Cellular Functions of HPV16 E5 Oncoprotein during Oncogenic Transformation

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ABSTRACT

The human papillomavirus (HPV) is recognized as the main etiologic agent associated with cervical cancer. HPVs are epitheliotropic, and the ones that infect the mucous membranes are classified into low-risk (LR) and high-risk (HR) types. LR-HPVs produce benign lesions, whereas HR-HPVs produce lesions that may progress to cancer. HR-HPV types 16 and 18 are the most frequently found in cervical cancer worldwide. E6 and E7 are the major HPV oncogenic proteins, and they have been profusely studied. Moreover, it has been shown that the HPV16 E5 (16E5) oncoprotein generates transformation, although the molecular mechanisms through which it carries out its activity have not been well defined. In contrast to *E6* and *E7*, the *E5* open reading frame is lost during the integration of the episomal HPV DNA into the cellular genome. This suggests that E5 acts at the early stages of the transformation process. In this review, we focused on the biochemical characteristics and functions of the HPV E5 oncoprotein, mainly on its association with growth factor receptors and other cellular proteins. Knowledge of the HPV E5 biology is important to understand the role of this oncoprotein in maintaining the viral cycle through the modulation of proliferation, differentiation, and apoptosis, as well as the alteration of other processes, such as survival, adhesion, migration, and invasion during early carcinogenesis. Finally, we summarized recent research that uses the E5 oncoprotein as a therapeutic target, promising a novel approach to the treatment of cervical cancer in its early stages.

Introduction

Cervical cancer has been recognized as a major public health issue of high priority. Globally, cervical cancer cases amount to 6.6% of all cancers reported annually among women. According to the 2018 GLOBOCAN report, approximately 569,847 new cases of cervical cancer were diagnosed in the world that year. From these incident cases, 85% were reported in developing countries, where cervical cancer is the second most common cancer (1).

The human papillomavirus (HPV) infection is considered a necessary factor for the development of cervical cancer, as it is detected in 99.7% of the cases. The most frequently detected types are HPV16, 18, 31, and 45, which are considered high-risk (HR; ref. 2). These HR-HPV infections have been associated with certain types of cancer, and the International Agency for Research on Cancer has classified HPV16 and 18 as carcinogens in the development of anal, cervical, oropharyngeal, penile, vaginal, and vulvar cancers (3).

Papillomaviruses (PV) are small viruses with nonenveloped icosahedral capsids, carrying double-stranded circular DNA, which belongs to the *Papillomaviridae* family and infects squamous epithelial cells inducing proliferative lesions (4). The PV DNA contains three regions: (i) an early region (E) with open reading frames (ORF) that codes for nonstructural proteins involved in viral DNA replication (E1), viral expression regulation (E2), virus assembly (E4), and immortalization and transformation of infected epithelial cells (E5, E6, and E7), in the case of HR-HPVs; (ii) the second one is the late region (L) that codes for L1 and L2 proteins of the viral capsid; and (iii) the third one is the long control region that contains the origin of viral replication necessary for the regulation of viral gene expression (4).

During productive infection, HPV genomes are established as autonomous replicating extrachromosomal elements, or episomes, with a high copy number that depends on cell differentiation. Interestingly, Scott and colleagues propose that the HPV16 E5 (16E5) protein helps the virus to avoid the integration of the host genome (5), which may ensure viral progeny. However, the progression from high-grade squamous intraepithelial lesions (HSIL) to cancer usually occurs in lesions that contain integrated copies of the viral genome. This leads to an abortive infection, and cells can no longer produce viral particles (6). The HPV DNA integration causes the loss of the *E2* and *E4* gene expression, which triggers a downreplication of the viral genome, a G_2 arrest, and an E6 and E7 overexpression that confers the cell a growth advantage over cells that maintain the HPV DNA episomally (7). These events result in the immortalization and possible transformation of infected epithelial cells (7).

The alteration of the *E2* region during viral integration is also believed to disrupt the *E5* gene, and for this reason, the main functions of E5 have been associated with the productive viral life cycle. However, recent evidence suggests that 16E5 could be expressed from integrated viral DNA, as is the case in the cervical cancer cell lines CasKi and SiHa (8). Another study showed that the expression of the E5 protein was observed in all of the HSIL and cervical cancer tissues, but only in 12% of the normal cervical tissues, which suggests that the expression of the E5 is important for the disease's development and progression (9).

This review will focus on recent information on the cellular and biological activities of the 16E5 oncoprotein, associated with different cellular processes that lead to transformation, and on its potential use as a target in therapeutic vaccination.

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Biochemical Characteristics of the HPV16 E5 Oncoprotein

E6 and E7 are the main HPV16 oncoproteins to have been characterized. More recently, the 16E5 oncoprotein has been considered oncogenic, as it can transform cells in culture (10). However, only the HR-HPV types codify for E5 proteins, whereas most low-risk (LR)-HPVs lack a defined homologous *E5* ORF and/or a translation start codon for this protein (11).

According to their *L1* gene sequence, HPVs are classified into alpha, beta, gamma, mu, and nu genera, and the *alpha-papillomavirus* genus is the one containing the HPVs associated with mucosal and genital lesions (12). In terms of the *E5* gene expression, the protein is observed in all HR-HPVs, although the amino acid sequences of E5 proteins are poorly conserved in those HPV types. To better characterize the E5 proteins phylogenetically, Bravo and Alonso used biochemical characteristics, principal restriction factor for protein evolution, such as hydrophobicity, the content of isoleucine, leucine, and valine, and presence of transmembrane regions, to finally classify the E5 into four different families: E5α, E5β, E5γ, and E5δ (11). According to this new classification, it was clear that the E5α protein type is associated with cervical and penile cancer, and it is encoded by HR-HPVs (**Fig. 1**). On the other hand, the E5 proteins encoded by LR-HPVs are E5β, E5γ or E5δ families (11). This knowledge allows us to better understand the functioning of this protein in the context of the cell.

The 16E5 is a small hydrophobic protein of 83 amino acids, usually localized in the membranes of the endoplasmic reticulum (ER), the Golgi apparatus, the nuclear membrane, and the endosomes (13, 14). Furthermore, the 16E5 protein was observed in the plasma membrane when transduced in HaCaT cells (immortal human keratinocytes), showing its N-terminus at the intracellular site and its C-terminus at the extracellular one (15). More recently, through a novel recombinant E5 expression system (FLAG-E5), the 16E5 protein was purified and characterized by transmission electron microscopy (TEM). It was found that it forms oligomeric assemblies organized in a channel with hexameric stoichiometry. This places the 16E5 oncoprotein within the virus-encoded "viroporin" family (16). Furthermore, by using an *in silico* model approach, Mahato and Fisher studied and confirmed the experimental model of the 16E5 viroporin. In this model, the researchers displayed both the hexameric structure and the pores formed by this viroporin. By using a simulation model, they also calculated a putative diameter of the pore (between 1.2 and 2.8 nm), and they suggested that this HPV viral channel has a weak ion selectivity as has been reported for other viroporins (17).

The amino acid sequence of 16E5 presents a high content of serine, threonine, and alanine residues, which can form polar interactions with adjacent side chains in transmembrane alpha-helices. Moreover, there are cysteine residues, which have been shown to induce alpha-helices via disulfide bonds or noncovalent interactions between its transmembrane domains (TMD), giving the E5 cysteines a potential role in stabilizing the transmembrane region (16, 18).

By using *in silico* predictions, circular dichroism, and hydropathic analysis, it was predicted that this protein has well-defined three-pass transmembrane hydrophobic domains, each with 15 to 22 hydrophobic amino acids (16, 18, 19). The amino acid sequences of the three 16E5 domains have the typical protein consensus motif with leucine-rich repeats that usually fold as a parallel β-pleated sheet. Alonso and Reed's model suggests that the 16E5 central hydrophobic domain folds as a β hairpin, whose ends are connected by a loop, whereas the central portion lies outside the membrane (19).

