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Mechanistic role of HPV-associated early proteins in cervical cancer: Molecular pathways and targeted therapeutic strategies

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ABSTRACT

Purpose: Cervical cancer (CC), one of the major causes of death of women throughout the world is primarily caused due to Human Papilloma Virus (HPV) 16 and 18. The early region (E) oncoproteins of HPV are associated with the etiopathogenesis and contribute to the progression of cancer. The present article comprehensively discussed the structural organization and biological functions of all E proteins of HPV and their contribution to progression of CC with an intent to decipher the pathological hallmarks and their relationship. Additionally, the role of E proteins in reference to therapeutics will also be presented.

Methods: A systematic search has been carried out for articles published in PubMed database by using combinations of different keywords with Boolean operators (AND, OR, NOT) including cervical cancer, HPV, E proteins, and signaling.

Results: From the analysis of literature review, it’s apparent that E proteins are the major contributor to disease progression. E1, E2, and E4 forms are mainly associated with viral integration, replication, and transcription whereas E6 and E7 act as an oncoprotein and are associated with the progression of cancer. E5 regulates cell proliferation, apoptosis, and facilitates the activity of E6 and E7. Additionally, E proteins were observed associated with numerous cell signaling pathways including PI3K/AKT, Wnt, Notch and reasonably contribute to the initiation of malignancy, cell proliferation, metastasis, and drug resistance. Knowing the role and interplay of each protein in initiation to progression of CC, their therapeutic significance has been elucidated. The present study observations demonstrate that E6 and E7 are the major cause of HPV-mediated CC progression. E1, E2, and
E5 also act as a backbone for E6 and E7 and most of the current approaches have targeted E6 and E7 mediated action only.

**Conclusion:** The present review illustrates the structural organization as well as function and regulation of all early proteins of HPV and their association with several cellular signaling pathways. The observations provide clues on the regulatory aspect of these proteins in initiation to progression and reasonably represent that targeting these proteins could be a novel therapeutic strategy for CC. In particular, its seemingly appears that inhibition of the activity of E6 and E7 oncoproteins may be a better selective target to delay the progression of CC. The review reaffirms the role of E proteins and encourages future studies on developing diagnostics, and most importantly therapeutics strategies targeting E6 and E7 oncoproteins to tackle CC related morbidity and mortality.

1. Introduction

One of the most common cancers in women is cervical cancer (CC). There were approximately 570,000 new cases of CC and 311,000 deaths occurred because of CC worldwide in 2018 according to WHO (Adiga et al., 2021; Arbyn et al., 2020; Bray et al., 2018; Galvao and Coimbra, 2020; Razavi, Z. S. et al., 2021). The average age at diagnosis and death of CC has been reported 53 years and 59 years, respectively. According to GLOBOCAN reports, it has been observed that approximately 85% of all CC deaths occurred in low-income countries. The death associated with CC in these countries are reported 18 times higher than the developed countries (Bray et al., 2018). Women from Eastern and Southern Africa has been reported to exhibit higher incidences (Arbyn et al., 2020). Whereas, more than a third of the CC cases globally were reported in Asian countries, China, and India (Bray et al., 2018; Ferlay et al., 2019). India reported approximately 74,000 deaths and 132,000 new cases per year. Majority of the causes of mortality due to CC in developing and underdeveloped countries has been found associated with the shortage of efficient treatment and diagnosis, lack of proper screening programs and awareness, along with increased exposure to the risk factors (Bobdey et al., 2016).

Human Papillomavirus (HPV) has been identified as a primary reason behind CC. Despite being a preventable disease by using therapeutic strategies, CC has been one of the major gynecological malignancies around the world (Paavonen, 2007). Recently, Adiga and co-workers have reviewed and discussed the various aspects of disease including pathogenesis, sequelae, major molecular mechanisms and possible therapeutic targets and associated challenges with CC (Adiga et al., 2021). Although persistent HPV infection is one of the primary requisite for induction of CC, but only HPV cannot develop the CC; early coitus, promiscuity, usage of oral contraceptives, early age pregnancy, tobacco-smoking, unhygienic conditions also contribute to the progression of CC. German virologist Harald Zur Hausen and his team have first established the relationship between HPV and CC in 1983 (Zur, 2002). Further, it has been reported that HPV infection is often seen in healthy women but rapid immune clearance commonly clears it within 1–2 years (Asial et al., 2014). However, sexually transmitted persistent HPV infection is the common causative agent of CC in most of the cases (84%) (Organisation, 2017). Among 300 different HPV genotypes, the strains that are mostly associated with the progression of CC and adenocarcinoma are HPV16 and HPV18 (Chaturvedi, 2010; Tian, R. et al., 2022). High risk HPV (HR-HPV) types have been shown associated to the progression of CC and precancerous lesions besides HPV16 and 18 include 68, 66, 59, 58, 56, 52, 51, 45, 39, 35, 33, 33 and 31 (Fani et al., 2020).

In this review article, the structural organization, and biological functions of all the E proteins of HPV and their role in the initiation and progression of CC and their therapeutic significance have been comprehensively discussed. Additionally, using synoptic tables and schematic illustrations we have also discussed the signaling networking cascade of the HPV infection for CC via early proteins and the crosstalk between early proteins. Table 1 summarizes the mechanism of early protein for CC progression, the pathogenic hallmarks, influence over cellular signaling and their therapeutic potential.

2. HPV genome

HPV, a papillomaviridae family virus, belongs to a category of double-stranded DNA, non-enveloped, small (50–55 nm) viruses. HPV is divided into five sub-categories (alpha, beta, gamma, mu, nu) based on the nucleotide sequence of the L1 ORF region (de Villiers et al., 2004). Among these, alpha categories were found to be carcinogenic. HPV16 is the most frequently detected genotype in CC cases, followed by HPV18 according to reports from the International Agency for Research on Cancer (IARC) (Serrano et al., 2018).

The HPV16 genome stretches to a length of 7.9 kb. The HPV genome is contained in an icosahedral capsid that is further sub-divided into three regions: the early (E) region comprising different types of precursor proteins including E1, E2, E4, E5, E6, and E7 that are required in the early stages of infection and associated with viral DNA replication, cell cycle control, and oncogenesis (Williams et al., 2011). The major and minor viral capsid structure is coded by the late (L) region proteins which comprise of two parts, L1 and L2. Viral replication and transcription are controlled by a non-coding region, the Long Control Region (LCR) (Graham, 2006). Among the six E proteins, E6 and E7 are the main regulators contributes to the pathogenicity of the virus. The cell repairing mechanisms, especially the functions of the tumor suppressor proteins – p53 and retinoblastoma are dysregulated by the expression of the E6 and E7 viral oncoproteins (Crook et al., 1991; Jones and Münger, 1996).

3. Role of E proteins in HPV integration

The initiation of the HPV infection is from the basal layer of the squamous epithelium. The viral replication process and transcription of other E proteins are regulated by E1 and E2 proteins (Pal and Kundu, 2020a). Initially, the copy number tends less in number; however, the copy number increase with the differentiation of basal cells to form the epithelial suprabasal layer and spread the infection to the adjacent cells. The integration of the HPV genome with the host genome is well reported (83% HPV-positive cases), but it has been also observed in an episomal form (Network, 2017). E2 disruption occurs due to the integration of the viral genome that activates the E6 and E7 oncoproteins. The viral genome is multiplied by the active assistance of E6 and E7 with the help of cellular machinery. During the process of viral replication, the cells can be tricked to become oncogenic (Pal and Kundu, 2020a). The collateral damage to viral replication is the development of HPV-mediated tumors.

