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Review

Human papillomavirus type 16 E5 oncoprotein as a new target for cervical cancer treatment

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ABSTRACT

Human papillomavirus (HPV) infection is considered to be the necessary cause of cervical cancer. E6 and E7 oncoproteins of HPV have been known to play major roles in malignant transformation of cervical cells, inhibiting the tumor suppressors p53 and Rb. However, the role of E5 oncoprotein has been relatively less defined.

HPV 16 E5 is a hydrophobic membrane-bound protein which associates with the Golgi apparatus, endoplasmic reticulum and perinuclear membrane. Accumulating evidences have suggested that E5 oncoprotein may also contribute to cervical carcinogenesis through modulating cellular signaling pathways in addition to augmenting the immortalization potential of E6 and E7. Multiple mechanisms, including activation of EGFR or inflammatory cell signaling pathway, have been implicated in malignant transformation by HPV 16 E5. Therefore, targeting E5 may be a rational approach for chemoprevention and treatment of cervical cancer, and understanding its oncogenic processes may help us to design novel therapeutic strategies. In this review, we discussed the roles of HPV 16 E5 in cervical carcinogenesis, altering several cellular signaling pathways involved in cell proliferation, angiogenesis and apoptosis.

Contents

1. Introduction

Cervical cancer is the second most frequent malignancy in women worldwide [1]. Although overall survivals of cervical cancer patients have been improved through wide-spread implementation of screening programs with increased proportions of patients being diagnosed with early lesions, prognosis for advanced cancer still remains poor despite several efforts to improve treatment outcomes [2,3]. Therefore, development of new therapeutic strategies through identifying potential targets is warranted.

Human papillomavirus (HPV) infection is considered to be the necessary cause of cervical cancer [4]. Of at least 14 high-risk HPV types, HPV 16 is the most predominant type identified in cervical

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cancer, accounting for about 50% of cases [5,6]. Recently, two recombinant HPV vaccines containing virus-like particles (VLPs), a quadrivalent HPV 16/18/6/11 vaccine (Gardasil®, MSD) and a bivalent HPV 16/18 vaccine (Cervarix®, GSK), have been developed and licensed to prevent persistent HPV infection and hence to reduce the incidence of cervical cancer. Phase III randomized clinical trials demonstrated that both vaccines can prevent over 90% of moderate and severe precancerous cervical lesions associated with the most common oncogenic HPV types 16 and 18 [7,8]. However, these vaccines are designed to prevent HPV infection, and are not designed to treat patients who have already been infected with HPV. Consequently, different strategies, such as targeting viral antigens or signaling pathways involved in cellular transformation, are needed for patients who already have HPV-associated cervical lesions.

Malignant transformation by HPV is primarily attributed to three oncoproteins: E5, E6 and E7 [9]. E6 and E7 have been extensively studied on their roles in cervical carcinogenesis. E6 and E7 genes are consistently expressed in cervical cancer, and are able to immortalize various cell types [9]. E6 and E7 oncoproteins mediate malignant transformation of cervical cells through degradation of p53 and inactivation of pRb tumor suppressor proteins, respectively [10,11]. However, the role of E5 has been relatively less defined compared to E6 and E7.

E5 is a hydrophobic membrane-bound protein of 83 amino acids that associates with the Golgi apparatus, endoplasmic reticulum and perinuclear membrane [12,13]. HPV 16 E5 gene is considered as an oncogene because it can transform murine fibroblasts and human keratinocytes [14–16], and contribute to skin carcinogenesis in transgenic mice [17,18]. A recent study on E5 transgenic mice demonstrated that HPV 16 E5 could contribute to cervical carcinogenesis alone or in combination with E6 or E7, and its potency seems similar if not greater than that of E6 [19]. Moreover, HPV 16 E5 can impair the gap junction-mediated cell–cell communication, rendering cells insensitive to normal growth regulation signals [20,21].

The role of HPV 16 E5 has been suggested mostly in early stage of cervical carcinogenesis because of the observations that E5 gene is frequently deleted when the HPV genome is integrated during the progression from low-grade to malignant disease [22,23]. When evaluating the expression of HPV 16 E5 by immunohistochemistry, E5 protein was expressed in approximately 80, 90 and 60% of HPV 16-infected low-grade squamous intraepithelial lesions (SILs), high-grade SILs and cervical carcinomas, respectively [24]. In invasive cancer, E5 was expressed in tumors which contained the episomal viral genome. Therefore, targeting E5 which is frequently expressed in earlier stages of malignant transformation may be a rational approach for chemoprevention of cervical cancer, preventing premalignant lesions from progressing into invasive cervical cancers.