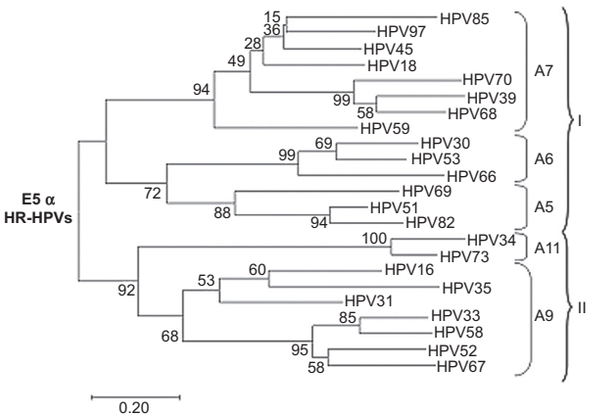

Figure 1.
Phylogenetic tree of the E5 protein sequences from HR alpha HPV. Phylogenetic tree for E5 proteins was created with the sequences of 19 alpha HPVs used by Bravo and Alonso (11) and the sequences of HPVs 53, 82, 85, and 97, recently described. Protein sequences were retrieved from the Los Alamos HPV sequence database and analyzed by the Mega X computing platform (20). The bootstrap values are indicated at the branch points (scale bar, 0.2 amino acid substitutions per site). The phylogenetic tree was generated by neighbor-joining and the JTT matrix–based methods and with a bootstrap test of 1,000 replicates. Two clusters I and II were differentiated by using these methodologies. Cluster I includes A7, A6, and A5 groups, and cluster II encloses A11 and A9 groups from E5 proteins described by Bravo and Alonso (11).

Our group used all this information, together with the knowledge that the N- and C-termini are not associated with the membrane and the recent finding on the orientation of the hydrophilic N- and C-terminal regions (15), introduced the data into the Hidden Markov Model for Topology Prediction (HMMTOP) version 2.0 and the RHYTHM programs, and analyzed it by the Mega X computing platform (20). To predict transmembrane helices and protein topology, we based our model on the principle that in sequence segments, the transmembrane protein topology is determined according to the maximum divergence in amino acid composition. The result of this hypothetical model for the 16E5 protein is shown in **Fig. 2**.

The predicted topological model for this protein displays three transmembrane alpha-helices (**Fig. 2A**) with an intracellular N-terminus and an extracellular C-terminus (**Fig. 2B** and **C**). This 16E5 hypothetical model is consistent with the work published by Wetherill and colleagues. Using TEM, they identified the presence of ring-like structures with an electron-dense center of approximately 2 nm in diameter that suggests the existence of a membrane-integrated channel structure. Wetherill and colleagues developed a model of the 16E5 oligomer by introducing the primary sequence into the membrane topology prediction software (PSIPRED and MEMSAT3), generating a three-TMD monomer with a triangular wedge-like arrangement. The final oligomerization model was constructed using a Maestro software that predicts a membrane-integrated hexameric channel structure reminiscent of other viroporins (**Fig. 2D**; ref. 16). Although most of this information is in agreement with the identification of the 16E5 protein as a membrane-associated protein, some controversies exist regarding the orientation of the C- and N-termini in the membrane.

Schlegel's group described the orientation of the 16E5 protein N- (cytoplasm) and C (extracellular)-termini; the results, however, contradicted those previously reported (21). These researchers generated

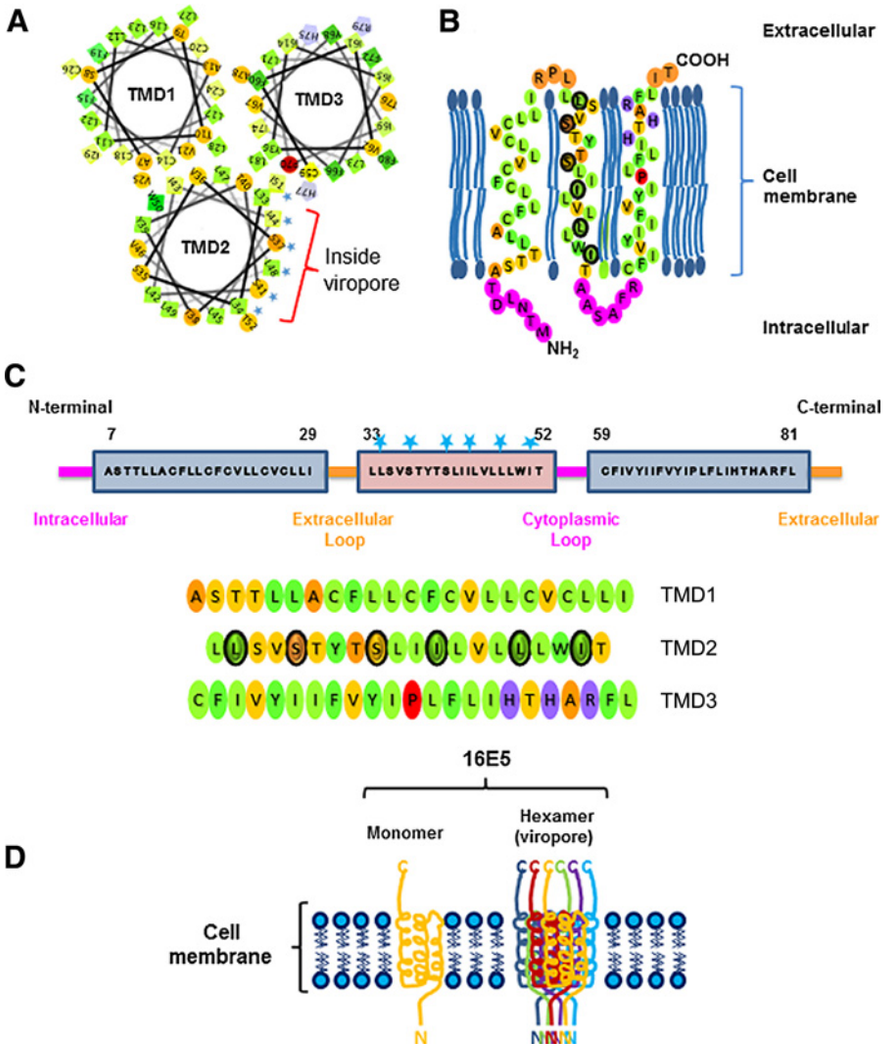

Figure 2.