4. Role and mechanism of HPV early-region proteins

4.1. E1 and E2 proteins

E1 protein has been reported highly conserved among the types of HPV and commonly decoded during the early expression of HPV infection (Bergvall et al., 2013). E2 proteins are multifunctional proteins mostly associated with viral replication and transcription. Depending on the promoter, it either acts as an activator of transcription or as a repressor of the viral genome (Grim et al., 2005). E2 proteins consist of two functional domains; the N terminus conserved domain, required for...
### Table 1

**Synopsis of the role of HPV Early proteins in CC and their therapeutic targets.**

| Early Protein | Pre-clinical | Pathways | Cancer Hallmarks | Mechanisms | Therapeutic Potential | References |
|---------------|--------------|----------|-----------------|------------|-----------------------|------------|
| **E1**        | *In vitro* and *in vivo* | NF-kB | NA              | Helps in initiation and regulation of HPV replication. Acts as a helicase and recruits replication factors. | ATP acts as an allosteric modulator of E1-E2 interactions. Indandione is a small class of molecules that act as a potential inhibitor of the E1-E2 interaction. | (Berg and Stenlund, 1997; Bergvall et al., 2013; Castro-Munoz et al., 2019; Chojnacki and Melendy, 2018; D’Abramo and Archambault, 2011; Goodwin and DiMaio, 2000; Moody and Laimins, 2010; White et al., 2001; White et al., 2003) |
| **E2**        | *In vitro* and *in vivo* | NA     | NA              | Aids in initiation and regulation of HPV replication. Enable the binding of E1 protein. Disruption of E2 leads to CC progression. Negative transcription regulator of E6. | ATP acts as an allosteric modulator of E1-E2 interactions. Indandione is a small class of molecules that act as a potential inhibitor of the E1-E2 interaction. | (Chojnacki and Melendy, 2018; Cruz-Gregorio et al., 2018; Goodwin and DiMaio, 2000; Gupta and Mania-Pramanik, 2019; Moody and Laimins, 2010; Zahra et al., 2021) (Berg and Stenlund, 1997; D’Abramo and Archambault, 2011; White et al., 2001; White et al., 2003) |
| **E4**        | *In vitro* and *in vivo* | NA     | NA              | Involved in viral release, transmission and PTM. Associates with keratin and could manipulate cytokeratin network, essential in the release of the virus. | Controlling the phosphorylation of E4 modulates its activity and is unable to affect the host. | (Doorbar; 2013; DOORBAR et al., 1996; Khan et al., 2011; Mcnintosh et al., 2010; Roberts et al., 2003; Rogel-Gaillard et al., 1993; Wang et al., 2009) (Kim et al., 2009) |
| **E5**        | *In vitro* | MAPK-ERK pathways | Angiogenesis, evading cell death, cellular proliferation | Major oncoprotein in HPV mediated carcinogenesis. Supportive role in E6 and E7 modulation. Suppresses p21 expression and initiates CC progression. | E5 inhibition by inhibiting the NF-kB pathway with inhibitors like narsin or flurosalan or celecoxib. Specific cytosolic T cells are designed specifically for HPV E5 proteins with CpG-oligodeoxynucleotide and targets and degrade them so that their function is lost permanently. | (Crock et al., 1991; Gangpally, 2012; Jones and Münger, 1996; Kabsch and Alonso, 2002; Kim et al., 2006; Regan and Laimins, 2008; Stoppler et al., 1990) (Dimmen and Ceresa, 2004) (Farley et al., 2006; Sima et al., 2008; Wang et al., 2010) |
| **E6**        | *In vitro* and *in vivo* | PE3K, AKT, Wnt, Notch pathways and EMT | Cellular proliferation, invasion metastasis, genomic instability, cellular immortality, cell cycle arrest, tumor-promoting inflammation, resisting cell death | Neoplastic effect on HPV-infected cells by promoting ubiquitin-dependent proteasome degradation of p53 and evades cell death. Targets degradation of apoptotic signaling cascade molecules through the ubiquitin-proteasome pathway leading to tumor development by inhibiting apoptosis. Causes dysregulated cell proliferation by downregulating transcriptional co-activator p300/CBP, essential for cell cycle. E6 is associated with the production of ROS that induces DNA break down and downregulates tumor suppressor genes. Downregulates IRF-3 of IFN-β and makes the immune response vulnerable against antigens of HPV. E6 inhibits the X-ray repair cross-complementing 1 is a scaffold DNA repair protein leading to increased mutation. | PDZ domains proteins including HDL protein, MUPP1, MAGI-1,2,3 are useful as therapeutic targets. Replacement of LxxΦ LxxΦ leucine motifs with alanine results in E6 binding being terminated. RNA interference (RNAi) technology simultaneously knockdown both E6 and E7 expression. | (Filippova et al., 2002; Henderson et al., 2014; Kelley et al., 2005; Lee et al., 1997; Li et al., 1999; Nakagawa and Huibregtse, 2000; Nguyen et al., 2002; Patel et al., 1999; Sanchez-Perez et al., 1997; Shah et al., 2015; Thomas et al., 2002; Vieira et al., 2014; Warren et al., 2017) (Al Moustafa et al., 2004; Be et al., 2001; Brehm et al., 1999; Brennan et al., 2009; DeMasi et al., 2005) |
| **E7**        | *In vitro* | PE3K/AKT, EMT | Replicative immortality, activating invasion and metastasis, evading growth suppressors, cell invasion, genomic instability, chronic inflammation | Promotes cervical dysplasia causing dysregulated cell cycle. Inactivates p73 and downregulates E2F for CC progression through conserved LXCXE motif of amino terminus 9. Causes deregulation of cyclin A/CDK2 and cyclin E/CDK2 by inactivation of p21 and p27 leading to increases in the Various HDAC inhibitors inactivate E7, p600 induces anchorage-dependent integrin modulated cellular interaction and is an important target for targeted therapy. RNAi technology simultaneously knockdowns both E6 and E7 expression. | (Alagii et al., 2014; Brehm et al., 1999; Brennan et al., 2009; Cardenal et al., 2012; DeMasi et al., 2005; Dinneen and Ceresa, 2004; Dooniger et al., 2001; Hasan et al., 2013; Hinds et al., 1992; Hsu et al., 2005; Iwakivik and Donlin, 2014; Kabsch and Alonso, 2002; Nguyen et al., 2002; Songock et al., 2017) | (continued on next page)
transactivation (TA) and DNA replication and the conserved C-terminal domain as a DNA binding domain that aids in the dimerization (Horner and DiMaio, 2007).