Recent studies have suggested that multiple mechanisms may be involved in malignant transformation by HPV 16 E5. Understanding oncogenic processes undertaken by HPV 16 E5 may help us to design novel therapeutic strategies. In this review, we summarized the roles of HPV 16 E5 in cervical carcinogenesis, altering several cellular signaling pathways involved in cell proliferation, angiogenesis and apoptosis.

2. HPV 16 E5 and epidermal growth factor receptor pathway

Several studies have suggested that HPV 16 E5 exerts its transforming effect mainly through interaction with epidermal growth factor receptor (EGFR) signaling pathway [14,17,22]. In vivo study on transgenic mice demonstrated that functional EGFR is required for E5-induced phenotypes, including epidermal hyperplasia and formation of spontaneous skin tumors [17]. Overexpression of EGFR signaling has been linked to the majority of cancers. Activation of EGFR regulates gene transcription and modulates cell proliferation, apoptosis, angiogenesis, tumor invasion and metastasis through Ras–Raf–MAP kinase pathway or PI3K–Akt pathway [25].

One of the mechanisms through which HPV 16 E5 regulates EGFR signaling is to increase the number of receptors expressed on the surface. E5 oncoprotein binds directly to the 16 kD subunit of vacuolar ATPase and inhibits its activity, interfering with the endosomal acidification and degradation of EGFR [22,26]. This impairs downregulation of the receptors and enhances turnover of the EGFR to the plasma membrane. In addition to increasing the number of receptors, HPV 16 E5 also increases EGFR phosphorylation in the presence of EGF [14,27–29]. Treatment of E5-expressing human keratinocytes with EGF results in an immediate increase in EGFR phosphorylation but not in an increase in the total amounts of EGFR, which indicates that increased EGFR activity cannot be solely due to an impaired downregulation of the receptors [28]. Furthermore, EGFR signaling pathway can be activated by HPV 16 E5 through either EGF-dependent or EGF-independent process. Transforming activity of E5, represented by increased cellular proliferation or anchorage-independent growth, is highly enhanced by the addition of EGF in a ligand-dependent manner in rodent fibroblasts and human keratinocytes [16,27]. By contrast, HPV 16 E5 can also activate mitogen-activated protein (MAP) kinase p38 and ERK1/2 in human keratinocytes in an EGF-independent manner [30]. ERK1/2 activation by sorbitol hyperosmolar shock in E5-expressing cells was shown to be EGF-independent and was only partially inhibited by tyrphostin AG 1478 (Sigma, München), which is an EGFR tyrosine kinase inhibitor.

Activation of MAP kinase by HPV 16 E5 involves two different pathways, a receptor tyrosin kinase-mediated and another, protein kinase C (PKC)-dependent pathway [31,32]. This, in turn, increases the transcription of early response genes such as *c-fos* and *c-jun* [15,33,34], which regulate the expression of other cellular genes and propel the cells through the cell cycle.

HPV 16 E5 was also reported to affect the activity of other ErbB family members such as ErbB2 or ErbB4 receptor [24,28,35,36], but less is known about the exact roles of the interaction between E5 and these receptors. Outside the EGFR pathway, HPV 16 E5 enhances G protein-coupled endothelin receptor (ET_A) pathway in response to endothelin-1 (ET-1) [37]. The presence of the E5 gene in growth factor-starved keratinocytes enhances the mitogenic activity of ET-1, and enables the E5-transfected cells to form a higher number of larger colonies compared to untransfected cells.

HPV 16 E5 also enhances cell proliferation through downregulation of tumor suppressor p21 and p27, both of which are cyclin-dependent protein kinase inhibitors (CKIs) and regulate the cell cycle progression. E5 was reported to suppress $p21^{Wafl/Sdil/Cipl}$ tumor suppressor gene at the transcriptional level in immortalized human keratinocytes [38]. The reduction of p21 promoter activity was correlated with transforming activity, which was manifested by increased expression of *c-jun*. In addition, HPV 16 E5 downregulates $p27^{Kip1}$, one of the most abundant CKIs, through reduction of the half-life of $p27^{Kip1}$ protein [39]. Downregulation of $p27^{Kip1}$, which is important for G1 cell cycle arrest, resulted in cell cycle progression and increase in DNA synthesis (S-phase). The effect of E5 on $p27^{Kip1}$ was enhanced in EGF-stimulated cells, but was abrogated by an EGFR inhibitor, suggesting that E5 activity on $p27^{Kip1}$ depends on EGFR signaling.