Membrane topology model for 16E5 oncoprotein. **A,** Helical wheel representation of transmembrane regions of 16E5 as analyzed by the program created by Don Armstrong and Raphael Zidovetzki. Amino acids shape code: ○ hydrophilic residues; ◇ hydrophobic residues; ⌂ positively charged residues. Amino acids color code: The scale from green to yellow is used to denote from the most hydrophobic residues (dark green) to the less hydrophobic residues (yellow). The scale from red to orange is used to denote from the most hydrophilic residues (red) to the less hydrophilic (orange). Light blue, charged residues. **B** and **C,** E5 protein was analyzed by two topology prediction programs HMMTOP version 2.0 and RHYTHM, with the N-terminus at the intracellular site and the C-terminus at the extracellular site. The residues 1–6 are part of the hydrophilic N-terminal cytoplasmic part; then residues 7–29 form the first helix in the membrane (TMD1) and connect with the second helix by a short loop (residues 30–32) located in the extracellular site. The second transmembrane helix (TMD2, residues 33–52) is linked with the third helix (TMD3, residues 59–79) by a short loop (residues 53–58), which is facing to the cytoplasmic site. Finally, a short hydrophilic tail at the C-terminal (residues 80–83) is localized at the extracellular side of the membrane. The numbers inside the helices show where each given sequence begins and ends. **D,** Representation of 16E5 monomer crossing a cell membrane with its three TMDs, with the N-terminus at the cytoplasmic side and C-terminus oriented to the extracellular side, respectively. According to Wetherill's model (16), the 16E5 oncoprotein is a viroporin because six monomers of it can ensemble as a hexameric structure that creates a pore through the membrane.

the 16E5 protein with the AU1 epitope tag at the N-terminus and the HA epitope at the C-terminus. Thus, they undoubtedly showed that in the ER, the C-terminus is at the cytoplasmic site. They also found that the double-tagged 16E5 protein retains its biological activity, binding the vacuolar H^+-ATPase-16-kDa subunit, and its ability to form koilocytes. However, the protein at the plasma membrane was not identified, and no binucleated cells were observed (21). Nevertheless, Schlegel's group suggested that these two activities attributed to the 16E5 protein could be a consequence of the overexpression of the E5 protein by the adenovirus system used in the HaCaT cells, rather than its activities *per se* (21). The lack of specific antibodies against the HPV E5 proteins has made it difficult to accurately describe its localization and its biochemical traits. More research is needed to resolve the structure of the 16E5 protein. This will be of great help in understanding its biological characteristics.

Role of E5 in the Viral Life Cycle and Cell Transformation

During the viral cycle, there is a differential spatial and temporal expression of HPV proteins in the infected epithelium. This event is highly dependent on the differentiation program of keratinocytes that, under normal conditions, are growth-arrested (22). For that reason, HPV has developed strategies, through E5, E6, and E7 oncoproteins, to alter cellular growth and differentiation pathways, to drive the infected cell into a survival stage that facilitates its replication and ensures a successful production of virions (23). However, if the HPV infection persists, instability of the cellular genome appears, the viral cycle is abandoned, and the viral DNA is integrated or could continue as mixed episome/integrated, allowing the initiation of the transformation process (24).

During the viral cycle, E5 protein expression and other early HPV proteins (E1 and E2) depend on the *E4* gene presence, but most important is that these two proteins (E4 and E5) seem to work together for viral DNA amplification (25). In this way, E5 protein expression is considered necessary for promoting proliferation by guiding the cell to re-enter the cell cycle, allowing viral genome amplification, and activating late viral functions (26, 27).

E5 protein expression is thought to be essential for the completion of the viral life cycle, promoting an adequate environment by stimulating the cellular proliferation through the regulation of the EGFR activity and retarding the differentiation process of keratinocytes, downregulating the keratinocyte growth factor receptor (KGFR; refs. 28, 29).

The HR-HPV tumorigenesis has been related mainly to the *E6* and *E7* oncogenes, but later on, it was demonstrated that *E5* also possesses oncogenic activity. The participation of HPV E5 in the development of cancer emerged from different studies, which showed that expression of this oncoprotein in murine fibroblasts induced the formation of colonies in soft agar and increased cellular proliferation of human cell lines, both activities strengthened by the presence of EGF (30–32). Besides, 16E5 protein expression showed to be tumorigenic in nude mice (31), and in cooperation with E6 and E7 improves its transforming activity (33, 34). In addition, in a transgenic mouse model expressing the 16E5 protein, it was demonstrated that EGFR activation was necessary to induce hyperplasia and spontaneous tumor formation (35).

The carcinogenic ability of 16E5 protein has been related to the EGFR activity and the signaling pathways. Overactivation of the EGFR pathway by E5 promotes proliferation and, eventually, transformation. These events could be driven through the interaction of E5 with the 16-kDa subunit of the vacuolar ATPase (v-ATPase) that disrupts the endosomes' acidification, which favors the recycling of EGFR to the cellular membrane, and enhances its mitogenic activity (**Fig. 3A**; refs. 24, 36, 37). Moreover, the EGFR is labeled for degradation by the ubiquitin ligase c-CBL protein (Casitas B–lineage lymphoma). This activity is inhibited by 16E5, reducing the degradation of this receptor (38). Therefore, the 16E5 protein increases the level of EGFR by controlling its degradation process and regulating the pH in the endosomes, either by inhibiting the v-ATPase activity or by introducing negative charges directly to the endosomes, or both.

Activation of EGFR allows the switch-on of mitogenic signals to the nucleus, such as the RAS-MAPK signal transduction pathway leading to the regulation of the transcriptional factor AP-1 (activating protein

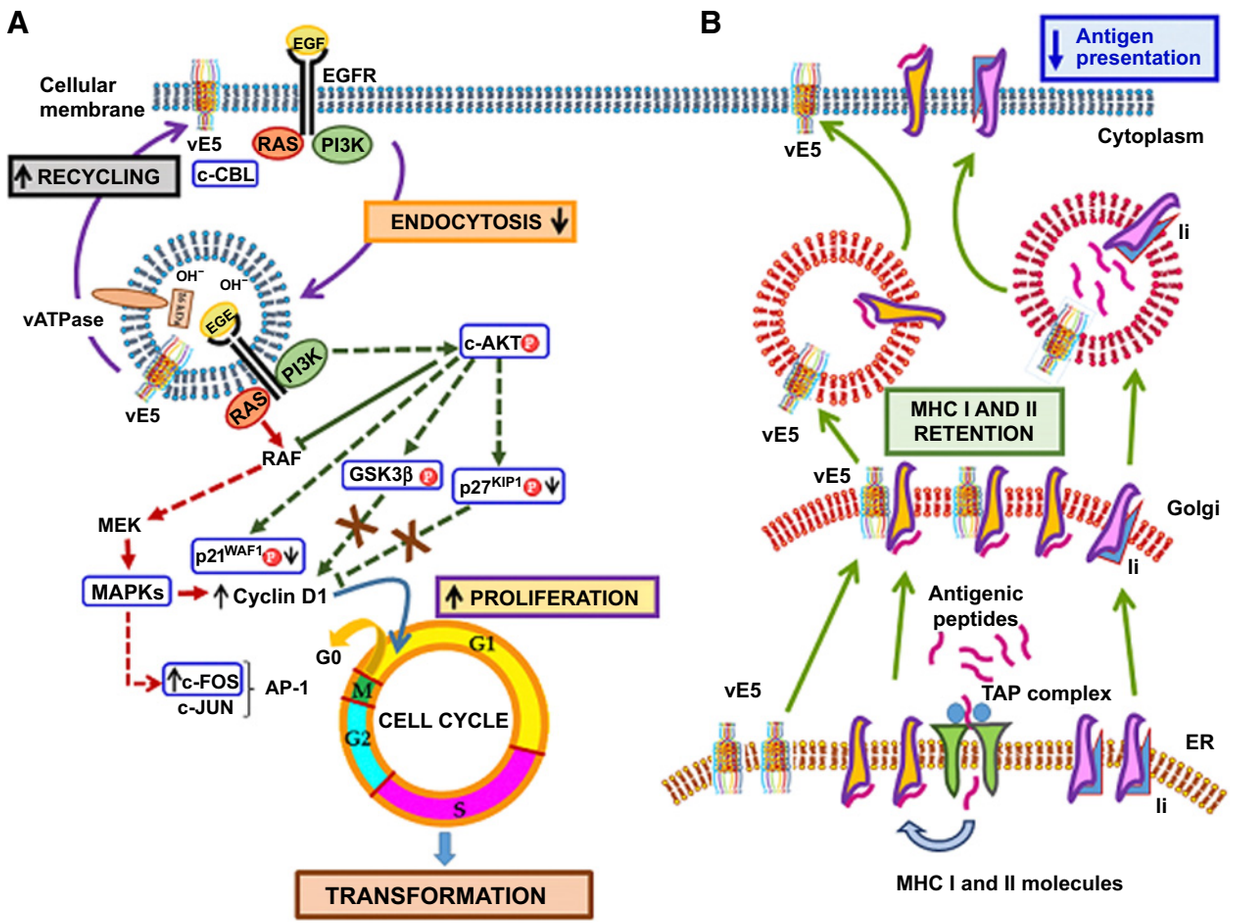

Figure 3.