E1 and E2 proteins play crucial role in the initiation and regulation of HPV replication as illustrated in Fig. 1. Initially, E2 proteins bind to their binding sites (E2BS11 and E2BS12) at the origin of replication, which recruits a pair of E1 proteins to form a complex. Further, the E1 tetramer formed by displacing the E2 dimer at the E1 dimer, in an ATP-dependent manner. Thereafter, two E1 monomers bind to each half of the replication origin and form an E1 double trimer complex. The subsequent binding of E1 monomers to the complex forms E1 double hexamer, which acts as a helicase to initiate the DNA unwinding for replication (Dinneen and Ceresa, 2004; Goodwin and DiMaio, 2000; Moody and Laimins, 2010). The E1 double hexamer complex starts unwinding the DNA in both directions and the replication process is initiated by recruiting the cellular replication factors like the single-stranded DNA binding protein RPA, topoisomerase I and DNA polymerase (Dinneen and Ceresa, 2004; Goodwin and DiMaio, 2000; Moody and Laimins, 2010). The E1 double hexamer complex starts unwinding the DNA in both directions and the replication process is initiated by recruiting the cellular replication factors like the single-stranded DNA binding protein RPA, topoisomerase I and DNA polymerase α-primase (Chojnacki and Melendy, 2018).

The functions of E2 proteins are disrupted by mutation or the integration of DNA. The disruption of E2 blocks the viral replication that resulted in the aberrant viral gene expression, loss of control on E6 and E7 proteins, and ultimately leading to CC progression (Gupta and Mania-Pramanik, 2019). Further, E2 proteins of HPV18 have shown the association with the crest proteins of the mitochondria like ATP synthase, cytochrome (Cyt) c1, or Cyt C oxidase subunit II and also induce oxidative stress and the damage of the crest (Cruz-Gregorio et al., 2018). Oxidative stress appears as one of the major culprit in the pathogenesis of CC due to its role in the DNA damage that increase the chances of the incorporation of the HPV genome (Zahra et al., 2021).

E1 protein has been observed to regulate epigenetic modulation in the cells along with controlling replication machinery and interacting with other cellular components (Burley et al., 2020). Earlier studies have shown that HR-HPV infection increases the immune tolerance which makes a more susceptible microenvironment for further infection and disease progression (Castro-Muñoz et al., 2019). A recent study, reported association of E1 proteins with immune response including TLR and interferon signaling (Castro-Muñoz et al., 2019). It has also demonstrated the inhibitory role of E1 proteins on the antiviral immune responses that is reasoned to suppress the expression of IFNβ1 and IFNβ1 through the NF-κb signaling pathway and infringe in disease progression (Castro-Muñoz et al., 2019).

4.2. E4 protein

Inside the E2 gene, in the region coding the flexible hinge domain of the E2 gene, is present the HPV E4 ORF. The HPV E4 protein is translated as an E1’E4 fusion protein by alternative splicing; as a result, the first few amino acids including initiation codon originate from the E1 (Wang et al., 2011). E4 associated with E1’E4 possesses a ‘leucine cluster’ motif in close proximity to the N-terminus of the protein, essential for the association with keratin. The C-terminus of the protein is involved in self-associations that produce amyloid fiber resembles structures and helps in the manipulation of the cyto keratin network, essential for the viral release or transmission (McIntosh et al., 2010).

Commonly, E4 proteins have been expressed in the late stages of infection. In the virus replication cycle, the expression of the E4 protein is mostly confined to the middle and the upper layers of the epithelium. This protein appears as granules in the infected cells cytoplasm. These granules are considered responsible for the cytopathic effect wherein variations in morphology attributed to the type of HPV which infects the host (Doorbar et al., 1996; Roberts et al., 2003; Rogel-Gaillard et al., 1993). The expression of E1’ E4 occurs when the host cell initiates late viral promoter activation (Doorbar, 2013). Along with replication, E4 proteins also participate in post-translational modifications including phosphorylation. Phosphorylation of E4 occurs at the amino acid Thr 57 by S-phase associated p42 ERK and other MAP kinases which increase the stability of the protein and keratin binding capacity (McIntosh et al., 2010; Wang et al., 2009). After the viral genome amplification stage, calpain dissociates the E4 protein at the 17th and 18th amino acid, which leads to E4 mediated keratin disorganizations that help in a viral release (Khan et al., 2011).

4.3. E5 protein

E5 is one of the major oncoproteins in HPV-mediated carcinogenesis, however, β-HPV does not encode for E5. It is a small size (10 kDa) 83 amino acids hydrophobic membrane-bound protein, associated with the endoplasmic reticulum and its gene located in the 3’ end of the early region of the viral genome expressed from a spliced mRNA (DiMaio and Petti, 2013). The protein contains three regions: the N-terminal domain, central domain, and C-terminal domain (DiMaio and Petti, 2013). The highly hydrophobic N-terminal is followed by the central and the C-terminal domain (Conrad et al., 1993; Moody and Laimins, 2010). E5 confers a supportive role in E6 and E7 modulation of both HR-HPV and LR-HPV and sometimes appears an oncogene as its lone presence may cause hyper-proliferation of the cells and cancer progression as illustrated in Fig. 2 (Crook et al., 1991). E5 oncoprotein from HPV-16 and – 18 is present in the Golgi bodies or apparatus (GA), in the nuclear envelope and the endoplasmic reticulum (Disbrow et al., 2003), whereas it localizes entirely to the ER and with a lesser amount to the GA and early endosomes at physiological level of expression, as observed in primary human foreskin keratinocytes (Regan and Laimins, 2008).

E5 protein plays an important role in cell growth and regulation of signal transduction pathways in HPV infected cells (Regan and Laimins, 2008). It is commonly expressed in the early stages of HPV infection that is evident by the E5 protein-mediated increased immortalization of human keratocytes by E6 and E7, suggesting that E5 plays an important role in the initial stages of CC (Ganguly, 2012; Stoppler et al., 1996). Additionally, it is reported to induce angiogenesis during prolonged periods of HPV infection. Angiogenesis is a major player in cancer progression and E5 achieves this mediating ERK and PI3k-AKT pathways and EGRF signaling thus increasing VEGF, a major contributor to angiogenesis (Kim et al., 2006). E5 binds to BAP31 (B cell receptor-associated protein 31) (Regan and
Laimins, 2008) which acts as a modulator of membrane transport and modulates the roles of the EGFR receptor (Leechanachai et al., 1992) and endosomal pH without affecting the glycosylation process (Oetke et al., 1992). E5 lowers the levels of MHC1 proteins, modulating MAPK regulation machinery and caveolin1 levels associated with attachment, transport, and apoptosis of cellular mechanisms (Suprynowicz et al., 2008). Due to its association with multiple target molecules, E5 becomes an important therapeutic target for HPV-induced CC. E5 suppresses p21 protein expression, a major tumor suppressor, and co-operates with E6 and E7 to initiate progression of CC (Tsao et al., 1996). E5 is also reported to curb cellular apoptosis and this is achieved by inhibiting Fas ligand (FasL) as well as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Kabsch and Alonso, 2002). E5 participates in TRAIL-mediated downregulation of FasL and manipulated the expression of the death-inducing signaling complex (DISC), which provides immortality to the CC cells and their mortality get reduced by the cleavage of procaspase 3, 8, and PARP (Kabsch and Alonso, 2002). Moreover, in HPV-16 and –18, E5 has various pro-carcinogenic activities including the stimulation of cell proliferation by EGF, tumor necrosis factor ligand (TNFL), and CD95 ligand (CD95L) mediated suppression of apoptosis and the alterations of genes associated with adhesion and motility of cells (Dinneen and Ceresa, 2004).

