



Unraveling the multifaceted molecular interactions of HPV E6 in carcinogenesis

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ABSTRACT

Background: Human Papillomaviruses (HPVs) are common sexually transmitted viruses that cause health problems, including genital warts and different type of cancers. There are over 200 different types of HPV, some of which are correlated with the progress of cervical cancer. One of the HPV genes responsible for its oncogenic potential is the E6 gene. E6 is a critical protein in the life cycle of HPV and a key contributor to the development of HPV-associated cancers. Its interactions with cellular proteins lead to disruptions in key cellular pathways and the promotion of cancerous cell growth. Overall, HPV E6 represents a reassuring target for the expansion of novel therapies for the treatment of HPV-associated cancers and understanding its molecular interactions with host proteins is critical for developing targeted therapies for HPV-associated cancers. In this article, we will focus on the cancer-related mechanism and cell interaction of HPV E6.

Keywords: Cancer, Viruses, Tumor suppressors, HPV, Cervical cancer, HPV E6.

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Introduction

Human papillomavirus (HPV) infection stands out as one of the most widespread causes of sexually transmitted diseases, impacting both men and women globally. It affects a substantial portion of the population at some point in their lives (1). HPV is characterized by its double-stranded circular DNA and icosahedral capsids (2). While some patients may eliminate an HPV infection within one to two years, around 10% to 20% of women with an HPV infection will experience persistent infection, which is responsible for almost all cervical cancers, and also contributes to the development of other anogenital cancers (1). HPV comprises a diverse group of over 200 closely related viruses, with some transmitted through vaginal, anal, or oral sexual contact. Sexually transmitted HPV types can be categorized into two main groups: low risk and high risk. Low risk group typically do not result in disease, although a few low-risk HPV types can lead to the formation of warts in the genital, anal, oral, or throat areas. On the other hand, high-risk HPVs are associated with the development of various types of cancer. Notably, HPV16 and HPV18 are responsible for the majority of HPV-related cancers (3-6).

The principal cause of HPV-induced cancers is the persistent expression of the viral oncogene, E6. E6, a formidable oncogenic factor found in high-risk HPV strains, has been under extensive investigation for its involvement in the path to malignancy (7). E6's function revolves around its ability to engage with and deactivate a multitude of crucial cellular proteins responsible for regulating processes like apoptosis, transcription of tumor suppressor genes, maintenance of epithelial structure, and regulation of cell growth (8, 9). The cumulative effect of E6's interactions with these proteins significantly enhances the oncogenic capacity of HPV. For instance, E6 hinders apoptosis by directing its attention towards p53, Bak, c-myc, FADD, and procaspase 8, leading to their degradation. As well, throughout the HPV infection process, E6 takes action by attaching to and rendering ineffective several crucial controllers of cell cycle advancement. This action aims to enable the uncontrolled multiplication of infected cells (8-11).

This article delves into the intricate molecular interactions involving the HPV E6 oncoprotein and its collaborative role in the advancement of cancer. We have discussed the multifaceted mechanisms by which E6 interacts with cellular components, shedding light on its role in driving the progression of cancerous conditions.

E6 structure

One of the three recognized oncoproteins linked to the advancement of cancer in HPV-infected cells is the E6 oncoprotein. The E6 oncoprotein, consisting of approximately 150 amino acids, is primarily situated within the cellular nucleus. It is synthesized from the initial open reading frame positioned downstream of the noncoding region of the HPV genome (11, 12). It has two zinc finger domains bordered by four Cys-X-X-Cys motifs located in its N and C terminus. These domains are separated by a brief linker region comprising approximately 35 amino acids. These patterns are consistently preserved in every E6 protein, and their completeness is crucial for the proper functioning of this oncogenic protein (9, 13). Moreover, situated after the C-terminal zinc finger is a concise PDZ-binding motif that eases interactions with proteins containing PDZ domains. The N-terminal and C-terminal zinc fingers are both constructed from a duo of CXXC motifs, while the PDZ-binding motif includes an XTXV/L sub-motif. Collectively, these binding domains equip HPV E6 with the capacity to effectively engage a broad spectrum of proteins, thereby exerting its influence over multiple cellular pathways (9, 14).

Molecular Targets and Functions of the E6 protein

1. Apoptosis inhibition

As illustrated in (Table 1), E6 establishes interactions with a wide range of cellular proteins, revealing insights into its functions and molecular targets. One of the main functions is to degrade the p53 protein in a ubiquitin-dependent manner. E6 interacts with the LXXLL motifs in ubiquitin ligase E6-Associated Protein (E6-AP), then create a heterotrimeric complex E6/E6AP/p53, which triggers p53 degradation (by targeting it for proteasome), resulting in apoptosis evasion (15, 16). Also, by changing the activity of p53 modifying enzymes, E6 prevents p53-mediated cell cycle arrest. It modifies p53 transactivation by prolonging the activation of ATR in response to DNA damage. Furthermore, E6 binds to p300 at several locations and stops p300 from transactivating p53 and also degrades the hADA3 enzyme, which can acetylate the protein p53. To reduce p53's activity and stability, it confines p53 in the cytoplasm by masking its nuclear localization signal, thereby preventing it from controlling gene transcription within the nucleus (9).

In addition, E6 has the ability to target other molecules that play a role in activating various death pathways like pro-apoptotic protein procaspase 8 (CASP8), Bak, tumor necrosis factor receptor 1 (TNFR1), and the adaptor molecule Fas-associated death domain (FADD). E6 accelerates the degradation of procaspase 8 and prevents TNF-mediated apoptosis by binding to TNFR1 and inhibits the Fas/Fas ligand death-triggered pathway to suppress apoptosis (17-20). E6 also activates NF- κ B, which can trigger the transcription of proteins that inhibit apoptosis, such as cellular inhibitor of apoptosis 2 (cIAP-2) (21).