3. HPV 16 E5 as an inducer of inflammatory cell signaling

Over the past decade, a series of studies have suggested that the cyclooxygenase-2 (COX-2) plays an important role in carcinogen-

esis and cancer progression in various malignancies, including cervical cancer [40–43]. COX-2 was demonstrated to be overexpressed in cervical intraepithelial neoplasia and cervical cancer, but not in normal cervical tissue [43–45]. The role of COX-2 in cervical carcinogenesis was further supported by two clinical trials showing the efficacy of COX-2 inhibitor in the treatment of premalignant lesions of cervix [46,47]. Moreover, COX-2 was associated with resistance to chemotherapy/radiotherapy and poor survival in cervical cancer patients [48,49]. We previously reported that COX-2 overexpression is correlated with decreased overall survival by increasing lymph node metastasis [50,51].

In addition to modulating p53 and pRb tumor suppressors, HPV 16 E6 and E7 oncoproteins were suggested to contribute to carcinogenesis through enhancing COX-2 transcription by activating EGFR–Ras–MAP kinase pathway [52]. HPV 16 E6 and E7 stimulate COX-2 transcription by enhancing the binding of activator protein-1 (AP-1) to the cyclic AMP (cAMP)-responsive element (CRE) of the COX-2 promoter and inducing a corepressor/coactivator exchange. Likewise, our group evaluated the effect of HPV 16 E5 on COX-2 expression and demonstrated that E5 upregulates COX-2 expression through the EGFR signaling pathway, with nuclear factor-kappa B (NF-κB) and AP-1 as critical factors [53]. In contrast to E6 and E7, HPV 16 E5 enhances COX-2 expression in a different pathway, in which NF-κB plays a larger role than AP-1.

Prostaglandin E_2 (PGE_2) is the principal enzymatic product of COX-2 and its levels are elevated in most epithelial malignancies. PGE_2 affects numerous mechanisms associated with carcinogenesis, including cell proliferation, cell survival, angiogenesis, migration and invasion [25,54]. Therefore, we evaluated the effect of HPV 16 E5 on PGE_2 production and found that E5 induces secretion of PGE_2 through COX-2 expression [53,55]. In addition, recent studies have suggested that PGE_2 receptors (EP receptors), components of the COX-2–PGE_2 pathway, also play an important role in the development and growth of tumors in various tissues, including the cervix [25,56,57]. G protein-coupled EP receptors include four subtypes (EP1–4). Of these, EP4 receptor has been implicated in carcinogenesis and cancer progression of breast and colon cancer [57–59]. We demonstrated that HPV 16 E5 induces EP4 expression in cervical cancer cells by stimulating the binding of CREB to a variant CRE site in the promoter of EP4 gene (Fig. 1) [55]. EP4 induction by E5 sequentially involves EGFR, COX-2, PGE_2, EP2 and EP4, cyclic AMP-dependent protein kinase A (PKA), CREB and CRE.

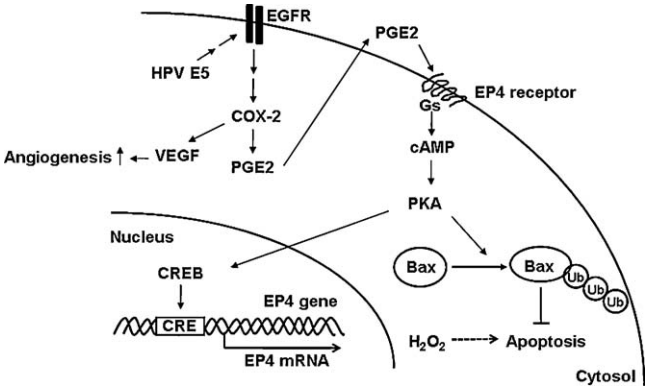

Fig. 1. A schematic diagram of COX-2–PGE_2 pathway induced by HPV 16 E5. E5 induces COX-2 expression through EGFR activation. The upregulated COX-2 enhances angiogenesis, activates EP4 expression, and inhibits hydrogen peroxide-induced apoptosis. COX-2 induces PGE_2 secretion, which, in turn, causes cAMP production. PKA which is the major downstream effector of cAMP mediates EP4 expression by E5 oncoprotein through enhancing CREB binding to variant CRE of the EP4 promoter. In addition, PKA mediates ubiquitin–proteasome-mediated Bax degradation.
Adapted from [55] with permission from Elsevier.