4.4. E6 protein

The E6 protein is a relatively larger protein of 18 kDa, spanning a stretch of about 150 amino acids containing two zinc finger domains flanked by four Cys-X-X-Cys motifs (Zanier et al., 2012). It is found mainly in the nucleus of the cells possessing both C-terminal and N-terminal ends. The PDZ-binding motif of the C-terminal domain interacts with several cellular proteins and affects several cellular processes (Ganti et al., 2015). The E2 gene, which is a negative transcription regulator of E6, is usually disrupted during the integration of the HPV genome into the host genome, and its absence increases E6 expression. By regulating all hallmarks of CC, E6 in combination with E7 may lead a normal cell to become malignant as illustrated in Fig. 3 (Sanchez-Perez et al., 1997).

The principal role of E6 oncoproteins is to promote the ubiquitin-dependent proteasome degradation of p53 in HPV-infected cells, that demonstrate a neoplastic effect. This is being done for escaping cell death, a hallmark feature in malignant cells. E6 protein binds to the LxxLL motifs in the conserved region of E6AP and forms a heterotrimeric complex (E6/E6AP/p53) that causes p53 degradation, leading to evasion of cell death (Colegato et al., 2022; De Nola et al., 2021; Kelley et al., 2005; Nguyen et al., 2002; Vats et al., 2022). Apart from p53, E6
also target the degradation of other apoptotic signaling cascade molecules like Bak, Fas-associated death domain (FADD), and Bax through the ubiquitin-proteasome pathway that by inhibiting apoptosis leads to the tumor development (Filippova et al., 2002). Apoptosis and cell death are inhibited by the activation of transcriptional factors like NF-κB, which leads to the synthesis of anti-apoptotic proteins such as cIAP-2 in an E6-dependent manner.

An uncontrolled proliferation caused by dysregulated cell cycle is another hallmark of cancer in malignant cells. This involves evasion of cell cycle checkpoints for uncontrolled cell proliferation. E6 causes dysregulated cell proliferation and carcinogenesis by downregulating transcriptional co-activator p300/CBP which is essential for cell-cycle (Patel et al., 1999).

Cell invasion and metastasis are two fundamental hallmarks of cancer that are mediated by the E6 protein in malignant cells. E6 is associated with the production of ROS that induces damage or breakdown of DNA by depleting the level of antioxidant enzymes including catalase PDZ domain proteins, which interact with E6 proteins of HR-HPV inducing normal invasion and metastasis in tumor cells by modulating cell polarity and differentiation (Lee et al., 1997). Scribbled planar cell polarity protein (SCRIB), membrane-associated guanylate kinase 1, 2, and 3 (MAGI-1, 2, and 3) are some of the tumor suppressor genes which are downregulated by E6 proteolytic machinery leading to cell invasion (Nakagawa and Huibregtse, 2000; Thomas et al., 2002). Additionally, the interaction between E6 and PTPN13 (a tyrosine phosphatase protein) modulate invasiveness of the tumor cells in HeLa

Fig. 2. Mechanism of action of Early Protein 5.

Fig. 3. Mechanism of actions of Early Protein 6 and 7.
cells (Kranjec et al., 2016; Lee et al., 2022). Moreover, downregulation of ECM accompanied with inhibition of cell adhesion protein Fibulin 1 and miR-23b in an E6 dependent manner result in tumor cell migration (Vats et al., 2021). E6 promotes metastasis and invasion in tumor cells by downregulating paxillin resulting in compromised actin fibers and consequently, the integrity of the epithelium (Wu et al., 2014).

Avoiding the host immune system is another hallmark in malignant cells, which demonstrated the inhibitory role of E1 proteins on the antiviral immune responses may take place to that for prolonged replication of HPV to remain undetected inside the host epithelium for initiation of the immune evasion mechanism. It is facilitated by downregulation of the Interferon Regulatory Factor-3 (IRF-3) of the IFN-β via E6 protein, thus making the immune responses vulnerable against antigens of HPV (Li et al., 1999). Furthermore, the E6 oncoprotein suppresses the immunological response induced by Interferon α (IFN-α) by inhibiting phosphorylation of Tyrosine Kinase 2 (TYK2) in a STAT/TYK2 dependent manner, preventing IFNs and its receptor from interacting, resulting in prolonged HPV replication (Li et al., 1999). The therapeutic approach for the management of CC, mainly immunotherapy, is likely affected by the translational consequences of tumor stroma and a proliferation of tumor-sensitive lymphocytes in tumor microenvironment. However, the diagnosis of CC is contrarywise associated with microvessel density, allowing inhibition of angiogenesis with therapeutic agents like bevacizumab, which play an important role in targeting advanced as well as recurrent CC (De Nola et al., 2021).

Immortalization of the cell is one of the hallmarks that are facilitated by HPV in CC via E6 protein. The unbalanced expression of E6 causes expression of hTERT resulting in immortalization of the cell via degradation of NFX1–91 (Nuclear transcriptional factor X box binding 1–91) (Shah et al., 2015). Moreover, the overexpression of proto-oncogenic protein c-Myc and transcriptional factor Sp1 in an E6 dependent manner leads to the recruitment of promotor that modulates epigenetic changes in hTERT along with phosphorylated DNA polymerase II ultimately leading to immortalization of cells altogether (Gewin et al., 2004; Zhang et al., 2017).

Genomic instability, a prominent hallmark of HPV is incorporated into the malignant cell by E6 protein by upregulating apolipoprotein B, an mRNA editing enzyme catalytic polypeptide 3 that caused replacing cytosine residue with uracil (Warren et al., 2017). This protein is prone to errors, and its overexpression leads to a greater mutation rate, resulting in genomic instability. E6 inhibits the scaffold DNA repair protein X-ray repair cross-complementing 1, causing an increase in mutation and thus increased genomic instability (Henderson et al., 2014; Vieira et al., 2014; Warren et al., 2017).

4.5. E7 protein

The E7 protein is smaller relative to E6 spanning across a length of around 100 amino acids, consisting of 3 conserved regions 1/2/3 (CR1/2/3). A minor portion of the CR1 region and more than a major portion of the CR2 region resembles the adenovirus in sequence and a large T antigen of SV40 (Pal and Kundu, 2020a; Phelps et al., 1988). The CR3 region is located at the carboxyl-terminal end of the protein. This area is conserved, and believed to encode a zinc finger domain bordered by two CXXC motifs separated by 29 amino acid residues (McINTYRE et al., 1993). Upon interaction with various host factors, the E7 protein is associated with various hallmarks in CC as illustrated in Fig. 5.