Table 1. Functions and Molecular targets of the E6 protein

Molecular target	Effect	Function	Ref
TNFR1	Blocking	Prevention of Apoptosis	(15, 17-21)
FAS/FASL			
NF-κB	Up-regulating		
CIAP-2			
P53	Down-regulating		
BAK Protein			
Procaspase 8			
IRF3	Down-regulating	immune system control	(22-25)
IFN-α			
IFN-κ			
STAT1			
TLR3			
P300/CBP	Down-regulating	Deregulation of the cell cycle	(26, 27)
NFX1-91	Down-regulating	Cell immortalization	(28, 29)
C-MYC	Up-regulating		
SP1			
SCRIB	Down-regulating	invasion and metastasis	(30-34)
MAG1-3			
PAR3			
Fibulin-1			
Paxillin			
XRCC1	Down-regulating	genomic instability	(35-37)
APOBEC3	Up-regulating		
DNMT1			

2. Innate immunity modulation

E6 reduces the immunological response to HPV infection by downregulating Interferon Regulatory Factor 3 (IRF-3), which is a transcription factor of Interferon β (IFN- β). This direct control of the immune system is one of E6's functions (22). Additionally, E6 blocks the interaction between IFN- α and its receptor, leading to extended HPV replication, by suppressing the phosphorylation of Tyrosine Kinase 2 (TYK2) (23). Moreover, the downregulation of Interferon- κ (IFN- κ) by E6, diminishes the signal transducer and activator of transcription 1 (STAT1), and Toll-like receptor-3 (TLR3) (24, 25).

3. Micro RNAs alteration

E6 is essential for controlling miRNAs linked to cell cycle regulation. For example, the downregulation of miR-34a leads to overexpression of p18^{Ink4c} and entry into the S-phase (38, 39). Furthermore, E6 reduces the amount of miR-23b, a miRNA associated with cell migration via the urokinase plasminogen activator gene (40, 41). By lowering the levels of miR-218, E6 regulates the expression of laminin subunit beta-3 (LAMB3) a protein required for cell growth and movement (42). Table 2 outlines microRNAs that are affected by E6. studies have demonstrated that miR-875 and miR-3144 can reduce the amount of unspliced E6 while promoting the expression of three E6* spliced transcripts (43). E6* is derived from spliced E6 mRNA and is non-oncogenic. However, it has the potential to trigger oxidative stress and elevate levels of free radicals, leading to genetic instability (44). Additionally, these microRNAs are known to decrease the expression of E6/E7 and epidermal growth factor receptor (EGFR) (43). Another survey revealed that the expression of miR-409-3p is notably reduced in cervical dysplastic tissues and is inversely associated with E6 mRNA levels. The function of miR-409-3p is to downregulate E6 in infected cells (45).

Table 2. MicroRNAs affected by E6 and its outcomes

MicroRNA	Effect	MicroRNA target	Outcome	Ref
miR-20a	Upregulating	PDCD6	growth-promoting effects	(46)
miR-218	Downregulation	LAMB3	Facilitate the spread of viral infection and progression of tumorigenesis	(42)
miR-22	Downregulation	histone deacetylase 6 (HDAC6)	promoting cervical carcinogenesis	(47)
miR-34a	Downregulation	p18Ink4c	Identifies p18Ink4c as a possible biomarker for cervical cancer.	(39)
miR-23b	Downregulation	urokinase-type plasminogen activator(uPA)	induce migration in human cervical cancer cells	(40)
miR-24	Upregulating	p27	elevate cell proliferation	(48)

4. Tumor suppressor inhibition

p21 tumor suppressor protein triggers cell cycle arrest at the G1/S checkpoint by acting as a general inhibitor of cyclin/cyclin-dependent kinase (CDK) complexes and DNA replication. The p53 protein controls transcriptionally the production of p21 (49). When DNA is damaged, the accumulation of p53

results in higher amounts of p21, which causes a G1 arrest and allows DNA repair before replication. Therefore, E6 hinders the accumulation of p21 protein in cells with DNA damage by breaking down p53 via the proteasome pathway and suppressing p21 transcription, thereby enabling continued cell growth (50). A study showed that E6 suppresses the transcription of p21^{WAF1} in a manner that is not dependent on p53, by deactivating p150^{Sal2}. This viral protein specifically targets p150^{Sal2}, which is a transcription regulator that promotes the expression of the p21^{WAF1} gene. This leads to the inhibition of G1/S arrest, allowing the cells to proliferate and facilitating efficient viral DNA replication (51). pRb regulates cell cycle by managing the G1 to S phase transition. In a stable state, pRb is unphosphorylated in early G1 and gets phosphorylated progressively as it approaches the S phase. When pRb is not phosphorylated, it interacts with the E2F transcriptional factors and inhibits transcription and cell cycle by inhibiting the E2F factor. E6 promotes the phosphorylation of pRb and the aggregation of the products of genes that are negatively controlled by pRb, including p16^{INK4a}, CDC2, E2F-1, and cyclin A. The E6 proteins significantly boosted the activity of cyclin A/cyclin-dependent kinase 2 (CDK2), which is implicated in pRb phosphorylation. In addition, in E6-expressing cells, the activity of kinases implicated in pRb phosphorylation such as cyclin D/CDK4, and cyclin D/CDK6, complexes are increased. As a result, it is extremely likely that E6 proteins promote proliferation by activating CDK complexes and, as a result, pRb hyperphosphorylation (52).

