

The Role of HPV 16 E5 Protein and Its Interacting Partners in Cervical Cancer Cases

Tanojit Sur

Tanojit Sur, National Institute of Biomedical Genomics (NIBMG), Kalyani, India

Correspondence to: Tanojit Sur, National Institute of Biomedical Genomics (NIBMG), Kalyani, India

Email: surtanojit.gen.cu@gmail.com

Telephone: +918961268900

Received: April 29, 2014

Revised: May 14, 2014

Accepted: May 19, 2014

Published online: July 18, 2014

ABSTRACT

Human papillomavirus (HPV) is one of the most common causal agents of sexually transmitted disease in both men and women worldwide. In developing countries, cervical cancer is the most common cancer in women and may constitute up to 25% of all female cancers. Cervical cancer is preceded only by breast cancer as the most common cause of death from cancer in women worldwide. The link between genital HPV infections and cervical cancer (CaCx) was first demonstrated in the early 1980s. From this link of HPV with CaCx researchers identified some oncogenes of HPV which are playing a major role in CaCx pathogenesis. These oncogenes are E5, E6 and E7. The role of oncogene E6 and E7 are extensively studied than E5. This article will review the role of HPV 16 E5 oncoprotein and its interacting partners in the progression of cervical cancer (CaCx).

© 2014 ACT. All rights reserved.

Key words: HPV 16; E5 oncoprotein; CaCx; E5 and EGFR; E5 mRNA

Sur T. The Role of HPV 16 E5 Protein and Its Interacting Partners in Cervical Cancer Cases. *Journal of Tumor* 2014; 2(7): 179-186 Available from: URL: <http://www.ghrnet.org/index.php/JT/article/view/776>

INTRODUCTION

Human papillomaviruses (HPVs) are small, double stranded DNA

viruses that infect cutaneous and mucosal epithelial tissues, more than 95% of cervical cancers are positive for HPV DNA and a subset of HPVs, known as high risk types, mainly HPV 16 and HPV 18. These two types are mainly associated with cervical cancer (CaCx) development^[1]. The viral genome of HPV 16 contains 7,904 bp and the HPV genome is composed of six early (E1, E2, E4, E5, E6, and E7) ORFs, two late (L1 and L2) ORFs, and a non-coding long control region (LCR). HPV 16 infects mucosal and cutaneous epithelia and form lesions that can persist and progress to cervical cancer^[2]. In HPV-infected basal cells, every early transcript encoding E6, E7, E1, E2, E4, and E5 are expressed with their specific functions. For example, viral E1 and E2 proteins are expressed to maintain the viral DNA as an episome and they also helps to facilitate the correct segregation of genomes during cell division^[3]. In these early ORFs HPV 16 encodes three oncoproteins E6, E7 and E5. The role of two oncoproteins E6 and E7 in the formation of cervical cancer (CaCx) has been extensively studied. E7 protein inactivates pRB to cause continuous division of infected host cells and E6 induces the degradation of p53 to prevent apoptosis in HPV infected cells. But the role of another oncoprotein E5 in cervical carcinogenesis is not clearly understood. This article will review my current understanding of the role of HPV 16 E5 oncoprotein and its interacting partners in the progression of cervical cancer (CaCx)^[4]. Papillomaviruses are a large group of viruses that have been found almost 20 different species in mammals. There are 100 different types of HPV already have been identified because of their medical importance^[1].

LIFE CYCLE OF HPV

HPV is a circular dsDNA virus with a genome of 7,904 bp. The genome is divided into three regions where six early (E) ORFs and two late (L) ORFs are expressed. The viral genome carries all ORFs on a single sense strand and carries one upstream regulatory region (URR) or non-coding region (Figure 1)^[5]. The virus shell of HPV is composed of two coat proteins L1 and L2, L1 is the primary structural element where L2 is the minor virion component and present at the center of pentavalent capsomers^[1]. During genomic amplification, the expression of L1 and L2 is upregulated both at