In HPV-induced CC, E7 oncoprotein plays a crucial role in promoting cervical dysplasia causing dysregulated cell cycle as illustrated in Fig. 4 (Moody and Laimins, 2010). The main targets of E7 are the inactivation of the pRb and the downregulation of E2F. It is well reported that E7 degrades the pRB protein, which is known to halt cell cycle by

Fig. 4. Events mediated and modulated by early protein 6 and 7 in CC.
suppressing the E2F factor induced unlimited cellular division and cancer progression. E7 induces proteasome degradation by ubiquitin-mediated pathways and low levels of E7 can also cause high damage leading to cancer progression through the conserved LXCXE motif of amino terminus 9 (Hinds et al., 1992). There are eight members in the Rb family and the majority of the proteins act like activators; (Chellappan et al., 1992; Liu et al., 2006), thus, E2F members can also be targeted for therapy with upregulation E2F repression. Whereas, p107 and p130 which are the pockets and the regulator of E2F are also degraded by E7 (Barrow-Laing et al., 2010). E7 causes deregulation of cyclin A/CDK2 and cyclin E/CDk2 by inactivation of p21 and p27 leading to increases in the spindle formation rate which causes uncontrollable cell cycle formation. Phosphorylation of these CDKs can cause inhibition of tissue transformation and morphogenesis (Huh et al., 2005). E2F6 is a transcriptional repressor of S phase-inducing genes which are inhibited by E7, leading to uncontrolled cell proliferation along the dysregulated cell cycle. Claspin belongs to a group of calcium-activated cysteine proteases and play role in a variety of cellular processes (Zhang et al., 2006). Overexpression of the cell cycle protein claspin in an E7 dependent manner leads to the facilitation of cell proliferation even during DNA damage.

Cell invasion is another hallmark modulated by E7 protein in an MMP-9 dependent manner. E7 promotes cell invasion by overexpression of MMP-9 leading to degradation of ECM and cell invasion (Cardeal et al., 2012). Genomic instability, another hallmark of CC is promoted by E7 through overexpression of centrosomes leading to aneuploidy. The dysregulated pRb and causes aberration at the centrosome through CDK2 resulting in degradation of the mitotic spindle and g-tubulin (Akagi et al., 2014; Duensing et al., 2001; Nguyen et al., 2002). Chronic inflammation is another hallmark modulated by E7 protein following binding to TLR9 that results in disrupted IFN-γ signaling and inhibition of the cyclic GMP-AMP-synthase (Hasan et al., 2013; Ivashkiv and Donlin, 2014; Songock et al., 2017). The interplay of E6 and E7 triggers inflammation process in a pro-inflammatory cytokine-dependent manner by IL-6 and IL-18 leading to tumor progression by chemokines, and metalloprotease (Hammes et al., 2007; Ren et al., 2013; Richards et al., 2014).

A large cross-talk occurs between E6 and E7 oncoprotein during cervical carcinoma progression as illustrated in Fig. 4. This cross-talk between oncoprotein E6 and E7 is crucial for HPV-induced carcinoma and these oncoproteins largely control various cell processes. The angiogenic switch get modulated in malignant cells in an E6 and E7 interplay-dependent manner. This leads to the downregulation of maspin and thrombospondin-1 which results in simultaneous overexpression of IL-8 and VEGF (Toussaint-Smith et al., 2004). Tumor invasion and metastasis are triggered by the interplay between E6 and E7 protein as it induces downregulation of TIM-2 and RECK proteins which are responsible for MMP activity (Cardeal et al., 2012). The interplay of E6 and E7 is also involved in the dysregulation of cellular energetics via upregulation of GLUT1 and pyruvate kinase (Zwerschke et al., 1999).

5. HPV signaling pathway and targets

HPV E5, E6, and E7 oncoproteins can regulate and alter several cell-signaling pathways to cause cancer. These molecular pathways play major roles in malignant transformation, thus targeted therapy against these key signaling pathways has potential therapeutic implications. E5 oncoprotein stimulates the MAPK-ERK pathway (Branca et al., 2004). E6 oncoprotein alters phosphatidylinositol 3-kinase and protein kinase B (PI3K/AKT) pathway, Wnt pathway, Notch pathway, and E7 regulates the PI3K/AKT pathway. PI3K/AKT is particularly important due to its relation to initiation of malignancy, cell proliferation, metastasis, and also drug resistance (Manzo-Merino et al., 2014). Moreover, these

![Hallmarks of Cancer](image_url)
oncoproteins also alter the NF-κB pathway, YY1 pathway (Guifen et al., 2011), and apoptotic pathways (intrinsic and extrinsic). Herein, we have discussed studies which suggest targeting the altered cell signaling pathways and epidermal growth factor receptor along with vascular endothelial growth factor as potential therapeutic approach against CC and illustrated in Fig. 6 and Fig. 7.

Activation of the PI3K/AKT signaling is the primary pathway implicated in cancer cell’s longevity involving the transduction of signals by phosphorylation of AKT (Gupta et al., 2018). HPV-induced infection followed by the expression of either E6 or E7 activates the signaling pathway of PI3K/AKT by altering various cellular mechanisms, thus leads to carcinogenesis (Rodon et al., 2013). Earlier investigations have well demonstrated that HPV 16/18 E6 and E7 proteins activate this pathway and thus in turn AKT activity results in the inactivation of pRB which play a role in causation of CC. p27 has been reported to be negatively regulated by the E7 protein of HPV 16 using AKT and increased retention of p27 in the cytoplasmic domains (Charette and McGance, 2007; Menges et al., 2006).

Cervical carcinomas and CIN lesions may be used to test ERK1 signaling in predicting CC incidence and it is suggested that the expression of ERK1 tends to be a early predictor of CC (Branca et al., 2004). The recently discovered proto-oncogene tyrosine-protein kinase SRC protein, together with phosphorylated ERK-1/2 and c-Fos, aided in the modulation of ERK signal transduction pathway expression to facilitate cell proliferation and prevent apoptosis in CC cells (Song et al., 2017). It has also been shown that ERK1/2 involves the expression of HIF-1α, VEGF, and IL-8 induced by HPV16 E6 oncoprotein (Gupta et al., 2018; Liu et al., 2016).

HPV16 oncoprotein E6 activates the Wnt /β-catenin pathway, vital for the transformation of HPV, epithelial cells immortalization, balancing tissue homeostasis and pathologic phenotype (Gupta et al., 2018; Moon et al., 2004). The altered Wnt/β-catenin signaling pathway is believed to play role in the onset and maintenance of HPV-associated malignancies, and could be utilized as a therapeutic target for CC (Ramachandran et al., 2012).

The Yin Yang complex1 protein binds to the URR of HPV 18, thus controlling transcription of HPV E6 and E7 protein (Bauknecht and Shi, 1998). It also behaves as a negative regulator of p53 by interacting with mdm2 (Park and Atchison, 1991). It has recently been reported that YY1 has been found over expressed in CC and thus may play an important role in the progression of HPV infection associated CC.

There are reports which suggest that the activity of HER3 overexpression results in the poor prognosis of CC (Ojesina et al., 2014). Studies of full-exome sequencing suggest that activated HER2 is associated with somatic mutation and incorporation of HPV into CC (Chavez-Blanco et al., 2004; Oh et al., 2015). HPV E6/E7 controls the expression of HER3 proteins and downstream PI3K signaling, that is suggestive of positive association between HER3 expression and HPV-induced CC (Brand et al., 2017). The knowledge of viral oncogene-driven methods reveal new molecular targets for tumors following infection with HPV.