5. Dysregulation of cell cycle, metastasis and genomic instability

Apart from the mentioned targets, E6 is associated with several other targets that lead to events such as deregulation of the cell cycle, invasion, genetic instability, and so on. E6 has the potential to dysregulate the cell cycle, prevailing its checkpoints and has the ability to deactivate the p300/CBP transcriptional coactivator complex, which is crucial for controlling cell cycle development (26, 27). Unbalanced E6 expression triggers hTERT overexpression, through NFX1-91 (Nuclear transcriptional factor X-box binding 1–91) degradation that leads to immortalization of the cell (28). Additionally, the activation of a promoter that regulates hTERT's epigenetic alterations, accompanied by phosphorylated DNA polymerase II, results in the overexpression of the c-Myc proto-oncogene and the transcriptional factor Sp1, which ultimately results in the cell immortalization (29). The E6 protein mediates two key characteristics of cancer including cell invasion and metastasis. It downregulates several tumor suppressor genes, resulting in cell invasion, including Scribbled Planar Cell Polarity Protein (SCRIB) and Membrane-Associated Guanylate Kinases (MAGI 1-3) (30, 31). Additionally, it has been discovered that each member of the PAR3 family is a target of E6 proteolytic degradation, which promotes cell invasion (32). Moreover, Fibulin1, a critical cellular adhesion protein found in the extracellular matrix and plasma, can be targeted and degraded by E6 (33). Also, it can disrupt the adhesion protein paxillin, which in turn reduces the development of actin fibers and, subsequently, the integrity of the epithelium, causing invasiveness and, eventually, the metastatic procedures (34). E6 protein incorporates genomic instability into the malignant cell by increasing apolipoprotein B, an mRNA editing enzyme catalytic polypeptide 3 (APOBEC3) which led to the replacement of the cytosine residue with uracil. The scaffold DNA repair protein X-ray cross-complementing 1 (XRCC1) is also inhibited by E6, which increases mutation and, consequently, genomic instability (36, 37). It can upregulate DNA methyltransferases DNMT1, which can increase epigenetic alterations and hence cause genomic instability (35). Figure 1 highlights the molecular targets of the E6 protein that are associated with invasion and genomic instability. E6 oncoprotein also can control and modify a number of cells signaling pathways to induce cancer. The Wnt/ β -catenin pathway is activated by E6, which is essential for HPV transformation, immortalization, and disrupting tissue homeostasis (53). The expression of E6 or E7 after an HPV-induced infection triggers the phosphoinositide 3-kinase/protein kinase

B(P13K/AKT) signaling pathway by changing various cellular mechanisms, which results in the development of cancer (54). It has also been revealed that the triggering of the ERK1/2 pathway by the E6 oncoprotein induces the expression of HIF-1 α , VEGF, and IL-8 in NSCLC cells, resulting in enhanced angiogenesis (55).

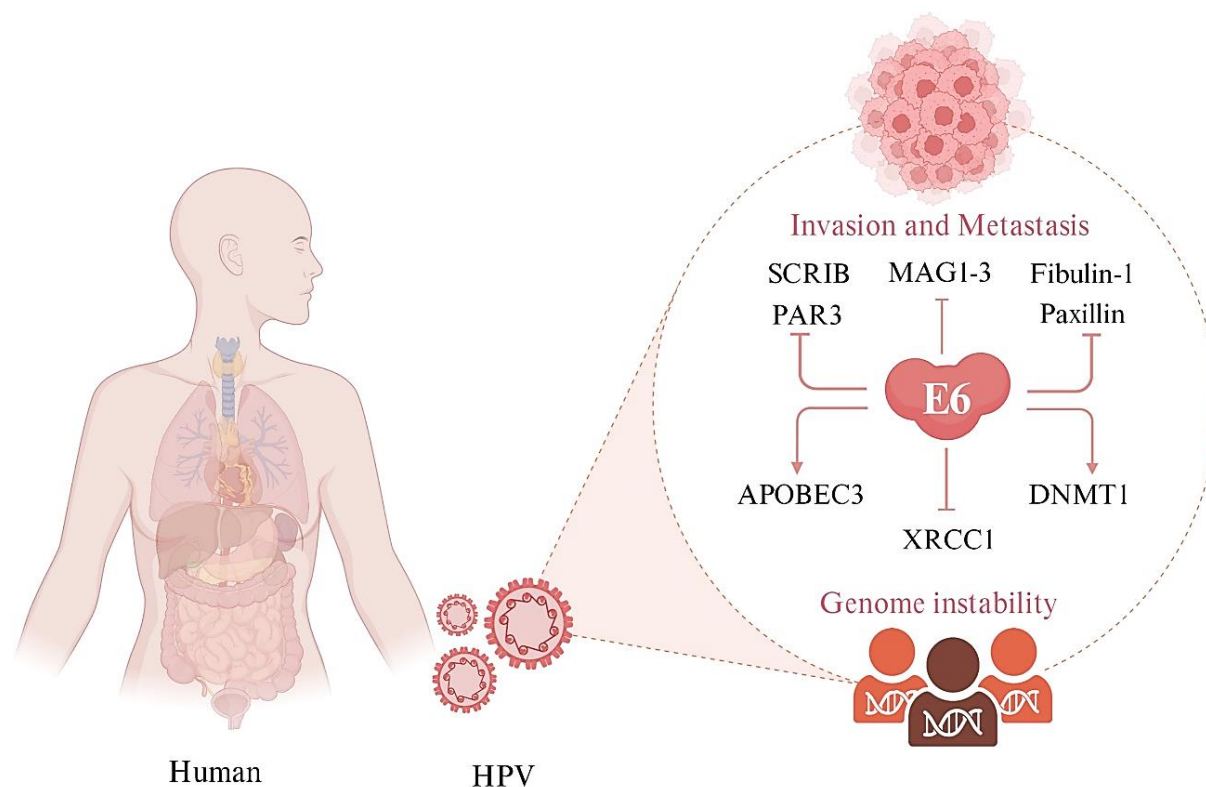


Figure 1. Molecular targets linked to invasion and genomic instability that are affected by the E6 protein. Abbreviation, scribbled planar cell polarity protein (SCRIB); family cell polarity regulator (PAR3); membrane-associated guanylate kinase 1, 2 and 3 (MAGI-1, 2 and 3); X-ray repair cross-complementing 1 (XRCC1); apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (APOBEC3); DNA -methyltransferase 1 (DNMT-1).

Additionally, the Sorting Nexin 27 (SNX27) protein, a crucial regulator of the endosomal transport pathway, can bind to E6. This binding alters the amount of the glucose transporter GLUT-1, which in turn increases glucose absorption by cancer cells (56). Elevated levels of Notch1 signaling lead to a significant reduction in HPV-triggered transcription of the E6/E7 viral genes, accomplished by suppressing the activity of AP-1. This indicates that Notch1 provides targeted protection against HPV-induced transformation by suppressing the expression of E6/E7. It is reported that constant HPV-E6/E7 expression and malignant transformation brought on by HPV require down-regulation of Notch1 signaling (57). Another study revealed that in the initial stages of cellular transformation, starting from mortal primary cells to HPV-immortalized cells and eventually to anchorage independent cells, there is an increase in Notch expression. This upregulation of Notch appears to assist in the early steps of transformation. However, once the transformation process is completed, Notch expression is subsequently downregulated. This downregulation is believed to promote events that induce tumorigenicity (58). Figure 2 highlights the molecular targets of the E6 protein.