RNA and protein levels. The expression of L1 is sufficient to achieve viral packaging but the presence of L2 increases this efficiency^[5]. In the basal layer of epithelium the viral infection begins and then established. The viral genome is maintained at about 100 copies per cell and the viral genome present itself as a stable episome in the cells of basal layer. E1 and E2 are viral replication protein and they play several roles during productive infection. They start the initiation of viral replication and genome segregation^[1,5]. The E2 protein binds to a palindromic motif which is AACCG (N4) cGGTT in the non-coding region, and then E1 helicase allows for its recruitment, E1 helicase carries a consensus motif (AACNAT) and shows a weak affinity to the consensus motif. This E1 helicase recruits host replication factors to the viral origin of replication^[6]. E2 can regulate the viral early promoter like p97 in HPV 16 and p99 in HPV 31 and can act as a transcription factor. E2 plays an antagonistic role at high and low levels, E2 act as a transcriptional activator at low levels, where at high levels E2 represses oncogenic expression^[1]. Primarily E2 associates with E1 through its N-terminus and through its C-terminus binds to DNA as a dimer^[5].

There are five stages present in HPV life cycle, they are (a) Genome maintenance (occurs at basal layer); (b) Genome maintenance and cell proliferation (occurs at suprabasal layers); (c) Genome amplification (occurs at suprabasal layers); (d) Virus assembly and release (occurs at granular layers); (e) Virus release (occurs at cornified layers)^[1].

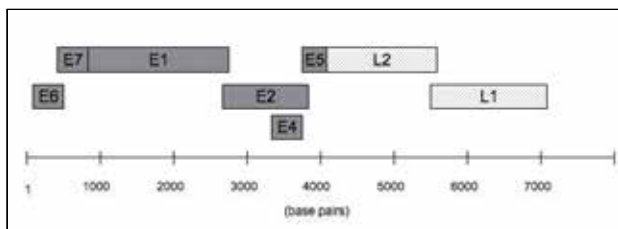


Figure 1 The linear representation of structure and organization of the HPV genome.

ONCOPROTEINS OF HPV

HPV 16 four oncogenic proteins: E6, E7, E5 and E4. The E6 and E7 oncoproteins are required for the inactivation of p53 and pRb tumor suppressors and to induce telomerase^[7]. The main function of E6 protein is to degrade p53, E6 forms a complex with p53 and the 100 kDa E6AP (ubiquitin ligase). This interaction results in the ubiquitination of p53 through the proteosomal pathway^[5]. The E7 protein inactivates and induces the degradation of the retinoblastoma protein (pRb), a cell cycle regulatory protein required for the G1/S transition and DNA synthesis^[2]. Besides modulating cell cycle control and inactivation of pRb, E7 protein has been implicated to downregulate the level of human toll-like receptors (hTLR) and the ER protein TAP-1^[5]. E6 and E7 both interact with many other cellular factors inducing tumor progression, immune evasion and genomic instability^[8]. The oncogenic activities of E6 and E7 are studied extensively and are well characterized. But the role of another oncoprotein E5 is not very clear to researchers. So, now we discuss about E5 oncogene with its special features, main functions and the role of CaCx development.

E5: THE SMALLEST ONCOGENE OF HPV

HPV E5 proteins are less well characterized and they are localized mostly to the Golgi apparatus, endoplasmic reticulum and they also present in the nuclear membrane^[9]. HPV E5 proteins are hydrophobic in nature and contain 83 amino acids; it is the smallest oncogene of HPV. E5 exhibits transforming activity and induces a malignant phenotype to cooperate with E6 and E7 oncoprotein^[10]. E5 is also able to stimulate several cellular pathways through the activation of epidermal growth factor receptor (EGFR) and can upregulated vascular endothelial growth factor (VEGF)^[11]. E5 has a primary role in proliferative and productive phase of viral life cycle. When the HPV genome is episomal, the E5-mRNA is the most abundant viral transcript and it plays an important role in the early phases of

Table 1 HPV proteins and their functions^[36].