Some reports showed that a possible binding site for STAT3 has been mapped to a 5’ region of HPV16 LCR that regulates the expression of viral oncogenes, which may play a key role in epithelial cancer (Arany et al., 2002). Therefore, more research are needed to decipher the role of STAT3 in CC. Although some studies have shown that STAT3 is present in stem cells, a subclass of CC lesion, this additionally supports that STAT3 may play a key role in viral gene expression and HPV16-mediated carcinogenesis (Manzo-Merino et al., 2014; Martinez-Zapien et al., 2016; Menges et al., 2006).

The most important factor in controlling the EMT signaling pathway is the transformation of the growth factor-beta (TGF-β1). HPV16-E6/E7 prompts EMT-induced by FGF-2 and FGF-4 to increase invasive cancer capacity (Cheng et al., 2012). Simultaneous expression of E6/E7 and ErbB-2 reduces the E-cadherin function enhancing the expression of transcription factors including SLUG, SNAIL, and TWIST which activate the EMT pathway and consequently leads to the overexpression of cyclin D1 and c-Myc genes and expression of SFRP1/2 (Al Moustafa et al., 2004; Jung et al., 2013).

Protein 1 (AP-1) is a transcription factor activator that plays a critical role in the progression of HPV-associated CC. The availability of AP-1, which binds to the HPV URR, is required for the production of two key viral oncogenes, E6 and E7 (Rosl et al., 1997). If there is an occurrence of mutation in the binding AP-1 sites inside URR, the expression E6 / E7 is completely abolished. Modified AP-1 complex with more binding c-Fos and c-Jun and absence of Fra-1 expression in CC cell line HeLa is associated with the tumorigenic phenotype. The overexpression of c-fos acts as the catalyst for CC induction by ectopic expression of c-Fos in HeLa fibroblast hybrid tumorigenic. The upregulation of transcription factor c-fos resulted in negligible or very little expression of TCF transcription factor in CC cell line whereas its absence results in dysregulation of activity of Notch 1 in CC cells (Talora et al., 2002). Furthermore, AP-1 is a key regulator of the expression of HPV oncogenes E6 and E7, as well contribute in the development of resistance to chemotherapy and radiotherapy (Dhandapani et al., 2007). In the absence of HPV, functional heterodimerization of c-Fos and Fra-2 in the presence of c-Jun has been demonstrated to promote aggressive tumor phenotype with poor prognosis.

Fig. 6. Role of early protein (E6, E7) in regulating cellular signaling for CC progression.
The notch signaling pathway is responsible for the up-regulation of leukemia and other forms of cancer. While it is difficult to identify protein–protein interaction inhibitors in the form of small molecules, for HPV one such potential inhibitor of the E1–E2 interaction has been established. The congestion of the complex depends on the E1–E2 interaction with DNA and its therapeutic potential. In the E1–E2–ori ternary alongside a critical protein’s threshold level, the interplay is established between E2’s TAD and E1’s C terminal ATPase domain (White et al., 2003). Indandione is a small class of molecules that act as a potential inhibitor of the E1–E2 interaction. The basic mechanism how the inhibitors achieve their role is by bringing about significant changes in the movement of amino acids at the binding site (Talora et al., 2005; Zagouras et al., 1995). The amino acids whose residues mostly undergo a conformational change include Tyr-19, His-32, Leu-94, and Glu-100, ultimately resulting in the creation of a deep hydrophobic pocket to which the inhibitor’s indandione entity would firmly bind to. Also, the mutation of Glu-100 with alanine has shown increased sensitivity of the E2 protein to the indandione molecules (Talora et al., 2005; Zagouras et al., 1995). The potential of these indandione compounds in restricting the replication of the HPV genome in vivo will pave a path for utilizing the capacity of E2 protein for further consideration as a novel therapeutic target as well as a target for small molecules targeting the interaction of HPV with proteins. Further, in E2 TAD, the presence and characterization of inhibitor binding pockets showed potential in recognition of a novel target for therapy towards enhancing HPV infection treatment (D’Abramo and Archambault, 2011).

E4 protein also play role in the cell cycle arrest as it evident from the observation that the epithelial cells which showed an expression of E4 from HPV 16 and HPV 18 witnessed mitotic arrest at the G2 phase owing to the restriction of nuclear accumulation of cdk1 protein in the nucleus. Thus, it can be concluded that when phosphorylation of the E4 is controlled, its activity can be modulated and it is unable to affect the host. The available data is suggestive of that the epigenetic modulation of the E4 protein may serves as a novel therapeutic target for future drug development of future therapeutic strategy (White et al., 2005).

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discovery in therapeutics (Knight et al., 2006).

It has been speculated that HPV E5 oncoprotein induces cyclooxygenases – 2(COX-2) with the help of the EGFR signaling pathway along with NF-kB and activator protein-1(AP-1) (Kim et al., 2009). E5 inhibition carried out by inhibiting the NF-kB pathway with inhibitors like narasin or flurosalan, mediating inhibition of AP-1 was much more effective in controlling the E5 oncoprotein function. Inhibitors that bring effect directly have shown toxic at higher levels and at lower levels they are non-functional (Kim et al., 2013). However, researches have suggested new inhibitor compounds like celecoxib (Farley et al., 2006). With this kind of inhibitor, E5 oncoprotein mediated HPV-induced carcinoma can be targeted following subsequent lowering of E5 levels. Thus, targeting E5 oncoprotein or its relevant pathways may inhibit HPV-induced carcinoma in cervical cells. Wang et al. reported that recombinant oncolytic adenovirus M6 with an antisense E6-E7 gene selectively replicated in cancer cells, inhibiting tumor development and inducing apoptosis in HPV16-positive CC cells without causing cross-resistance or other problems (Wang et al., 2010). Other remedies include therapies like specific cytotoxic T cells which are designed specifically for HPV E5 proteins with CpG-oligodeoxynucleotide and may target and degrade them so that their function is lost permanently and leads loss of function of the viral E5 (Chen et al., 2004). RNA interference (RNAi) technology is another therapeutic strategy that target the HPV16-E7 region and help in the modulation of E6 and E7 proteins and knockdown both E6 and E7 expression simultaneously (Simà et al., 2008). This approach along with radioimmunotherapy have shown visible effects and showed potential of suppressing HPV-induced carcinoma along with a significant rise in p53 and Rb proteins (Simà et al., 2008; Wang et al., 2010).

HPV E6 oncoproteins are shown associated with p53 and degradation of p53 which is dependent upon the activity of the E6/E6AP complex. The inhibition of this complex can be a promising therapeutic approach to reactivate the function of p53 tumor suppressor protein. PDZ domains proteins including HDL (human homolog of Drosophila discs large tumor suppressor protein, MUPP1 (tight junction) (Brennan et al., 2009), and MAGI-1,2,3 membrane-associated guanylate kinase (MAGUK) proteins (Thomas et al., 2002) are considered useful as novel therapeutic targets. It is associated with E6 and maintains cellular polarity, adherence, and disrupting the cause of neoplasm formation. Among numerous approaches, one of them is to target the sequence alignment of the E6 binding motif Lxx Φ Lsh where L is leucine, s the small amino acid (glycine or alanine), Φ denotes a dipeptide where one of the residues is Glu, Asp, Gln or Asn, Lsh is the hydrophobic residue (commonly leucine) and h usually glutamine, glutamate, asparagine or aspartate (Baleja et al., 2006). The studies of mutagenesis have shown that the replacement of each of these leucine motifs with alanine may result in E6 binding being terminated (Baleja et al., 2006; Be et al., 2001).