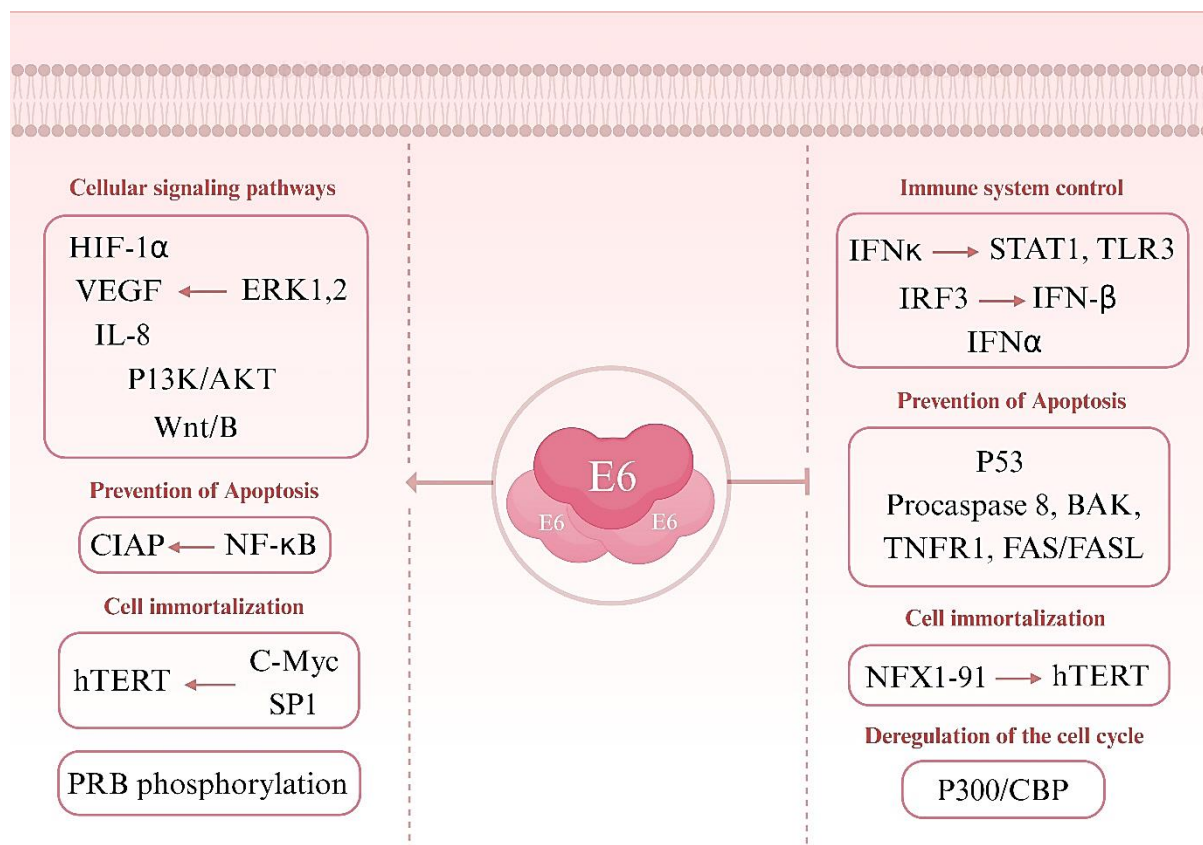


Figure 1. Molecular targets that affected by E6 protein. Abbreviations, Hypoxia-inducible factor-1 alpha (HIF-1α); Vascular endothelial growth factor (VEGF); cellular inhibitor of apoptosis 1 (cIAP1); Human Telomerase Reverse Transcriptase (hTERT); Specific protein 1(SP1); retinoblastoma protein (pRB); Toll-like receptor 3 (TLR3); Interferon regulatory factor 3 (IRF3); Tumor necrosis factor receptor 1 (TNFR1).

HPV E6 as a Potential Target in Innovative Therapeutic Strategies

The initiation of HPV-induced carcinogenesis primarily hinges on E proteins, with E6 emerging as the pivotal oncoprotein among the various forms. This underscores the potential effectiveness of focusing therapeutic efforts on the E6 oncoprotein. Therapeutic strategies focused on targeting HPV E6 represent a crucial frontier in the battle against cervical cancer and related malignancies. HPV E6 is a viral protein notorious for its role in degrading the p53 tumor suppressor protein, facilitating uncontrolled cell growth (15). To combat this insidious mechanism, researchers are exploring a diverse range of interventions, from gene-editing techniques to immunotherapies and Phytotherapy. These innovative approaches offer the potential to disrupt E6's influence, ultimately providing new avenues for the treatment and prevention of HPV-associated cancers (59-61). Numerous gene-editing methodologies are available for the purpose of directing their focus towards E6. The inception of gene editing methods in therapeutic strategies initially relied on tools like antisense oligonucleotides, ribozymes, DNazymes, small interfering RNA (siRNA), and short hairpin RNA (shRNA). However, recent research has ushered in more potent approaches to effectively suppress E6/E7 expression. These advanced techniques encompass zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat-associated nuclease (CRISPR/Cas9) RNA-guided endonuclease, representing a significant evolution in gene-editing capabilities (60-62). In one of the revolutionary efforts to counteract the influence of E6 and E7