HPV Proteins	Molecular Weights	Functions
L1	55 kda	Major viral capsid protein self-assembly in capsomers and capsids and interacting with L2 Interacting with cell receptor
L2	70 kda	Minor viral capsid protein Interacting with DNA Facilitating virion assembly Interacting with cell receptor
E1		Viral genome replication DNA helicase and ATPase activity Binding to specific DNA elements in the viral origin of replication and assembly into hexameric helicases with the assist of a second viral protein, E2. Site specific DNA binding protein.
E2	42 kda	Viral genome replication. Viral genome expression regulation Viral genome segregation during cell division. Interacting with and recruits E1 to the origin. Playing a role in regulating viral transcription from the early promoter.
E4		Facilitating virus assembly and release Binding and collapsing the cytokeratin network. Preventing the progression of cells into mitosis by arresting them in the G2 phase of the cell cycle.
E1^E4	10 kda	Binding to mitochondria. Inducing the detachment mitochondria from microtubules. Induction of apoptosis.
E5	83 residues	Cellular transformation and initiation of neoplasia. Being able to active epidermal growth factor receptor (EGFR) and other protein kinases. Inhibiting apoptosis and interacting with gap junction proteins.
E6	150 amino acids	Immortalization and transformation of infected epithelial cells. Disrupting normal cell growth and proliferation by binding to protein p53. Inducing telomerase and preventing cell differentiation.
E7	100 amino acids	Immortalization and transformation of infected epithelial cells. Disrupting normal cell growth and proliferation by binding to protein pRb.

tumorigenesis^[12]. There are multiple cellular effects found, which are associated to the expression of HPV16 E5. E5 binds the 16 K proteolipid subunit of the membrane proton pump and this binding may be play an essential role in EGFR over activation^[13]. Some reports said that the binding region of E5 has been present to amino acids 54-78 or amino acids 41-54^[14]. The HPV E5 ORF itself has been classified into four groups' alpha, beta, gamma and delta which have oncogenic potential with different clinical manifestations. The E5 alpha protein is encoded by high risk HPV, whereas, the E5 gamma and delta proteins are encoded by low risk HPVs. The expression and amino acid sequences of HPV 16 E5 were detected by Chang *et al.* In cervical intraepithelial neoplasia (CIN), low-grade squamous intraepithelial lesions (LSILs) and high-grade SILs. They reported that there are three anchors like α -helices present in amino acid sequence of E5 at residues 8-30, 37-52 and 58-76.

INTERACTION OF E5 WITH OTHER PROTEINS

Interaction with MAP Kinases

E5 protein of HPV 16 upregulates the signal cascade initiated by EGFR through mitogen activated protein kinases, which are serine/threonine protein kinases in nature and are involved in directing cellular responses to a diverse array of stimuli such as osmotic stress, heat-shock, mitogens and this MAP kinase pathway is associated with both cell proliferation and differentiation^[15]. Activated MAP kinase is translocated into the nucleus upon activation, where it can potentially phosphorylate transcription factors, such as c-Jun, c-Myc, and c-Tail^[16]. Many oncogenes such as ras, raf, src and mos transform cells by prolonging the activated stage of MAPKK. Some reports have described E5 gene is often deleted from the viral genome during viral DNA integration in the more advanced cervical neoplastic lesions, similarly several reports have described E5 does participate in transformation process when the HPV DNA is episomal and it has a potential to be act as an oncogene which can show its transforming activity by downregulating the EGFR and increase the number of EGFR-Rs at cell surface^[17]. Several reports observed that HPV 16 oncogene products could alter cellular MAP kinase activity and E5 gene was able to induce an increase in the MAP kinase activity. Some assays are help to determine the effect of E5 on MAP kinase signaling pathway, both E5 and mutant E5 are showing their activity in this assay conditions. The mutant E5 used this assay was increased the translation efficiency of the corresponding E5 mRNA by introducing Kozak sequence around the initiation ATG of the E5 gene. The result of this in vitro transcription-translation assay showed that mutant E5 was at least five times more efficiently translated in-vitro, but both wild type and mutant E5 demonstrated a similar increase in the MAP kinase activity^[37].