E5 activities during the viral cycle and transformation. **A,** E5 protein modulates the EGFR activity by inhibiting endosome acidification and allowing its recycling to the cell membrane. These events are through the interaction with the v-ATPase H^+ and/or interfering with c-CBL. Over-activation of the MAK signal pathway occurs, which maintains high levels of cyclin D1 to keep the cells into the cell cycle. Transcriptional factor AP-1 activity is also increased in this cascade. The PI3K-AKT signal transduction pathway is also modulated by E5 protein, by increasing c-AKT activity, which modulates by phosphorylating the activity of various proteins. Phosphorylation of GSK3β leads to the accumulation of nuclear cyclin D1. Also, phosphorylation of $p21^{WAF1}$ and $p27^{KIP1}$ avoids translocation into the nucleus, unable to inhibit the CDK–cyclin D1 complexes permitting the constant re-entry to the cell cycle and eventually allowing cell transformation. Proteins enclosed in blue rectangles denote those that experimentally are modulated by E5 protein. Dashed arrows indicate known pathways modulate by E5 protein. **B,** E5 evades the immune response through the reduction of antigen presentation. Direct interaction of E5 with the heavy chain of MHC I retains these molecules in Golgi and reduces MHC I complexes on the cellular surface. In the case of MHC II, the E5 protein prevents the degradation of the invariant chain (Ii) associated to these molecules, resulting in the retention of these complexes intracellularly and unable to load peptides for antigen presentation.

1) composed of c-FOS and c-JUN (**Fig. 3A**; ref. 39). Several studies have confirmed that E5 upregulates MAPK and c-Fos activities in an EGFR-dependent manner (29, 32), suggesting that E5 may potentiate viral gene expression through the activation of *c-fos* because HPV has an enhancer that contains AP-1–binding sites (40). Thus, E5 may contribute to transformation by keeping up *E6/E7* oncogenes and other cellular genes (41).

On the other hand, the EGFR signaling transduction pathway activates the *RAS*-MAP kinase cascade to enter the cell cycle. However, this receptor also requires the activation of the PI3K/AKT pathway to complete it. Activation of AKT allows the progression from G_1 to S phase, increasing the nuclear translocation of cyclin D1 due to the inactivation by phosphorylation of the GSK3β protein. Also, AKT directly phosphorylates $p21^{WAF1}$ and $p27^{KIP1}$ at their nuclear localization signal, leading to cytoplasmic accumulation and rendering them unable to function as cyclin-dependent kinase (CDK) inhibitors in the nucleus (42). In this way, 16E5 showed to downregulate the expression of these CDK inhibitors in an EGFR-dependent manner (**Fig. 3A**; refs. 37, 43). Interestingly, $p27^{KIP1}$ can downregulate EGFR transcription (44). Therefore, it is suggested that the maintenance of low levels of this CDK inhibitor by 16E5 protein also allows the re-expression of EGFR, ensuring positive feedback of this pathway.

Finally, another activity that has been attributed to E5 protein is its participation in the evasion of the immune response against HPV infection. One mechanism is through direct interaction of the hydrophobic region of the E5 protein with the heavy chain of the MHC I, reducing these molecules on the cell surface and accumulating in the Golgi apparatus and the ER (**Fig. 3B**; refs. 24, 45). Remarkably, the chaperone BAP31 protein that regulates the quality control of the MHC I molecules is also altered by the E5 protein (46). These events cause a reduction in the recognition of infected cells by HPV-specific $CD8^+$ T cells (45, 47). At the same time, 16E5 also decreases the expression of MHC II on the cell surface, preventing degradation of the invariant chain that blocks peptide binding (**Fig. 3B**; ref. 48). This event could be driven by inhibition of acidification of the endosomes, avoiding the loading of peptides to the MHC II complex (49). Furthermore, some research suggests that E5 decreases the surface expression of the nonclassical MHC CD1 molecule through direct interaction with calnexin and CD1 in the ER, altering the cytosol proteolytic pathway and preventing the activation of natural killer T cells (50). All together suggest that E5 protein activity has a crucial role in helping HPV-infected cells to escape from the immunologic surveillance and, in the long term, generates cellular alterations that lead to transformation and cancer.

E5 Presence in Cervical Cancer and Other HPV-Associated Cancers

According to different studies, HPV DNA has been identified in the host cells as episomes or integrated forms. Integration into the genome of the cell suggests a critical step in cell transformation and the development of cancer (51, 52). During this event, it seems that the viral genome integration disrupts the *E2* gene and, with this, the loss of the *E5* oncogene transcription (52). For this reason, it is believed that E5 may contribute to the onset of carcinogenesis, as the expression of this protein is lost prematurely during cell transformation (53). Nevertheless, *E5* gene transcripts have been detected *in vitro* and in animal models showing transformation activity (8, 54). These studies also demonstrated that the loss of the *16E5* ORF is a rare event in cervical cancer–derived cell lines (9, 55).

On the other hand, little is known about the *in vivo* transcriptional activity of the *E5* oncogene. In this sense, some studies have shown that *E5*-specific transcripts were found with high frequency (60% to 75%) in low squamous intraepithelial lesions (LSIL) and with a more heterogeneous frequency (30% to 70%) in HSILs samples, an event that could be carried out through episomal or mixed episomal/integrated HPV16 forms (56–58). Other research groups, using a highly sensitive technology such as RNA sequencing, demonstrated the presence of *16E5* gene transcripts in LSILs and HSILs, and to less extent in carcinomas (59, 60). All this information suggests that the *E5* oncogene has a role in the early stages of the transformation process, and the maintenance and progression of the cervical cancer, as also the presence of *E5* transcripts has been reported in cervical cancer cell lines (8).

Due to the hydrophobic character of the HPV E5 proteins, it has been difficult to generate anti-E5 antibodies. This has hampered the detection and characterization of this HPV viral protein in uterine cervical lesions. However, some efforts have been made by Kell and colleagues. By using polyclonal antibodies against 16E5, they were able to identify the protein in a low proportion of cervical scrapes from women with LSILs (61). In addition, another group of researchers generated monoclonal anti-16E5 antibodies and detected the 16E5 protein in the lower third of the LSIL epithelium (80%). It was present in most of the HSIL epithelium (90%) and in only 60% of the squamous cell carcinomas, in which the viral genome appeared in episomal stage (33). Unfortunately, the instability of the anti-E5 monoclonal antibodies and the reduced number of samples tested have made these results difficult to corroborate.

Employing mass spectrometry, another group of researchers identified a unique four-amino-acid peptide (FLIT) at the C-terminus of the E5 protein. This peptide is generated during trypsin-cleavage treatment, and it serves as a marker of E5 expression. Using the FLIT peptide marker, this group showed that CasKi cells express the 16E5, whereas SiHa cells do not (8). This difference could be explained by the fact that CasKi cells retain a complete viral genome, whereas SiHa cells present a disruption at the *E1* and *E2* ORFs (62). Another approach to measuring the presence of 16E5 with the FLIT peptide was carried out with organotypic raft cultures in cell lines that replicate the LSIL and HSIL pathology. Sahab's group showed that the HSIL culture expressed a 2.2-fold higher copy number of the 16E5 protein than the LSIL tissue culture. This suggests that the 16E5 protein expression can be present in the late stages of cervical cancer (8).