oncoproteins, a research group utilized CRISPR-Cas9 technology to manipulate HPV16-infected SiHa cells. Their intervention disrupted the E6 and E7 genes, leading to the restoration of critical tumor-suppressor proteins, specifically p53 and p21. This restoration, in turn, activated apoptosis mechanisms controlled by p53 and arrested the cell cycle. Furthermore, the edited cells were transplanted into immune-deficient mice, resulting in significantly reduced tumor growth compared to untreated cells (59). An alternative strategy for E6-directed treatment involves the field of immunotherapy. Immunotherapies encompass a diverse range of approaches. For instance, they include therapeutic HPV vaccines, which have undergone extensive assessment in various preclinical models and clinical trials. These vaccines employ different modalities, such as live vectors, peptides, proteins, DNA, RNA replicons, and dendritic cell (DC)-based (63). Numerous vaccines, including ProCervix and PDS0101, have been designed with a particular focus on addressing persistent HPV-driven infections and lower-grade squamous intraepithelial lesions (SIL). These vaccines are specifically designed to target the HPV E6 and E7 oncoproteins. In addition, various vaccines have been developed to specifically address advanced-stage cervical cancers by targeting the E6 and E7 proteins associated with HPV16/18. These vaccines include ADXS11-OO1 (Lm-LLo-E7), INO-3112, HPV16-SLP, and TA-CIN + GPI-0100 (60, 62). Another avenue in E6-targeted therapy involves a Phyto-therapeutic approach, which relies on harnessing the potential of natural substances for combating various deadly forms of cancer. Numerous natural compounds have been discovered to effectively counteract HPV-E6/E7 activity. For instance, a group of flavonoids has been pinpointed for their capacity to attach to E6, impeding the degradation of p53 and thereby reducing the survival of cervical cancer cells infected with HPV (64). Additionally, research has demonstrated that Curcumin not only inhibits E6 and E7 transcripts and proteins but also prevents the translocation of NF- κ B and AP1, ultimately inducing apoptosis (65, 66).

In conclusion, the pursuit of E6-targeted therapy represents a dynamic and promising frontier in the battle against HPV-associated cancers. Through various innovative strategies, ranging from CRISPR-Cas9 gene editing to the utilization of natural compounds like flavonoids and Curcumin, significant strides have been made in disrupting the harmful effects of the E6 oncoprotein. These approaches not only restore vital tumor-suppressor mechanisms but also hold the potential to significantly reduce tumor growth and enhance the prospects for cancer treatment.

Discussion

Viruses are responsible for many diseases such as autoimmune disorders and many types of cancers such as cervical, liver and inducing various tumors within the body such as brain tumors (67-69). Diseases caused by the HPV have been associated with a range of malignancies, including cervical, vaginal, vulvar, head and neck, anal, and penile cancers (70). One of the most frequent type of cancer in women globally is cervical cancer, which poses a serious threat to global health (71).

The initiation of HPV replication is significantly influenced by the E1 and E2 proteins. These proteins are recognized for their ability to control both the replication of the viral genome and the transcription of early proteins. For instance, the repression of E6 and E7 is attributed to the E2 gene (62). Mutation, HPV genome integration, and methylation typically inhibit the E2 gene expression. This disruption inhibits viral replication, resulting in abnormal viral E6 expression, which results in cancer progression following virus integration (60, 72).

The primary driver behind HPV-induced cancers stems from the persistent expression of the viral oncogene, E6. E6, a potent oncogenic factor predominantly found in high-risk HPV strains, exerts its influence by engaging with and inactivating a myriad of critical cellular proteins that oversee essential regulatory processes (11, 60). One of its pivotal functions is inhibiting apoptosis, achieved through

interactions with various cellular targets such as TNFR1, FAS/FASL, NF- κ B, CIAP-2, P53, BAK Protein, and Procaspase 8 (15, 17-21). Simultaneously, E6 wields control over the immune system by downregulating factors like IRF3, INF α , INF κ , STAT1, and TLR3 (22-25). Moreover, E6 possesses the capability to disrupt the cell cycle, induce cell immortalization, promote invasion and metastasis, and induce genomic instability (26-29). These multifaceted functions underscore the crucial role of E6 in the development of HPV-related carcinogenesis.

Given the pivotal role of E6 in HPV-related carcinogenesis, it emerges as a compelling candidate for therapeutic intervention in HPV-associated cancers. Diverse strategies have been explored to target E6 proteins, with genome editing technologies offering a potent avenue (61). These techniques specifically aim at the HPV-E6/E7 region of the viral genome or their corresponding mRNAs, presenting a targeted approach for cervical cancer treatment (61, 62). Another approach involves immunotherapies, exemplified by therapeutic HPV vaccines. These vaccines, utilizing live vectors, peptides, proteins, DNA, RNA replicons, and dendritic cells, target the HPV E6 and E7 oncoproteins. Clinical trials and preclinical models have evaluated their potential in combatting HPV-related cancers (63). As well, T-cell mediated immunotherapy represents a promising and emerging treatment modality for eliminating tumorigenic cells infected with HPV. As an example, novel techniques involve the activation of the immune system through the introduction of synthetic HPV 16/18 E6 and E7 DNA sequences. This activation is achieved using an innovative plasmid known as VGX-3100 (73, 74). Another approach combines synthetic plasmids, collectively referred to as MEDI0457 (formerly known as INO-3112), to specifically target the HPV16 and HPV18 E6/E7 antigens. This combination also includes recombinant IL-12, an interleukin-12 variant, serving as a molecular adjuvant (INO-9012). IL-12 plays a crucial role in promoting the maturation and activity of T cells, thereby enhancing immune response effectiveness. MEDI0457 has demonstrated its ability to induce enduring antibody responses, stimulate robust production of HPV-specific IFN- γ by T cells, and facilitate the generation of antigen-specific cytotoxic T cells. Consequently, this enhanced cellular and humoral immune response, when integrated with other treatment modalities, has proven to be effective in reducing the incidence of local recurrence and metastasis rates (62, 75). Finally, a phytotherapeutic approach harnesses natural products for treating various deadly cancer types. Compounds such as curcumin and flavonoids are well-known players, but numerous plant extracts, including *Ficus carica* latex, flax-seed oil, and *Cudrania tricuspidate* stem extracts, have also demonstrated anti-oncogenic properties by inhibiting the expression of E6, offering a promising avenue for therapeutic exploration (66, 76-78). Utilizing natural products for treatment presents a simpler, readily accessible, and cost-effective alternative when contrasted with genome editing technologies or immunotherapeutic approaches. Prophylactic and therapeutic vaccines, T-cell-based therapies, and various genome editing techniques tend to be financially burdensome, making them inaccessible to economically disadvantaged populations.