Interaction with Calpactin I

Calpactin I is a heterotetrameric protein complex that consists of two annexin A2 or lipocortin II subunits and two p11 subunits^[18]. Annexin A2 is a 36 kD calcium binding proteins, associates with phospholipids in a calcium dependent manner and regulates different cellular processes such as membrane fusion and endocytosis^[19]. Several reports observed that HPV 16 E5 binds calpactin I and induces perinuclear membrane fusion events. E5 is present in vacuole membranes, and its C terminus is required for vacuole formation. Krawczyk *et al* 2011 reported that E5 associates with calpactin I after transfection in COS cells, they done immunoprecipitation where they observed a 36 kDa polypeptide that coimmunoprecipitates with E5 was excised from a Coomassie blue-stained sodium dodecyl sulfate-

polyacrylamide gel, digested with trypsin, and microsequenced. After this experiment they confirmed that there was a strong association present between E5 and annexin A2, E5 and p11 and annexin A2 and p11 in COS cells. They also observed that E5 promotes perinuclear membrane fusion with calpactin I, to confirm this hypothesis they used HEC stable cell line which express HPV 16 E6 and it infected with retrovirus encoding E5. After 3 days they pulse-labeled the cell line for 30 min with Alexa Flour 594 (red)-conjugated CTB and six hours later the same cells were pulse labeled with an Alexa Flour 488 (green)-conjugate of CTB. Then they performed fluorescence colocalization and they found a small number of cells exhibited perinuclear vacuoles that showed two distinct populations of membrane vesicles had undergone fusion. It is already demonstrated that E5 induces a perinuclear redistribution of calpactin I and the binding interaction with E5 and perinuclear localization are mediated by the C terminus of E5, which is known to be exposed to the cytoplasm^[38]. In this study Krawczyk *et al*^[20] identified calpactin I as a new cellular target for the HPV 16 E5 oncoprotein and E5 have high affinity for calpactin I.

Interaction with ER stress pathway proteins

HPV 16 E5 protein has the ability to bind several cellular proteins. The presence of viral proteins may activate cellular defense mechanisms and in particular the ER stress response. HPV 16E5 can suppress three main proteins of the ER stress pathway and they are cyclooxygenase-2 (COX-2), XBP-1 and IRE1a. It can alter splicing of XBP-1 which is a part of ER stress response pathway. In 2010 Sudarshan *et al* reported that high risk HPV 16 E5 protein has an effect on XBP-1 splicing. To study gene expression they used microarray approach and tried to capture alterations in the ER stress pathway. At first they constructed 16 E5 and E5 mutant in pLXN vector and then used EcoRI and BamHI to clone the construct into the vector for stable expression. On the other hand they prepared human foreskin keratinocytes (HFKs) and human ectocervical cells (HECs) and treated them with chemical inducers of ER stress. During microarray each E5 cell lines was run against the corresponding pLXN cell lines. Next they performed reverse-transcriptase PCR (RT-PCR) and prepared cDNA by denaturing the RNA for 3 minutes at 80°C with oligod (T) and random hexamers. cDNA samples were diluted and used in real time RT-PCR. After real time RT-PCR, they quantified the ratio of spliced to unspliced XBP-1 transcripts in HFKs and HECs cell lines and also found E5 protein reduce the expression of XBP-1 in ER stress pathway. Then they looked to the mutant E5 and studied its role in these cell lines, in high risk HPVs the region of E5 protein where the mutations were made, were highly conserved. So, all mutant E5 proteins also showed reduction of XBP-1 expression. After all these experiments they reported that in ER stress pathway 179 genes were significantly altered by HPV 16 E5 protein^[21].

In 2009 Kim *et al* reported that COX-2 expression is downregulated in cervical cancer lines C33A and SiHa. They concluded that HPV 16 E5 is able to lower COX-2 expression in cells co-expressing E6/E7, during viral replication^[43]. However, other studies suggested that COX-2 protein levels did not correlate with the disease severity of HPV-induced cervical lesions or with HPV-positivity in primary and metastatic cervical cancer tissues^[44].