Several studies have shown that E7 oncoprotein can influence the E2F factor-induced transcription by interacting with various cellular proteins as well as class I histone deacetylases as illustrated in figure 14 (Bremer et al., 1999; DeMasi et al., 2005). Various HDAC Inhibitors are being investigated in clinical trials to deactivate E7 as the upregulation of HDAC has a significant role in cancer progression (Baleja et al., 2006). In the reverse genetic experiments, it has been shown that mutations in E7-HDAC binding sequences in the HPV 31 genome can be diminished to facilitate the epismal maintenance and also regulate the proliferation of the primary keratinocytes (Longworth and Laimins, 2004). Taken together, the results of the interaction between E7 and HDAC inhibitors can be considered as a promising antiviral target and can be used for future remedies. Another protein, p600, (600 kDa) induces anchorage-dependant integrin modulated cellular interaction and becomes an important target both for therapy as well as for the E7 oncoprotein mediated action (Akhatova et al., 2021; Huh et al., 2005).

7. Therapeutic strategies for targeting E proteins

E proteins are largely responsible for instigating the process of HPV-mediated CC, however, amongst all forms of E proteins, E6 and E7 are the most crucial oncoproteins. This is suggestive of that targeting E6 and E7 oncoproteins may be important in significant eradication of all CC cells by inhibiting these cancer hallmarks. Currently, the focus is on developing various therapeutic strategies for targeting E proteins as a treatment approach in HPV-mediated CC.

Nucleic acid-based therapeutic strategies depend on the usage of antisense RNA or DNA moieties to intrude the appearance and/or activities of E6 and E7 oncogenes, which can efficiently avoid the progression of CC. Studies have shown that the hammerhead ribozymes significantly targeted the HPV16 E6/E7 transcripts and causes potent inhibition of the cell growth following apoptosis in vitro and also tumor growth in vivo in nude mice models (Zheng et al., 2002, 2004). In another study, it has been reported that a ribozyme targeting E6AP in the HeLa cells enhanced the process of apoptosis and mitomycin-C triggered DNA impairment (Kim et al., 2003). Reschner and co-workers developed antisense oligonucleotide conjugated with a ruthenium (photoreactive) complex that targeted E6 protein in HPV16-infected SiHa cells and significantly inhibited cell growth in monolayers as well as three-dimensional specimens (Reschner et al., 2013). Hu et al. first reported TALEN (transcription activator-like effector nucleases)-based genome editing of the E6/E7 oncogenes and demonstrated that it leads to the potent inhibition of the tumor growth, induce apoptosis, and decrease tumorigenic competence of HPV-infected cells, including HPV16 (SiHa) and HPV18 (HeLa) (Pal and Kundu, 2020b).

Conventionally, preventive vaccines exhibited a potential role in activating the immunity of the body to avoid the initiation and progression of CC. However, recent clinical studies are directing the development of targeted therapeutic vaccines that can be useful and efficient in treating CC, even at progressive malignant phases. Certain preventive vaccines which exhibit significant effects against L1 or L2 capsid proteins, get deactivated or removed during the combination of the HPV genome within the cellular genome. On the other hand, the therapeutic vaccines efficiently target the E6 and E7 oncoproteins, and get expressed for a prolonged time in HPV’s life cycle, therefore, exhibit significant hallmarks of HPV-mediated CC (Chabeda et al., 2018).

Numerous therapeutic vaccines are being investigated and are presently under different stages of clinical trial (phase I/II/III) for CC. Few vaccines, such as ProCervix and PDS0101, have been developed specifically for persistent HPV-mediated infections and inferior grade squamous intraepithelial lesions (SIL). In addition, few vaccines such as pNGVL4a-CRT/E7 (detox), GX-188E, PepCan + Candin have been developed for targeting cervical intraepithelial lesions or higher grade SILs. Moreover, few highly modified vaccines such as TA-CIN + GPI-0100, INO-3112, ADXS11-O01, HPV16-SLP, and others have been developed for targeting HPV16/18-E6/E7 oncoproteins and treating CC of advanced stages (Yang et al., 2016). Akhatova et al. (2021) assessed and compared the prevailing DNA therapeutic vaccines under clinical trials (phase I/II), that expressed HPV E6 and E7 proteins for the treatment of CC relying on the viral clearance, clinical efficiency, immunogenicity, and adverse effects. It has been observed that 5 different DNA therapeutic vaccines (VXG-3100, GX-188E, pNGVL4α–Sig/E7(detox)/HSP70, pNGVL4a-CRT/E7(detox), and MEDIO457) possess anti-viral activity to be effective against CC and exhibit high endurance against CC (Akhatova et al., 2021).

The advancements in the genome editing tools have assisted the researchers in specifically targeting the HPV-E6/E7 regions of the HPV genome or associated mRNAs, that results in improved CC therapy. In recent studies, various approaches have been shown for competently silencing the E6/E7 expressions, including zinc finger nucleases, TALENs, and the clustered regularly interspaced short palindromic repeat-associated nuclease (CRISPR/Cas9) RNA-guided endonucleases. These
approaches may aid in reducing the mortality rate associated with CC, particularly in the advanced stages of CC (Pal and Kundu, 2020b). One of the most important genome-editing tool, CRISPR-Cas9, has been initially detected as a part of adaptive immunity in the microbial systems to defend against extraneous nucleic acids (Makarova et al., 2015; Tenailleau et al., 2016; Wright et al., 2016). As per the reported studies, the CRISPR-Cas9 mediated E6 or E7 gene silencing is found highly applicable in inhibiting disease progression in CC. Moreover, the guide RNAs can be efficiently programmed following the specificity of the targeting exon segments of the E6 and E7 oncogenes. CRISPR-Cas9 facilitated silencing of E6/E7 oncogenes in HPV16-infected CaSkI or SiHa carcinogenic cells and HPV18-infected HeLa cells exhibited reduced growth, arrest of cell cycle and cell death mediating regeneration of p53 and pRB (Hsu et al., 2014; Kennedy et al., 2014). A recent study showed that the gene knockout chain reaction (GKCR) Cas9/guide RNA (gRNA) cassettes can significantly integrate within the targeted loci through homology-independent targeted insertion (HTI). The study demonstrated that the GKCR method led to a higher proportion of insertions or deletions (indels) in the E6 and E7 oncogenes of HPV18. Furthermore, the targeting of HPV18 E6/E7 oncogenes using GKCR method resulted in upregulation of P53/BB proteins and inhibition of the propagation of HeLa cells. Thus, this technique may be promising in the treatment of HPV infections and associated CC (Tian, Rui et al., 2022).