In conclusion, E6's capability to contribute to HPV carcinogenesis by interacting with various cellular targets highlights its pivotal role in the development of HPV-related cancers. Exploring therapeutic strategies targeting E6 represents a promising frontier in the context of oncology and cancer treatment. Genome editing technologies such as CRISPR/Cas9 enable precise disruption of E6 expression, while immunotherapies like therapeutic HPV vaccines leverage the immune system's potential against HPV-infected cells. Additionally, phytotherapeutic approaches utilizing natural products offer accessible alternatives for treatment. These diverse strategies provide hope for enhanced management and prevention of HPV-related cancers. This is particularly crucial for economically disadvantaged populations disproportionately affected by these diseases. Ongoing research underscores the commitment to finding innovative solutions to combat these challenging health concerns.

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References

1. Scott-Wittenborn N, Fakhry C, editors. Epidemiology of HPV related malignancies. Seminars in Radiation Oncology; 2021: Elsevier.
2. Luria L, Cardoza-Favarato G. Human papillomavirus. 2017.
3. HPV and Cancer - NCI. 2019.
4. Burd EM. Human papillomavirus and cervical cancer. Clinical microbiology reviews. 2003;16(1):1-17.
5. Dunne EF, Park IU. HPV and HPV-associated diseases. Infectious Disease Clinics. 2013;27(4):765-78.
6. Brianti P, De Flammoneis E, Mercuri SR. Review of HPV-related diseases and cancers. New Microbiol. 2017;40(2):80-5.
7. Yeo-Teh NS, Ito Y, Jha S. High-risk human papillomaviral oncogenes E6 and E7 target key cellular pathways to achieve oncogenesis. International journal of molecular sciences. 2018;19(6):1706.
8. Tungteakkhun SS, Duerksen-Hughes PJ. Cellular binding partners of the human papillomavirus E6 protein. Archives of virology. 2008;153:397-408.
9. Wallace NA, Galloway DA. Novel functions of the human papillomavirus E6 oncoproteins. Annu Rev Virol. 2015;2(1):403-23.
10. Szymonowicz KA, Chen J. Biological and clinical aspects of HPV-related cancers. Cancer biology & medicine. 2020;17(4):864.
11. Wallace NA, Galloway DA. Novel functions of the human papillomavirus E6 oncoproteins. Annual review of virology. 2015;2:403-23.
12. Zanier K,ould M'hamed ould Sidi A, Boulade-Ladame C, Rybin V, Chappelle A, Atkinson A, et al. Solution structure analysis of the HPV16 E6 oncoprotein reveals a self-association mechanism required for E6-mediated degradation of p53. Structure. 2012;20(4):604-17.
13. Tomaić V. Functional roles of E6 and E7 oncoproteins in HPV-induced malignancies at diverse anatomical sites. Cancers. 2016;8(10):95.
14. Ganti K, Broniarczyk J, Manoubi W, Massimi P, Mittal S, Pim D, et al. The human papillomavirus E6 PDZ binding motif: from life cycle to malignancy. Viruses. 2015;7(7):3530-51.
15. Martinez-Zapien D, Ruiz FX, Poirson J, Mitschler A, Ramirez J, Forster A, et al. Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53. Nature. 2016;529(7587):541-5.
16. Kelley ML, Keiger KE, Lee CJ, Huibregtse JM. The global transcriptional effects of the human papillomavirus E6 protein in cervical carcinoma cell lines are mediated by the E6AP ubiquitin ligase. Journal of virology. 2005;79(6):3737-47.
17. Filippova M, Parkhurst L, Duerksen-Hughes PJ. The human papillomavirus 16 E6 protein binds to Fas-associated death domain and protects cells from Fas-triggered apoptosis. Journal of Biological Chemistry. 2004;279(24):25729-44.
18. Filippova M, Song H, Connolly JL, Dermody TS, Duerksen-Hughes PJ. The human papillomavirus 16 E6 protein binds to tumor necrosis factor (TNF) R1 and protects cells from TNF-induced apoptosis. Journal of Biological Chemistry. 2002;277(24):21730-9.
19. Filippova M, Johnson MM, Bautista M, Filippov V, Fodor N, Tungteakkhun SS, et al. The large and small isoforms of human papillomavirus type 16 E6 bind to and differentially affect procaspase 8 stability and activity. Journal of virology. 2007;81(8):4116-29.
20. Thomas M, Banks L. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. Journal of General Virology. 1999;80(6):1513-7.
21. James MA, Lee JH, Klingelutz AJ. Human papillomavirus type 16 E6 activates NF- κ B, induces cIAP-2 expression, and protects against apoptosis in a PDZ binding motif-dependent manner. Journal of virology. 2006;80(11):5301-7.