More recently, it was demonstrated that transgenic mice carrying the *16E5* oncogene developed cervical cancer when they received prolonged treatment with estrogen, a cofactor in cervical carcinogenesis (54, 63). Furthermore, the transforming activity of 16E5 is enhanced in the presence of the E6 and E7 proteins. This was demonstrated with *in vivo* studies in transgenic mice expressing all three oncoproteins: they developed larger tumors than mice expressing only the E6 and E7 oncoproteins (63). This suggests that the 16E5 oncoprotein plays a predominant role in triggering the transformation process, as well as in the development and maintenance of cervical cancer.

All these results together suggest that the expression of the 16E5 protein in the different stages of the disease depends mainly on the presence of episomal genomes, but also a mixture of episomal/integrated HPV DNA is present at late stages of the disease. This could cause the E5 protein to be expressed at high or low levels, depending on the number of copies of the genomes present in the cell, as well as on the type of promoter under which the transcription of the E5 is regulated. Therefore, the idea that the expression of the E5 protein

is not required for late events of HPV-mediated carcinogenesis should be reviewed.

The association of HPV infection with other types of cancer such as anal, vulvar, oral, and head and neck cancer has been demonstrated (3, 64). However, little is known about the role of E5 in the initiation and progression of these types of cancer. In this way, two studies analyzed the expression of HPV16 E5 in oropharyngeal cancer samples and showed that between 72% and 77% of them were positive for this oncogene (65, 66). It was also identified that high levels of 16E5 correlated with better survival and recurrence-free, but not with overall survival (65). Similarly, in an *in vitro* model for anal cancer (AKC2, HPV16-immortalized, anal epithelial cell line), the researchers showed that blocking the expression of *E5* oncogene by siRNA reduced the levels of total and phosphorylated EGFR, but as well reduced the invasion characteristic of this AKC2 cell line (67). These experiments suggest the critical role of *E5* expression in the progression of this type of cancer. However, more *in vitro* and *in vivo* studies need to be carried out to characterize better the role of the *E5* oncogene in the development and progression of HPV-associated cancers.

In contrast, another way to detect E5 oncoprotein is through the indirect measurement of antibodies against this protein. These studies were carried out with sera from women with cervical cancer, but no conclusive results have been described (68). Currently, our group is working on the evaluation of human serum antibodies against 16E5 as a biomarker for early cervical lesions (D.A. Salazar-Piña; unpublished data). In oropharyngeal cancer, antibodies against 16E5 were detected using chemiluminescence ELISA. However, the results did not show the association of E5 antibodies with any stages of this type of cancer (69).

Direct detection of E5 oncoprotein in uterine cervical lesions and cancer has been hampered because of the absence of anti-E5 antibodies available for research. Several efforts have been made to determine the role of E5 in different HPV-associated cancers. However, more studies are required to assess the utility of this oncoprotein as a marker of disease stage and in the development of cancer.

Koilocytosis and Cell Fusion, Important HPV16 E5–induced Events in Carcinogenesis

Koilocytosis is a cellular change that is used as a pathologic diagnosis marker of the HPV infection in Papanicolaou (Pap) smears and cervical biopsies (70). This cellular morphology trait has been attributed to the HPV infection, but it was only recently that Krawczyk and colleagues showed that E5 from LR-HPV6 and HR-HPV16 induces koilocytosis in cooperation with E6, which is abrogated when the 20 C-terminus amino acids of E5 are removed in human cervical cell lines *in vitro* (71).

The recent description of the 16E5 viroporin structure and a large number of hydrophobic residues from the E5 protein could explain the tension and destabilization of the membrane, thus displaying a different conformation. This could be a possible mechanism through which membrane fusion and the appearance of large vacuoles in the HPV-infected cells are induced.

In contrast, it is well documented that Annexin II promotes membrane fusion in endosomal vesicles (72); and just recently, Krawczyk and colleagues showed that the E5 protein helps to relocalize and translocate calpactin I (heterotetramer that contains two molecules of Annexin II) to the perinuclear membrane, thus contributing to the formation of koilocytic vacuoles (**Fig. 4**). As yet, the biological role of koilocytosis in HPV-infected cells is unclear, but these authors suggest that the perinuclear vacuolization may increase cell fragility, which could in turn facilitate the liberation of viral particles and the spread of the infection (73).

Another characteristic observed in precancerous lesions is the appearance of tetraploid cervical cells, a prognostic factor used to predict progression to advanced cervical lesions (74). This is a feature specifically associated with the presence of an HPV infection (75). Hu and colleagues showed that cells expressing the 16E5 protein give rise to heterokaryons, which cause changes in cell morphology, such as an increase in the number of binucleated cells generated through the induction of a cell–cell fusion process (76). However, the E5 protein's fusogenic characteristic was observed only in the 16E5, but not in the 6bE5 protein. This observation was related to a distinct structural conformation between the HR- and LR-E5 proteins. In the case of the 16E5 protein, the N- and C-termini are hydrophilic and nonmembrane associated (15, 16). Moreover, Hu and colleagues showed that the E5 protein must be expressed in both cells for fusion to occur, which suggests that interaction has to occur in an E5–E5 protein region (76). Wetherill's *in silico* model of the 16E5 hexameric channel suggests that the first 6 to 10 hydrophilic amino acids of the C-terminal of this protein could be involved in this fusogenic process (16). However, this hypothesis has to be revised in more detail in the future.

In this sense, the presence of koilocytosis and the cell fusion trait of the carcinogenic process just recently attributed to the E5 protein seems to correspond to two parts of the same process (**Fig. 4**). The E5 protein is localized in the ER, the endosomes, and the plasma membrane, but how can this protein travel from the ER to the plasma membrane is still unknown. A possibility is that the E5 protein travels through the cellular vesicular system like other proteins, such as Ras, do (77). However, due to the E5 protein's membrane fusogenic trait, it is possible that, during its transport to the plasma membrane, the fusion of the vesicles that transport the viral protein takes place. Thus, this event could allow the presence of bigger vacuoles in E5-expressing cells, which could result in the appearance of the koilocytic morphologic characteristic of the HPV-infected cells. Nevertheless, experiments need to be carried out in this field to prove this theory.

On the other hand, characteristics such as enlarged nuclear size, high DNA content, and tetraploidy are observed in the clinical detection of cervical cancer precursor lesions during Pap test screening (70). Besides the occurrence of tetraploid cells, due to the cell fusion caused by 16E5 activity, enlarged nuclei have also been observed in 16E5-expressing cells. Experiments to address this observation demonstrated that the change in nuclear size in the 16E5-expressing cells was accompanied by an increase both in cellular DNA content and in chromosomal number through a process of endoreplication, an effect that is dependent on the level and duration of 16E5 expression in the cells (78). Under normal conditions, the emergence of polyploid cells induces the activation of apoptosis, or quiescence systems, as can be seen in cells with a sustained 16E5 expression, where a reduction in cell viability was observed. However, coexpression of HPV16 E6/E7 rescues the system and enhances the proliferation of the polyploid cells (76), by regulating the cell-cycle checkpoint proteins (p53 and pRb, respectively) and freeing the entrance to the cell cycle (6).

Cell–cell fusion has recently been reported in carcinogenesis, and special emphasis was put on the fusogenic activity associated with viruses, an activity that has been related to cancer development (hepatitis B and C viruses, Kaposi sarcoma virus, and Epstein–Barr virus; ref. 79). Duelli and Lazebnik proposed the tetraploid model of carcinogenesis, in which the cells first become tetraploid, then chromosomal instability occurs, and the final result is aneuploid cells.

