevertheless, further research is necessary to fully understand the marketing and utilization of these vaccines. This is because it remains uncertain whether the antitumor effect is a result of the immune response triggered by the vaccine or the suppression of E6/E7 expression facilitated by the E2 protein.

At present, there is a lack of commercially accessible therapeutic HPV vaccines, and the creation of such vaccines requires the identification of new targets. The E2 protein is currently under investigation as a potential target for the creation of vaccines due to its significant importance. Nevertheless, due to the significant upregulation of the E2 gene during the initial phase and its subsequent removal following chromosomal integration, a vaccine that specifically targets the E2 protein is more beneficial for treating precancerous conditions rather than cancer itself [4].

## 5. Summary

The HPV genome is structured as double-stranded DNA, containing both early and late genes responsible for regulating viral replication and transcription alongside structural proteins composing viral particles. HPV infections are closely linked to various diseases, notably cervical cancer. Continued research into the HPV genome and its pathology offers promising avenues for addressing the associated cancer burden, with a particular focus on strategies targeting the E2 protein. The E2 protein holds a pivotal regulatory role in the HPV life cycle and is actively involved in governing viral replication, transcription, and gene expression. Disruptions or deletions in E2 function can result in genomic instability and abnormal expression of the HPV genome, potentially contributing to cellular transformation and the oncogenic process. Therapeutic approaches aimed at the HPV E2 protein are in development, aiming to hinder HPV replication and the persistence of viral infection by disrupting E2 binding or function.

Additionally, inhibiting the transcription of E6 and E7 oncoproteins shows promise in curtailing cancer development. Nevertheless, while targeting E2 presents a promising avenue, further understanding and research are required to develop more effective and safer therapeutic strategies for treating HPV-associated diseases. This ongoing effort is essential for advancing the management and prevention of HPV-related conditions, ultimately improving patient outcomes.

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