22. Shah M, Anwar MA, Park S, Jafri SS, Choi S. In silico mechanistic analysis of IRF3 inactivation and high-risk HPV E6 species-dependent drug response. *Scientific reports*. 2015;5(1):1-14.
23. Li S, Labrecque S, Gauzzi MC, Cuddihy AR, Wong AH, Pellegrini S, et al. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon- α . *Oncogene*. 1999;18(42):5727-37.
24. Rincon-Orozco B, Halec G, Rosenberger S, Muschik D, Nindl I, Bachmann A, et al. Epigenetic Silencing of Interferon- κ in Human Papillomavirus Type 16-Positive Cells IFN- κ Regulation in HPV16-Positive Cells. *Cancer research*. 2009;69(22):8718-25.
25. Reiser J, Hurst J, Voges M, Krauss P, Münch P, Iftner T, et al. High-risk human papillomaviruses repress constitutive kappa interferon transcription via E6 to prevent pathogen recognition receptor and antiviral-gene expression. *Journal of virology*. 2011;85(21):11372-80.
26. Xie X, Piao L, Bullock BN, Smith A, Su T, Zhang M, et al. Targeting HPV16 E6-p300 interaction reactivates p53 and inhibits the tumorigenicity of HPV-positive head and neck squamous cell carcinoma. *Oncogene*. 2014;33(8):1037-46.
27. Patel D, Huang S-M, Baglia LA, McCance DJ. The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300. *The EMBO journal*. 1999;18(18):5061-72.
28. Gewin L, Myers H, Kiyono T, Galloway DA. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. *Genes & development*. 2004;18(18):2269-82.
29. Zhang Y, Dakic A, Chen R, Dai Y, Schlegel R, Liu X. Direct HPV E6/Myc interactions induce histone modifications, Pol II phosphorylation, and hTERT promoter activation. *Oncotarget*. 2017;8(56):96323.
30. Kranjec C, Tomačić V, Massimi P, Nicolaides L, Doorbar J, Banks L. The high-risk HPV E6 target scribble (hScrib) is required for HPV E6 expression in cervical tumour-derived cell lines. *Papillomavirus Research*. 2016;2:70-7.
31. Thomas M, Laura R, Hepner K, Guccione E, Sawyers C, Lasky L, et al. Oncogenic human papillomavirus E6 proteins target the MAGI-2 and MAGI-3 proteins for degradation. *Oncogene*. 2002;21(33):5088-96.
32. Facciuto F, Valdano MB, Marziali F, Massimi P, Banks L, Cavatorta AL, et al. Human papillomavirus (HPV)-18 E6 oncoprotein interferes with the epithelial cell polarity Par3 protein. *Molecular oncology*. 2014;8(3):533-43.
33. Du M, Fan X, Hong E, Chen JJ. Interaction of oncogenic papillomavirus E6 proteins with fibulin-1. *Biochemical and biophysical research communications*. 2002;296(4):962-9.
34. Wu D-W, Chuang C-Y, Lin W-L, Sung W-W, Cheng Y-W, Lee H. Paxillin promotes tumor progression and predicts survival and relapse in oral cavity squamous cell carcinoma by microRNA-218 targeting. *Carcinogenesis*. 2014;35(8):1823-9.
35. Au Yeung CL, Tsang WP, Tsang TY, Co NN, Yau PL, Kwok TT. HPV-16 E6 upregulation of DNMT1 through repression of tumor suppressor p53. *Oncology reports*. 2010;24(6):1599-604.
36. Iftner T, Elbel M, Schopp B, Hiller T, Loizou JI, Caldecott KW, et al. Interference of papillomavirus E6 protein with single-strand break repair by interaction with XRCC1. *The EMBO journal*. 2002;21(17):4741-8.
37. Vieira VC, Leonard B, White EA, Starrett GJ, Temiz NA, Lorenz LD, et al. Human papillomavirus E6 triggers upregulation of the antiviral and cancer genomic DNA deaminase APOBEC3B. *MBio*. 2014;5(6):10.1128/mbio.02234-14.
38. Zhu Y, Han Y, Tian T, Su P, Jin G, Chen J, et al. MiR-21-5p, miR-34a, and human telomerase RNA component as surrogate markers for cervical cancer progression. *Pathology-Research and Practice*. 2018;214(3):374-9.
39. Wang X, Meyers C, Guo M, Zheng ZM. Upregulation of p18Ink4c expression by oncogenic HPV E6 via p53-miR-34a pathway. *International journal of cancer*. 2011;129(6):1362-72.
40. Au Yeung C, Tsang T, Yau P, Kwok T. Human papillomavirus type 16 E6 induces cervical cancer cell migration through the p53/microRNA-23b/urokinase-type plasminogen activator pathway. *Oncogene*. 2011;30(21):2401-10.
41. Yeung CLA, Tsang TY, Yau PL, Kwok TT. Human papillomavirus type 16 E6 suppresses microRNA-23b expression in human cervical cancer cells through DNA methylation of the host gene C9orf3. *Oncotarget*. 2017;8(7):12158.
42. Martinez I, Gardiner A, Board K, Monzon F, Edwards R, Khan S. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene*. 2008;27(18):2575-82.
43. Li Y, Cai Q, Lin L, Xu C. MiR-875 and miR-3144 switch the human papillomavirus 16 E6/E6* mRNA ratio through the EGFR pathway and a direct targeting effect. *Gene*. 2018;679:389-97.