Interaction with p21^{Waf1/Cip1}

p21 or cyclin dependent kinase inhibitor 1 is a protein which is encoded by the CDKN1A gene located on chromosome 6 (6p21.2)^[22]. The p21 protein binds and inhibits the activity of cyclin-CDK2,

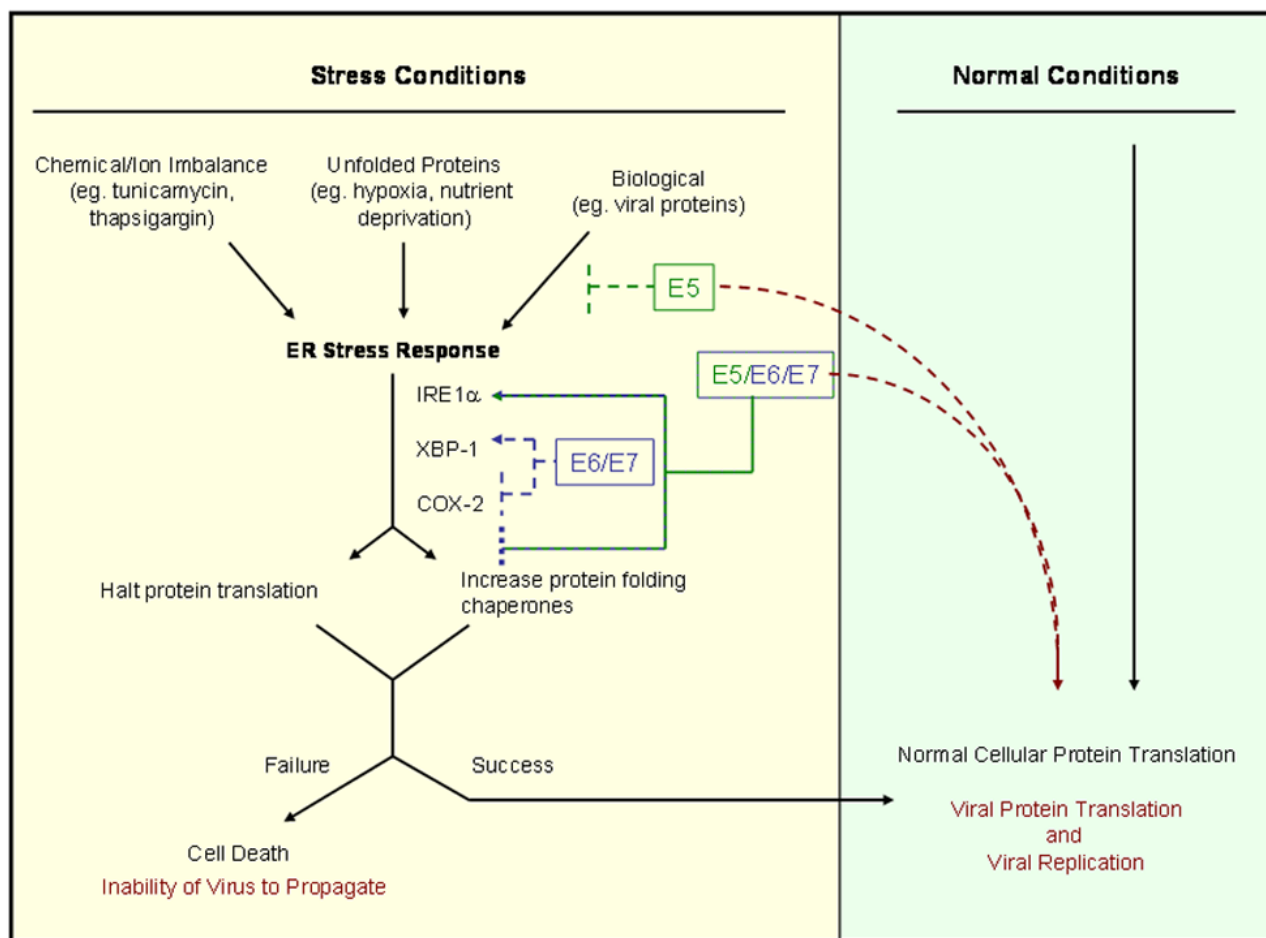


Figure 2 Potential model of HPV 16 E5 ER stress repression^[21].