Activation of EP4 by HPV 16 E5 increased anchorage-independent colony formation and vascular endothelial growth factor (VEGF) expression, both of which are required for tumor growth, angiogenesis and metastasis.

Taken together, these results suggest that HPV 16 E5 may play an important role in carcinogenesis, progression and metastasis of cervical cancer by inducing inflammatory cell signaling pathways.

4. HPV 16 E5 and angiogenesis

Angiogenesis is an essential step in tumor growth and metastasis [60,61]. Vascular endothelial growth factor (VEGF), a major mediator of tumor angiogenesis, promotes mobilization of endothelial progenitor cells, cell proliferation, migration, survival and vascular permeability [62,63]. Overexpression of VEGF has been observed in most solid tumors including lung, colon and ovarian cancer [64–66]. VEGF was also found to be overexpressed in cervical cancer and associated with poor prognosis of cervical cancer [50,67].

HPV 16 E6 and E7 oncoproteins have been shown to induce VEGF expression in a HIF-1α-dependent manner [68,69]. We also found that HPV 16 E5 induces VEGF expression in various cell lines. E5-mediated overexpression of VEGF involves phosphorylation of EGFR, resulting in activation of MEK-extracellular signal-regulated kinase1/2 (ERK1/2) and phosphatidylinositol 3-kinase (PI3K)–Akt pathways [70]. These two pathways regulate VEGF expression through change in VEGF transcriptional activity rather than alteration in mRNA stability. COX-2–PGE_2 pathway is also implicated in VEGF expression by HPV 16 E5 (Fig. 2) [55]. Overexpression of VEGF mRNA and protein by E5 oncoprotein was blocked by EP4 receptor antagonist, suggesting that E5 may contribute to angiogenesis through activation of EP4.

Collectively, HPV 16 E5 enhances angiogenesis by inducing VEGF expression through EGFR signaling and COX-2–PGE_2 pathway. The ability of HPV 16 E5 to induce angiogenesis further supports its role in cervical carcinogenesis.

5. HPV 16 E5 and apoptosis

Acquired resistance to apoptosis is a hallmark of most and perhaps all types of cancer, leading to cancer growth, metastasis

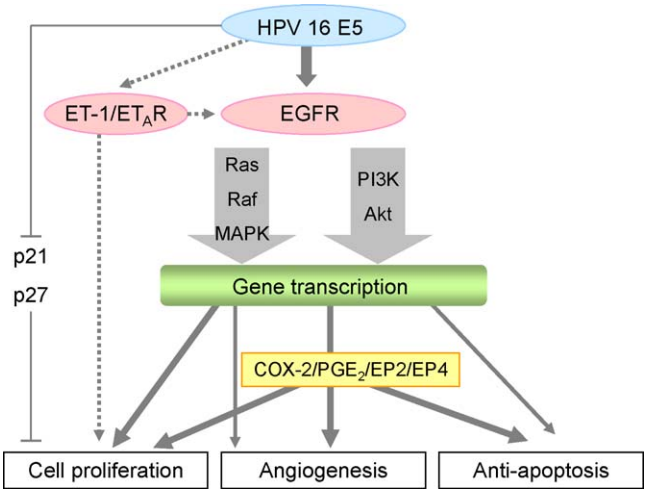

Fig. 2. HPV 16 E5-induced signaling pathways. HPV 16 E5 exerts its transforming effect mainly through EGFR signaling pathway. Activation of EGFR regulates gene transcription and modulates cell proliferation, angiogenesis, and anti-apoptosis through Ras–Raf–MAP kinase pathway or PI3K–Akt pathway. E5 protein also enhances cell proliferation through activation of G protein-coupled endothelin receptor (ET_A) pathway or through downregulation of tumor suppressor p21 and p27.