An ideal tumor/carcinogenic progressive microenvironment necessitates deactivation of the immune system of the body, which comprises of the low amount of T cells and their repressed actions, alterations in the cytokine expressions and damaged antigen performance (Mandal et al., 2016; Sun et al., 2014; Varilla et al., 2013). E6/E7-specific humoral and cellular immunity are clinically effective leading to the incessant activities of these oncogenes during the disease development and progression. In one of the study, synthesized HPV-16/18 E6 and E7 DNA sequences were familiarized to trigger the immune system responses employing a novel plasmid VGX-3100 (Bagarazzi et al., 2012; Trimble et al., 2015). Jin and co-workers showed that the T cells can be genetically modified and engineered for targeting the HPV16 E7, leading to the deterioration of HPV16-infected CC in mouse xenograft models (Jin et al., 2018). In another study, it has been demonstrated that the metastatic CC undergo complete deterioration in response to a solo permeation of T cells targeting HPV16 E6/E7 (Stevanovic et al., 2015).

Galvao and Coimbra discussed the role and significance of long non-coding RNAs (lncRNAs) in various types of cancer, including CC. They also summarized the facts associated with lncRNAs and recognized overall outline of the transcripts according to their role which they exhibit in CC (de Carvalho Galvao and Coimbra, 2020). Recently, it has been shown that non-coding RNAs (ncRNA), including lncRNAs, microRNAs, piRNAs (PIWI-interacting RNAs), and circular RNAs play significant role in monitoring various cellular function in the gynecological cancers, including CC. Furthermore, numerous clinical trials are ongoing to identify the biomarkers and potential therapeutic effects of ncRNAs (Razavi, Zahra Sadat et al., 2021).

Celegato et al. developed a small moiety (Cpd12) that showed potent inhibition of the E6-mediated deprivation of p53 by disrupting the E6/p53 binding in HPV16- and HPV18-positive CC cells. The authors reported that Cpd12 actively targeted CC cells altered through other high-risk-HPV (HR-HPV) strains, including HPV68 and HPV45, and also against a HPV16-altered head/neck carcinogenic cell line. Thus, it was concluded that Cpd12 could be used in targeting a wide range of HPV-induced carcinomas (Celegato et al., 2021). Lee et al. reported direct interface between the PDZ binding motif (PBM) of HPV16 E6 and the PSD-95/Dlg/ZO-1 (PDZ) domain of human protein tyrosine phosphatase non-receptor type-4, established through structural and biochemical studies. Also, it was confirmed that the equivalent motifs from various other HR-HPV genotypes, such as HPV-18/31/33/45, bind to PTPN4-PDZ with similar affinities, signifying that PTPN4 acts as a common target of several pathogenic HPV genotypes (Lee et al., 2022).

Numerous phototherapeutics-based approaches have been reported for targeting HPV-mediated CC, both in vitro and in vivo. Studies showed that various herbal compounds directly abrogated HPV-E6/E7 activities and these compounds play a crucial role as a potential drug candidate which efficiently knockdown the oncoproteins and assist in CC regression. Furthermore, numerous flavonoids have been recognized that specifically bind to the E6 proteins, leading to the inhibition of the p53 degradation which results a decrease in the viability of HPV-infected CC cells (Cherry et al., 2013). Numerous plant extracts and products such as latex from Ficus carica, flax-seed oil, Cudrania tricuspidata stem extracts reported to exhibit anti-oncogenic properties by inhibiting the expressions of E6 and E7 oncoproteins, following apoptosis (Deshpande et al., 2019; Ghanbari et al., 2019; Ghanbari et al., 2019; Pertietta et al., 2017).

8. Conclusion and future perspectives

CC is a burgeoning issue plaguing the lives of many women across the world. While the causative organisms for the occurrence of CC remain to be a double-stranded DNA virus, the major aim of which is to develop effective therapeutic approaches. Even though there has been the development of vaccines, the cost-effectiveness and accessibility continue to be a major issue. Here, we comprehensively reviewed the oncoproteins of HPV, their roles in various signaling pathways, cross-talk among the major oncoproteins, and targeting of cellular pathways for plausible development of novel therapeutic strategies.

The enzymatic role of E1 protein helps in HPV induced carcinoma with severe immunosuppressive nature concerning the immunological molecules which help in administration of HPV induced carcinoma with progression towards carcinogenesis. It can also be deduced that when E1 oncoprotein is targeted to control the prognosis of the HPV by regulating the enzymatic activity of E1 protein as the lead molecule for new drug molecules as if the enzymatic activity is dysregulated almost all the down-regulation of E1protein is controlled. The available data demonstrate that E2 has a dual role, therefore negative regulation of the E2 can easily be used to control the HPV and it can also serve as a potential target site. E4 may also play a role in arresting cell cycle as it has been evident that the epithelial cells which showed expression of E4 from HPV 16 and HPV 18 undergoes mitotic arrest at the G2 phase owing to the restriction of nuclear accumulation of CDK1 protein in the nucleus. This is suggestive of that if the phosphorylation of the E4 is controlled its activity is modulated and it is unable to affect the host. Thus, the epigenetic modulation of the E4 protein may serve as a novel therapeutic target for future drug discovery. The inhibition of the E6/E6AP complex may represent a novel therapeutic approach to reactivate the function of p53 tumor suppressor protein. The interaction between E7 and HDAC inhibitors is also suggestive of promising therapeutic target for antiviral drugs in future.

HPV is regulated by several E proteins of which E5, E6, and E7 oncoprotein can regulate and alter several cellular signaling pathways to cause cancer. These molecular pathways play major roles in malignant transformation. E5 oncoprotein stimulates the MAPK-ERK pathway. E6 oncoprotein alters PI3K/AKT pathway, Wnt pathway, Notch pathway, and E7 regulates the PI3K/AKT pathway. Moreover, these oncoproteins also alter the NF-kB pathway, Y11 pathway, and apoptotic pathways (intrinsic and extrinsic). These altered pathways are the major cause of CC initiation. Targeting altered cell signaling pathways, as well as epidermal growth factor receptor and vascular endothelial growth factor, has been recommended in several studies as a viable treatment method. Some inhibitors of these altered pathways have also been investigated in animal models and clinical studies. So, in essentiality, E5, E6, and E7 oncoproteins are the key players in cellular proliferation and cancer progression. Therefore, targeting these proteins and inactivating them in some aspects of their signaling pathways can prove to be promising anti-oncogenic therapeutic approaches.

Even though various advancement has been achieved to develop therapeutic strategies and techniques for targeting HPV-mediated CC,
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that influence the work reported in this paper.

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However, translations of these approaches in clinics are yet a major concern. The establishment of targeted delivery systems remains a crucial issue due to irregular particle size, surface charge, and lower membrane permeation (Bharti et al., 2018). Moreover, the application of genome editing tools in clinical usage have been a major concern due to safety, ethical and economic issues. Nevertheless, the research findings are promising but more efficient research should be performed for the exploration of effective, targeted and affordable therapeutics to assist the populations affected with HPV-mediated CC.

Author contribution

KKK, NKJ and RB conceptualized, wrote, and edited the manuscript. RB, SSB, AN, DD, AB, SM, LK, SK, and SR performed the literature survey and drafted and edited the manuscript. NKJ ideated the schemes and performed artwork. SSD, SKS, VJJ, DI, SA, SO and JR significantly contributed in editing and revision of the manuscript. KKK, NKJ and JR supervised the work. All authors contributed to the article and approved the submitted version.

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