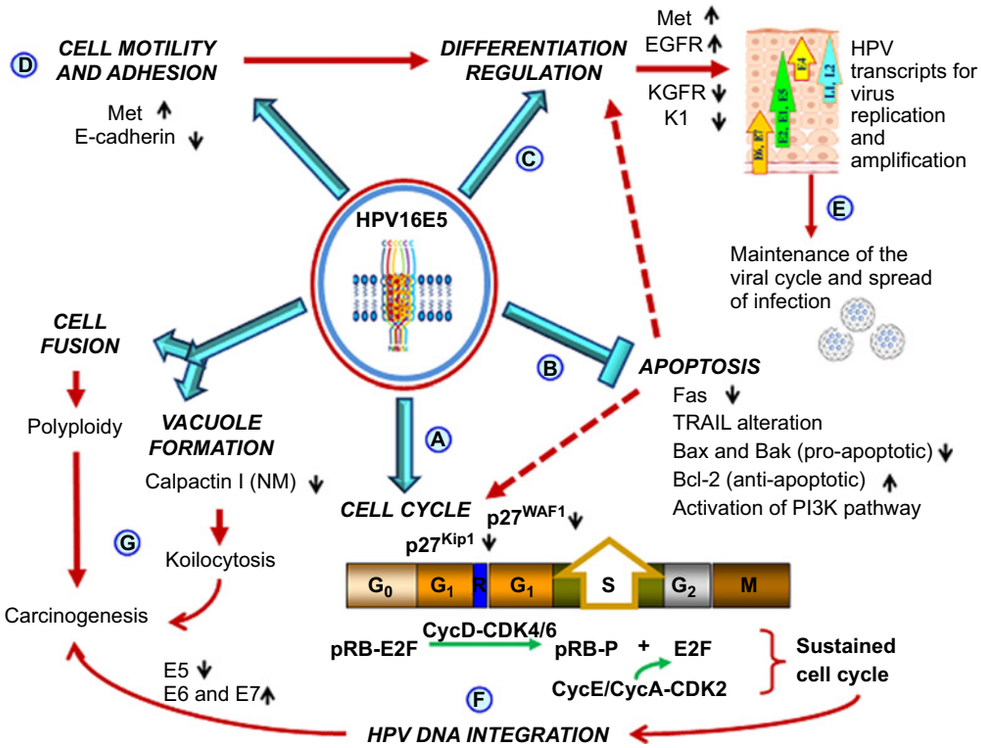

Figure 4.

Cellular pathways are altered by the 16E5 oncoprotein during carcinogenesis. Different pathways are modified by the 16E5 oncoprotein during the progression to cellular transformation. **A,** During the early stages of the HPV16 infection, the E5 oncoprotein can modulate the EGFR signal pathway, and it achieves to decrease the levels of the cell-cycle inhibitors $p21^{Waf1}$ and $p27^{Kip1}$. **B,** At the same time, there is an abrogation of apoptotic signals derived from Fas or Bax/Bak. **C,** These events together keep the cells into the cell cycle with a concomitant delay of the differentiation process driven by the KGFR, which is downregulated by 16E5. **D,** The 16E5 protein favors the cell detachment from the extracellular matrix through the downregulation of E-cadherin and modulation of Met expression, which contributes to an increase of motility of the HPV-containing cells through the epithelium. **E,** Maintenance of the cell cycle allows the viral replication and the spread of the HPV16 infection into neighboring cells. **F,** Persistent HPV16 infection and sustained cell cycle may allow integration of the viral DNA. This event permits the overexpression of the *E6/E7* oncogenes, which results in the cell genome instability and, with it, the initiation of the carcinogenesis process. **G,** The carcinogenic process is supported collaterally by the phenomena of cell fusion and koilocytosis; both activities are also regulated by 16E5 oncoprotein. These features of 16E5 make this protein a promising early target to attack viral infection and also to inhibit cancer development. NM, nuclear membrane.

However, for tetraploid cells to survive, they have to overcome cell-cycle checkpoints, and this could happen when p53 or apoptosis is disrupted (74, 79). Finally, the fused cells still have to go through a reprogramming process associated with epigenetic changes that generate hybrids, either with a stable genome or as quiescent heterokaryons (80, 81). Still, only those cells with stable genomes would be able to survive, but only as transformed cells with growing advantages. In the case of HPV, the recent observation that 16E5 is a fusogenic protein makes this virus fit perfectly in the tetraploid model. For carcinogenesis to happen according to this approach, several steps are required. Firstly, the E6 and E7 oncoproteins deregulate the cell cycle. Secondly, a cell fusion to generate tetraploid cells is carried out by the E5 oncoprotein, allowing for chromosomal instability and the appearance of cancerous cells (**Fig. 4**). This hypothesis is partially supported by the work of Lambert's group with HPV16 E5 transgenic mice. They demonstrated that 16E5 alone could generate cervical carcinogenesis, although mice need to be previously treated with estrogens, a cofactor for carcinogenesis, for 6 months (54). However, transgenic mice expressing the *E5/E6/E7* oncogenes jointly developed a more severe neoplastic disease than mice that expressed one oncogene at a time (63). This argues in favor of the hypothesis of the cooperation of the three HPV oncogenes as a necessary event for the development of HPV-associated cervical cancer.

Role of E5 in Adhesion, Migration, and Invasion

Once it was demonstrated that the 16E5 protein expression is present during the cervical lesion progression that generates the hyperkeratinization of the cells, as well as their defective differentiation, it becomes important to identify whether some other activities or functions can be attributed to this oncoprotein during the cell transformation process.

Adhesion and invasion are cell activities that have also been altered by the E5 oncoprotein, although the signaling pathway through which this happens is not clear yet. It has been reported that 16E5 upregulates the levels and activity of paxillin, a downstream target of focal adhesion kinase, and downregulates the lamin A/C protein levels, altering the signaling pathways that regulate the actin cytoskeleton network and allow cell migration (82, 83). E-cadherin, a Ca^{2+}-dependent protein that is associated with cancer progression, likewise affects cell adhesion, cytoskeleton rearrangement, and invasion. The loss of E-cadherin

in transformed cells reduces cell adhesion, which affects not only the cell–cell and the cell–extracellular matrix adhesion but also subsequently influences migration and invasion (84). Another possible mechanism could be through the action of matrix metalloproteases (MMP), cancer-associated proteases produced by transformed cells that digest the extracellular matrix, setting the cells free both to invade surrounding tissues and to move to distant sites of the organism (85). Therefore, it is possible that in 16E5-expressing cells, the E-cadherin protein levels could be regulated through the MMP-7 that is overexpressed in them, facilitating the detachment of the transformed cells from the tumor and the subsequent invasion of surrounding tissues (82). In this sense, the E5 oncoprotein seems to be deregulating adhesion through the downregulation of E-cadherin and increasing invasion through the overexpression of MMP-7 in a late step during the carcinogenesis and metastatic processes.

The reduced cell adhesion of the E5-expressing cells also affects their motility and invasiveness, which are higher than in non–E5-expressing cells (83). One of the mechanisms of increased motility and invasive ability of the transformed cells is the cross-talk between the signaling pathways of the monomeric G proteins, RAS, and RHO. For this to happen, elevated levels of the Rho protein are required to downregulate the $p21^{WAF1}$, thus allowing the cells to proliferate (**Fig. 4**). At the same time, the RAS signal pathway increases the MAPK signal that prevents RHO-GTP from inducing the formation of actin fibers, thus increasing the motility of the RAS-transformed cells (86).

In the case of 16E5, it has been shown to decrease the expression of $p21^{WAF1}$ and to modulate the activity of MAP kinases (ERK1/2) in keratinocytes (10, 43). This suggests that 16E5 could crosstalk with the RHO signaling pathway enhancing the motility and invasiveness of the 16E5-expressing cells, as the RAS oncoprotein does during the transformation process.