44. Williams VM, Filippova M, Filippov V, Payne KJ, Duerksen-Hughes P. Human papillomavirus type 16 E6* induces oxidative stress and DNA damage. *Journal of virology*. 2014;88(12):6751-61.
45. Sommerova L, Anton M, Bouchalova P, Jasickova H, Rak V, Jandakova E, et al. The role of miR-409-3p in regulation of HPV16/18-E6 mRNA in human cervical high-grade squamous intraepithelial lesions. *Antiviral Research*. 2019;163:185-92.
46. Liu X. Up-regulation of miR-20a by HPV16 E6 exerts growth-promoting effects by targeting PDCD6 in cervical carcinoma cells. *Biomedicine & Pharmacotherapy*. 2018;102:996-1002.
47. Wongjampa W, Ekalaksananan T, Chopjitt P, Chuerduangphui J, Kleebkaow P, Patarapadungkit N, et al. Suppression of miR-22, a tumor suppressor in cervical cancer, by human papillomavirus 16 E6 via a p53/miR-22/HDAC6 pathway. *PloS one*. 2018;13(10):e0206644.
48. McKenna DJ, Patel D, McCance DJ. miR-24 and miR-205 expression is dependent on HPV onco-protein expression in keratinocytes. *Virology*. 2014;448:210-6.
49. Al Bitar S, Gali-Muhtasib H. The role of the cyclin dependent kinase inhibitor p21cip1/waf1 in targeting cancer: Molecular mechanisms and novel therapeutics. *Cancers*. 2019;11(10):1475.
50. Yi JW, Jang M, Kim SJ, Kim SS, Rhee JE. Degradation of p53 by natural variants of the E6 protein of human papillomavirus type 16. *Oncology Reports*. 2013;29(4):1617-22.
51. Parroche P, Touka M, Mansour M, Bouvard V, Thépot A, Accardi R, et al. Human papillomavirus type 16 E6 inhibits p21WAF1 transcription independently of p53 by inactivating p150Sal2. *Virology*. 2011;417(2):443-8.
52. Malanchi I, Accardi R, Diehl F, Smet A, Androphy E, Hoheisel Jr, et al. Human papillomavirus type 16 E6 promotes retinoblastoma protein phosphorylation and cell cycle progression. *Journal of virology*. 2004;78(24):13769-78.
53. Lichtig H, Gilboa DA, Jackman A, Gonen P, Levav-Cohen Y, Haupt Y, et al. HPV16 E6 augments Wnt signaling in an E6AP-dependent manner. *Virology*. 2010;396(1):47-58.
54. Gupta S, Kumar P, Das BC. HPV: Molecular pathways and targets. *Current problems in cancer*. 2018;42(2):161-74.
55. Liu F, Lin B, Liu X, Zhang W, Zhang E, Hu L, et al. ERK signaling pathway is involved in HPV-16 E6 but not E7 oncoprotein-induced HIF-1 α protein accumulation in NSCLC cells. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*. 2016;23(3):109-18.
56. Ganti K, Massimi P, Manzo-Merino J, Tomaić V, Pim D, Playford MP, et al. Interaction of the human papillomavirus E6 oncoprotein with sorting nexin 27 modulates endocytic cargo transport pathways. *PLoS pathogens*. 2016;12(9):e1005854.
57. Talora C, Sgroi DC, Crum CP, Dotto GP. Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. *Genes & development*. 2002;16(17):2252-63.
58. Henken FE, De-Castro Arce J, Rösl F, Bosch L, Meijer CJ, Snijders PJ, et al. The functional role of Notch signaling in HPV-mediated transformation is dose-dependent and linked to AP-1 alterations. *Cellular oncology*. 2012;35:77-84.
59. Estêvão D, Costa NR, da Costa RMG, Medeiros R. Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*. 2019;1862(2):153-62.
60. Bhattacharjee R, Das SS, Biswal SS, Nath A, Das D, Basu A, et al. Mechanistic role of HPV-associated early proteins in cervical cancer: Molecular pathways and targeted therapeutic strategies. *Critical Reviews in Oncology/Hematology*. 2022:103675.
61. Zhen S, Li X. Oncogenic human papillomavirus: application of CRISPR/Cas9 therapeutic strategies for cervical cancer. *Cellular Physiology and Biochemistry*. 2018;44(6):2455-66.
62. Pal A, Kundu R. Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. *Frontiers in microbiology*. 2020;10:3116.
63. Knoff J, Yang B, Hung C-F, Wu T-C. Cervical cancer: development of targeted therapies beyond molecular pathogenesis. *Current obstetrics and gynecology reports*. 2014;3:18-32.
64. Cherry JJ, Rietz A, Malinkevich A, Liu Y, Xie M, Bartolowits M, et al. Structure based identification and characterization of flavonoids that disrupt human papillomavirus-16 E6 function. *PloS one*. 2013;8(12):e84506.

65. Divya CS, Pillai MR. Antitumor action of curcumin in human papillomavirus associated cells involves downregulation of viral oncogenes, prevention of NFkB and AP-1 translocation, and modulation of apoptosis. *Molecular Carcinogenesis*: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2006;45(5):320-32.
66. Letafati A, Sakhavarz T, Khosravinia MM, Ardekani OS, Sadeghifar S, Norouzi M, et al. Exploring the correlation between progression of human papillomavirus infection towards carcinogenesis and nutrition. *Microbial Pathogenesis*. 2023;106302.
67. Ghorbani S, Letafati A, Khatami A, Farzi R, Shabani S, Moradi P, et al. Association between human herpesvirus-6 and primary brain tumors: a systematic review and meta-analysis. *Future Virology*. 2022;17(5):305-14.
68. Khalesi Z, Tamrchi V, Razizadeh MH, Letafati A, Moradi P, Habibi A, et al. Association between human herpesviruses and multiple sclerosis: A systematic review and meta-analysis. *Microbial Pathogenesis*. 2023;106031.
69. Ghorbani S, Tambrchi V, Farzi R, Khatami A, Samiei RN, Saadati H, et al. Association between human Epstein-Barr virus and brain cancer: a systematic review and meta-analysis. *Future Virology*. 2023(0).
70. Bansal A, Singh MP, Rai B. Human papillomavirus-associated cancers: A growing global problem. *International Journal of Applied and Basic Medical Research*. 2016;6(2):84.
71. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *The Lancet Global Health*. 2020;8(2):e191-e203.
72. Simanaviciene V, Popendikyte V, Gudleviciene Z, Zvirbliene A. Different DNA methylation pattern of HPV16, HPV18 and HPV51 genomes in asymptomatic HPV infection as compared to cervical neoplasia. *Virology*. 2015;484:227-33.
73. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. *The Lancet*. 2015;386(10008):2078-88.
74. Bagarazzi ML, Yan J, Morrow MP, Shen X, Parker RL, Lee JC, et al. Immunotherapy against HPV16/18 generates potent TH1 and cytotoxic cellular immune responses. *Science translational medicine*. 2012;4(155):155ra38-ra38.
75. Akhatova A, Chan CK, Azizan A, Aimagambetova G. The efficacy of therapeutic DNA vaccines expressing the human papillomavirus E6 and E7 oncoproteins for treatment of cervical cancer: systematic review. *Vaccines*. 2021;10(1):53.
76. Ghanbari A, Le Gresley A, Naughton D, Kuhnert N, Sirbu D, Ashrafi GH. Biological activities of *Ficus carica* latex for potential therapeutics in Human Papillomavirus (HPV) related cervical cancers. *Scientific reports*. 2019;9(1):1013.
77. Deshpande R, Raina P, Shinde K, Mansara P, Karandikar M, Kaul-Ghanekar R. Flax seed oil reduced tumor growth, modulated immune responses and decreased HPV E6 and E7 oncoprotein expression in a murine model of ectopic cervical cancer. *Prostaglandins & other lipid mediators*. 2019;143:106332.
78. Kwon S-B, Kim M-J, Yang JM, Lee H-P, Hong JT, Jeong H-S, et al. *Cudrania tricuspidata* stem extract induces apoptosis via the extrinsic pathway in SiHa cervical cancer cells. *PLoS One*. 2016;11(3):e0150235.