and resistance to chemotherapy or radiotherapy [71]. HPV-positive cervical cancers and cell lines display a differential expression of several caspases and downregulation of Fas expression, leading to impaired apoptosis [72,73]. HPV 16 E6 has been widely studied, and reported to inhibit intrinsic, p53-dependent apoptosis by inactivating pro-apoptotic proteins such as p53, Bak, FADD, or c-Myc through the ubiquitin proteasome pathway [74–77]. In contrast, HPV 16 E5 was reported to impair Fas ligand (FasL)- and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in HaCaT cells by downregulation of Fas expression and altering the formation of the death-inducing signaling complex (DISC) triggered by TRAIL [78]. Through impairing FasL- and TRAIL-mediated apoptosis, E5 protects cells from extrinsic ligand-mediated apoptosis. HPV 16 E5 also protects tumor cells from UV-irradiation-induced apoptosis by enhancing the PI3K–Akt and ERK1/2 MAP kinase signaling pathway [79]. In addition, we showed another mechanism of inhibiting apoptosis in cervical cancer cells by HPV 16 E5. We found that E5 protein inhibits hydrogen peroxide-induced apoptosis by stimulating ubiquitin–proteasome-mediated degradation of Bax, which is a pro-apoptotic protein. The degradation of Bax is mediated by COX-2–PGE_2 signaling pathway, which sequentially involves COX-2, PGE_2, EP2, EP4 and PKA (Fig. 1) [80]. In contrast, E5 was also reported to sensitize human keratinocytes to apoptosis induced by osmotic stress [81]. However, this may be due to cell membrane modifications caused by highly hydrophobic E5 protein.

By modulating apoptosis, HPV 16 E5 enables HPV 16-infected cervical cells to survive apoptotic stimuli from physical or chemical insults. In addition, HPV 16 E5 may contribute to evasion of host immunosurveillance by protecting infected cells from apoptotic stimuli derived from immune effector cells through impairing FasL- and TRAIL-mediated apoptosis. These activities of E5 may ultimately lead to cervical carcinogenesis and progression.

6. HPV 16 E5 as a target for cervical cancer treatment

HPV 16 E5 has emerged as a new potential target for treatment of cervical cancer based on the observations that E5 contributes to cervical carcinogenesis by modulating several cellular signaling pathways involved in malignant transformation. Moreover, since E5 is more consistently expressed in precancerous lesions, E5 oncoprotein or its relevant pathways may be targeted to prevent precancerous lesions from progressing into invasive cancers.

Immunologically targeting E5-expressing cells with tumor vaccines against HPV 16 E5 is one of the potential therapeutic strategies. In animal experiments, vaccination with recombinant adenovirus expressing HPV 16 E5 gene or cytotoxic T lymphocyte (CTL)-specific E5 peptide with CpG-oligodeoxynucleotide could significantly reduce the tumor growth [82,83]. The regression of HPV-associated lesions was dependent on cell-mediated immune response, and CD8+ CTLs were the most effective immunological effectors.

Other potential therapeutic strategies targeting HPV 16 E5 include oncolytic adenoviruses, radioimmunotherapy, gene silencing using short interfering RNA (siRNA) and others, which have been originally used to target E6/E7 oncoproteins. Transfection of HPV 16-related cancer cells with oncolytic adenovirus in combination with radiotherapy significantly suppressed the cell growth and induced apoptosis in vitro [84]. The same group also demonstrated that treatment of cervical tumors in mice with radiolabeled monoclonal antibodies to E6 protein resulted in dose-dependent retardation of tumor growth [85]. Selective silencing of HPV oncogenes using siRNA is another promising therapeutic approach to HPV-associated cancer. In vitro and animal experiments using siRNA targeting E6/E7 showed a significant inhibition of tumor growth by restoring normal Rb and p53 function [86,87].

Since HPV 16 E5 oncoprotein is involved in several mechanisms associated with cervical carcinogenesis, including upregulation of EGFR signaling pathway, angiogenesis and anti-apoptosis, targeting the relevant pathways can also be a feasible therapeutic strategy. If verified as effective and safe in future clinical studies, E5-targeted therapies or in combination with standard therapeutic modalities could improve the treatment outcomes in cervical cancer.

7. Conclusion

HPV 16 E5 gene has been regarded as another potential oncogene apart from E6 and E7. HPV 16 E5 plays an important role in cervical carcinogenesis through multiple mechanisms, including upregulation of EGFR signaling pathway, angiogenesis and anti-apoptosis (Fig. 2). In addition, HPV 16 E5 contributes to malignant transformation through augmenting and supplementing the roles of E6 and E7. We also found that HPV 16 E5 induces inflammatory cell signaling pathway, COX-2–PGE_2 pathway, which is involved in various oncogenic processes such as angiogenesis and anti-apoptosis. Targeting these E5-induced signaling pathways or E5 itself could be a new therapeutic strategy in HPV-associated cancers including cervical cancer.

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