Another mechanism that regulates cell motility takes place through the Met tyrosine kinase receptor, which participates in the survival and migration of epithelial cells as well as during wound healing, allowing the mobilization of keratinocytes (87). Just recently, the 16E5 oncoprotein was shown to promote cell motility by upregulating the expression of *Met* at the mRNA and protein levels in an EGFR-dependent manner (88). Thus, the 16E5 oncoprotein may use the MET signaling pathway to allow cell survival during the early stages of the viral cycle (**Fig. 4**). Whereas in the late stages of the disease, it modulates the E-cadherin and the MMP-7, which could allow invasion and metastasis.

Regulation of Cell Cycle, Differentiation, and Apoptosis by E5 Expression Levels

It is well documented that the transformation process elicited by the 16E5 oncoprotein is dependent on an active EGFR signal transduction pathway and that this receptor pathway is necessary to enter the G_1 phase of the cell cycle (35, 37). Recently, it was suggested that the activity of 16E5 accelerates this phase, so transit to the S-phase is faster. Pedroza-Saavedra and colleagues showed that 16E5 uses the EGFR signal transduction pathway to downregulate the cell-cycle inhibitor $p27^{KIP1}$ and keeps the cell in the S-phase longer, thus allowing the production of viral progeny (**Fig. 4**). However, prolonged permanence in the cell cycle may cause instability in the cellular genome and finally transform the cells (37). This is in agreement with what has been observed in epithelial cancers, where 65% of the tumors have reduced levels of the $p27^{KIP1}$ protein compared with normal tissue; this fact correlates with pathologic tumor grade and level of invasion (89). On the other hand, $p21^{WAF1}$ protein plays an important role in preventing the G_1–S transition, by controlling the phosphorylation of the pRB–E2F complexes, as well as the transcription of genes, which are essential for cell proliferation (90). The 16E5 protein can suppress the $p21^{Wafl}$ gene expression (43), which facilitates the activation of cyclin D/CDK4 complexes, allowing the release of the E2F transcriptional factor from the pRB–E2F complex, leading to the cell-cycle progression (**Fig. 4**).

On the other hand, it is well understood that the HPV life cycle is tightly bound to keratinocytes differentiation (6). Late stages of this cycle are restricted to the more differentiated upper layers of the epithelium, where the viral particles are assembled (6). During a normal differentiation process, the EGFR signaling pathway is required at the basal layer to promote cell-cycle progression and suppression of keratinocyte differentiation, which avoids hyperproliferation at the basal layer of the epidermis (22, 91). When the keratinocyte reaches the suprabasal layer, a set of signals is switched-on to activate the terminal differentiation program (92), and a critical regulator of this process is the KGFR/FGFR2IIIb (93). In the case of HPV, the infected cells that migrate from the basal to the suprabasal layer fail to undergo differentiation and re-enter the cell cycle to allow amplification of viral DNA (6, 94). This event has been associated with a prolonged G_2 phase characterized by high levels of cyclin B1 in the cytoplasm (95). Recently, it was demonstrated that E5 from HPV18 keeps active the EGFR signal transduction pathway in the suprabasal layer to regulate the levels of cyclin B1 in the cytoplasm to maintain the cells in G_2 and at the same time to disrupt the KGFR signaling pathway by reducing the AKT activity to allow proliferation and to delay differentiation (29). In this sense, Barbaresi and colleagues demonstrated that the 16E5-expressing HaCaT cell line can form an epithelium with dysplastic/neoplastic characteristics when grown in organotypic raft cultures (96).

Furthermore, in a 16E5 transgenic mouse model, it was observed that this oncoprotein can alter the growth and differentiation of epithelial cells through a pathway that depends on a functional EGFR (35). Other altered characteristics observed in these 16E5 transgenic mice include aberrant differentiation in their skin, epidermal hyperplasia, hyperkeratosis, enhanced DNA synthesis, and long-term spontaneous skin tumors (54). For this reason, it has been suggested that the 16E5 oncoprotein modulates the cell differentiation process through the downregulation of the KGFR, which delays epithelial cell differentiation. The regulation of the KGFR expression plays a crucial role in controlling the proliferation/differentiation processes during the transition of cells from the basal to the suprabasal cell layers, an event that has been demonstrated to be regulated by 16E5 (97). Apart from the modulation of the KGFR expression by E5, which triggers the early differentiation of the epithelial cells, the downregulation of the keratin 1 transcripts (K1, an early differentiation marker) has also been observed in this process (28). Because of this, Belleudi and colleagues postulated that 16E5 might exert its biological activity in two ways: (i) enhancing the growth of undifferentiated cells at the suprabasal layer by increasing the function of the EGFR, and (ii) regulating proliferation and differentiation by regulating the expression and signaling of KGFR and K1 in keratinocytes of the suprabasal layer (**Fig. 4**; ref. 28). Although there is not a complete understanding of how this signaling between the EGFR and KGFR pathways changes, there is sufficient evidence that EGFR functions as an inhibitor of KGFR expression (22). Thus, this information suggests that the proliferation/differentiation processes are finely modulated by the E5 oncoprotein.

In contrast, the transcription factor family of p63, p73, and p53 is involved in the cell's response to stress and development, although they work in combination to stimulate different processes. For instance, p53 and p73 can induce cell death, whereas p63 and p73 together can be associated with differentiation (98). In this sense, it has been shown that, during an HPV infection, the 16E5 protein strongly upregulates the p63 and p73 proteins, ensuring that the differentiating cells keep their replication machinery active (5, 97). It is clear that control in the expression of molecules such as KGFR, p63, and p73 exercised by 16E5 reinforces the observations that this oncoprotein effectively modulates differentiation.

Finally, apoptosis is one of the mechanisms of programmed cell death following virus infection. Several viruses modulate the apoptotic pathway to block or delay this process to allow DNA viral replication and a successful progeny (99). There are two possible pathways to trigger apoptosis, intrinsic and extrinsic, but HPV alters the latter to benefit the viral cycle (100). The extrinsic apoptotic pathway is triggered through the activation of death receptors such as the tumor necrosis factor receptor, the factor-related apoptosis (Fas/CD95) receptor, and the TRAIL receptor. They are specifically activated by their ligands [TNF family, Fas ligand (FasL), and TRAIL, respectively; ref. 101]. The process is triggered by the binding of the ligands to the death receptors and their subsequent trimerization. This event prompts the recruitment of the adapter protein FADD (Fas-associated DD) and procaspase-8, allowing the formation of the death-inducing signaling complex (DISC). This complex mediates the cleavage of procaspase-8, which in turn activates caspase-3 and -7, leading the cell to apoptosis (99, 101).

In the case of HPV, the 16E5 protein is responsible for protecting the HaCaT cells from TRAIL- and FasL-mediated apoptosis, and this effect correlates with the level of 16E5 expression (102). Decreased Fas levels reduce the possibility of the FasL reaching its target, which makes the cells less sensitive to apoptosis and allows tumor cells to escape immunosurveillance in the early stage of viral infection (103). Reduced cell surface expression of Fas in 16E5-expressing HaCaT cells suggests that 16E5 exploits this principle to protect cells from apoptosis (102). Furthermore, 16E5 was found to inhibit TRAIL signaling by interfering with the formation of the TRAIL DISC and the subsequent cleavage of procaspase-8 and -3, as well as of PARP (102). The localization of the 16E5 oncoprotein in the ER and Golgi apparatus (13, 14) may allow it to bind to death receptors during their posttranslational processing and to transport them to the cell surface, thus disabling them from binding to TRAIL or from transmitting the apoptotic signal via the FADD protein (102).

On the other hand, in the process of DNA damage by UV-B irradiation, the 16E5 oncoprotein can protect cells from apoptosis by enhancing the PI3K-AKT and ERK1/2 MAPK signal pathways, activating the two main survival pathways. This seems to be the result of the increase in the phosphorylation of AKT and ERK1/2, leading to the inhibition of BAD and the activation of the anti-apoptotic BCL-XL and BCL-2 signals (104).

Similarly, it was identified that the intrinsic apoptotic pathway in C33A (non–HPV-harboring human cervical cancer cells) is also altered by the transfection of the 16*E5* oncogene when the cells are subjected to stress conditions with hydrogen peroxide. This inhibition causes a decrease in the expression of pro-apoptotic molecules, such as Bax and Bak proteins, and a greater expression of the antiapoptotic protein BCL-2, which allows for a change in the balance toward antiapoptotic and survival responses (105).

Therefore, it is postulated that E5 can protect HPV-infected cells from apoptosis through different molecular mechanisms (**Fig. 4**). In other words, this oncoprotein could contribute to carcinogenesis, tumor progression, metastasis, and therapeutic resistance by inhibiting the apoptotic process of transformed cells.

E5 Oncoprotein as a Therapeutic Target in the Control of Cervical Cancer

In recent years, the study of the 16E5 oncoprotein has developed, allowing us to understand some of its activities, as well as the cell signaling pathways that it modulates (i.e., positive activation of the EGFR pathway, angiogenesis, and anti-apoptosis). Therefore, it has turned into an important therapeutic target. The E5 oncoprotein is essential in the early stage of infection, and the lack of its expression has been associated with malignancy. This has led to the proposition that E5 could be a target in the early treatment of HSILs and even of cancer when it is not invasive (106).

For that reason, it has been suggested to address 16E5 with strategies already used against other HPV oncogenes, such as radioimmunotherapy, oncolytic adenoviruses, and gene silencing using siRNA (107, 108). Moreover, some therapies originally designed to inhibit E5-regulated signaling pathways have been also developed. Considering how 16E5 inhibits the interaction between the c-CBL protein and the EGFR, avoiding the latter's degradation and ubiquitination, and increasing its recycling and mitogenic signals, it is thought that EGFR inhibitors may be able to prevent the signal transmitted by E5 and thus be useful in reversing its carcinogenic effects (38).

As previously mentioned, the 16E5 protein is a viroporin, which let lipid membranes permeable to ions and small molecules (16, 17). Several other viroporins have been identified in many RNA and DNA viruses. They allow modulation of a wide range of cellular processes such as autophagy, trafficking, inflammation, transformation, and survival, among others (109). The first viroporin identified was the M2 protein of influenza A virus, which was able to form tetramers and increased the intracellular pH, which gave some evidence about its role as an ion channel, and this characteristic has been the target for the development of antiviral drugs such as amantadine and rimantadine (110). Recent experiments showed that 16E5 is also susceptible to inhibition by these compounds and other recognized viroporin inhibitory molecules such as alkylated iminosugars (16, 111).

Treatment of 16E5-expressing cells with rimantadine or iminosugars prevented the phosphorylation of ERK–MAPK and reduced the expression of cyclin B1, all of which stopped cell proliferation. These observations provide evidence of E5′s key role as a viroporin during the HPV life cycle and point to this activity as a potential target for the development of antiviral therapies that can prevent low-grade lesions from progressing to cervical cancer (111).

Until now, most of the research has focused on the development of prophylactic vaccines to prevent an HPV infection, but these do not clear previous infections. For this reason, it is necessary to develop therapeutic vaccines that stimulate the immune response, induce the regression of the HPV infection, and limit its sequelae. In this sense, E6 and E7 have been used as targets for therapeutic vaccines, due to their role in disrupting the cell cycle as well as to their presence in HSILs and cervical cancer (112). Different strategies have been used for the development of these types of vaccines, including live vectors, nucleic acids, peptides, proteins, the transfer of syngeneic cells, and the stimulation of the immune response against HPV proteins. Some of these approaches are currently being tested in clinical trials (112).

HPV-infected cells can function as antigen-presenting cells inducing an immune response against the virus. However, in the case of an HPV infection, a weak immune response is generated, accompanied by

mild inflammation, so the immune system is not able to eliminate the infection (113). Part of the HPV immune response evasion occurs because the E5 oncoprotein indirectly retains the MHC I molecules in the Golgi complex through the interaction of the di-leucine motifs (LL1 and LL3) and the MHC I, and the chaperone calnexin and the di-leucine motifs (LL2 and LL4; ref. 46). Moreover, E5 prevents the acidification of late endosomes and blocks the proper loading of antigenic peptides to the MHC II as well as their subsequent transport to the cell surface (48). In this way, it impairs antigen processing and presentation, and it supports HPV evasion of the immune system, which is crucial for the establishment and persistence of an HPV infection and, in the long term, for cell transformation and progression to cancer (114).

Therefore, blocking E5′s function is an interesting alternative to activate the immune response against HPV-induced tumors and to avoid persistent infection. Thus, several groups have used the E5 antigen to study the immune response and to evaluate this oncoprotein as a therapeutic target in the treatment of HPV-associated cancers. Liu and colleagues, for instance, demonstrated that delivering 16E5 through an adenovirus vector in an E5-expressing murine tumor model produced a 90% reduction of tumor growth, an effect associated with the stimulation of specific $CD8^+$ cells (115). These researchers also mapped the specific CD8 epitope between amino acids 25 and 33 (VCLLIRPLL) of the E5 oncoprotein, and this proved to be a better vaccine than the adenovirus-based E5 vector used previously (116). Another research group identified an immunodominant 16E5 peptide between amino acids 34 and 42 (LSVSTYTSL) using bioinformatics prediction of CD8 epitopes, and they evaluated it as a vaccine in the C57BL/6 E5-expressing mice tumor model. They observed that mice immunized with this E5 peptide showed a reduction in tumor size, which was associated with an increase in the $CD8^+IFN\gamma^+$ cells at the tumor-draining lymph nodes. Prolonged mice survival time was also observed (117).

Until now, there is no successful therapeutic vaccine against HPV-associated cancers. Therefore, the early expression of E5 during the carcinogenesis process, its important role in the maintenance of the viral cycle, and its role in evading the immune response make this oncoprotein an important target for therapeutic vaccines that could prevent the progression to high-grade lesions and cancer.

Perspectives

The knowledge of the HPV E5 biology is important to understand its role in maintaining the viral cycle through the modulation of vital cellular processes such as proliferation, differentiation, and apoptosis. Moreover, during early carcinogenesis, it also modulates other processes such as survival, adhesion, migration, and invasion. In this context, the E5 protein keeps the CDK inhibitors $p21^{WAF1}$ and $p27^{KIP1}$ in balance to allow the proliferation of the cells, but at the same time, it turns on the PI3K-AKT cascade to allow cell survival and to reduce apoptosis by increasing the BCL-2 anti-apoptotic system. Also important, it is the regulation of the proliferation/differentiation process through the negative regulation of the KGFR by increasing the EGFR activity that delays the differentiation process. In the long term, the persistence of the HPV infection and the stress around the cell generate the instability of the cellular genome. This causes mutations in important genes, which are necessary to maintain the cell under continuous proliferation. It also leaves the cells without the capacity of DNA repair, which leads to their transformation. The identification of activities and protein targets related to the 16E5 oncoprotein has demonstrated that it functions at the start of the transformation process. This has led to the development of drugs that can inhibit the transformation process associated with E5. This oncoprotein has also been the target of novel treatments to stop the early stages of the carcinogenesis process associated with HPV.

Authors' Disclosures

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