CDK1, CDK4/6; p21 can also mediate cellular senescence. The expression of p21 is controlled by tumor suppressor gene p53^[23]. Several reports have described that p21 concentration can be affected by HPV E5. Tsao *et al* 1996 reported that p21 is repressed by both HPV 11 and 16. They also studied the relation between the transformation activity and p21 suppression effect of HPV by using a series of E5 mutant. In the beginning of their experiment, they used pC11E5 and pC16E5 plasmids which contain an ORF frame of HPV 11 and HPV 16 E5. In the 5' end of this ORFs were cloned with T7 epitope which is a major capsid protein contains a sequence of 11 amino acids. Both plasmids were used the vector pCEP4 and plasmids pC11E5 and pC16E5 and the pCEP4 vector were separately transfected into keratinocytes by Lipofectin transfection with selection for resistance to hygromycin. After 3 weeks, at least 80 hygromycin-resistant colonies were pooled and stable cell lines were generated. They found that cells expressing epitope-tagged HPV-11 E5 and HPV-16 E5 fusion proteins were named Z11E5 and Z16E5. Transforming activity was characterized by anchorage-independent growth. They revealed that the concentration of p21 RNA and protein were decreased in Z11E5 and Z16E5 cells, which could express HPV-11 and -16 T7-tagged E5 fusion proteins. The decreased concentrations of p21 RNA and protein in cells containing the E5 gene could be due to repression of p21 promoter activity. Next they investigated the promoter activity of p21 gene by transient-transfection assays. Luciferase reporter analysis showed that both HPV-11 E5 and HPV-16 E5 repressed p21 promoter activity in NIH 3T3 cells and keratinocytes^[24].

Interaction with HLA Class I

HPV infect mucosal and cutaneous epithelia and induce a mildest form of HPV disease, known as warts or papillomas. Most of the cases this warts are removed after several months by the activation of host immune system against HPV's antigen (viral antigen)^[25]. Sometimes certain types of HPVs (mainly HPV 16) avoids the immune clearance and the lesions can progress to cancer (CaCx by HPV 16)^[26]. There are several factors present in HPV life cycle which helps to avoid this immune clearance, for the removal virally infected cells requires cytotoxic T lymphocytes (CTL) which can recognize and kill the infected cells by MHC I (HLA I in humans). HPV 16 E5 protein prevents the transport of major histocompatibility complex or MHC class I to the cell surface and keeps the complex in the Golgi apparatus^[27]. The main effector of HPV 16 is viral oncoprotein E5 which helps to escape from immune clearance. Several report observed that HPV 16 E5 downregulates the expression of surface HLA I by retaining the complex in Golgi apparatus^[28]. In 2006, Ashrafi *et al* worked on the interaction of E5 with the heavy chain of HLA I. At the beginning of their study they constructed HPV 16 E5 mutants by using the plasmid pcDNA-Neo (Invitrogen), encoding G418 resistance and containing the universal IE promoter of CMV. All the wild type and mutant E5 were tagged with hemagglutinin (HA) epitope at their N-terminus. They created four HPV 16 E5 deletion mutants: R79, A54, V36 and R30 by introducing double stop codons at the specific position of nucleotides of HPV 16 E5 sequence. They made HPV 16 E5 Del 1 mutant protein by deletion of first hydrophobic domain by PCR amplification using forward

primer from nt 91 to 105 of the HPV-16 E5 ORF and reverse primer from nt 252 to 231 including sites for Eco RI and Nhe I. They used pEGFP-C1 eukaryotic expression plasmid which encodes the GFP. They used it for full length cloning of HPV 16 E5 and its mutants to produce GFP-E5 wt and GFP-E5 mutant fusion proteins by PCR. Then they transfected the HaCaT cell line and the RNA was isolated from it and removed the residual DNA by Dnase I treatment, they performed the real-time RT-PCR using Taqman EZ RT-PCR kit for E5, E5-mutant and β -actin and then done a semi-quantitative RT-PCR because semi-quantitative Real time RT-PCR did not work for E5 Del 1 due to lack of appropriate forward primer in the first hydrophobic domain. Next western blotting was performed by Ashrafi *et al*, they took fifty microgram of protein lysates from HaCaT cell harboring empty vector (Control), expressing Del1 or E5, were electrophoresed and transferred to nitrocellulose membrane. The membranes were incubated with mAb HA 11 for 1 h. and incubated with anti-mouse IgM-HRP and after washing finally the bound antibody was detected by chemoluminescence staining. To detect HLA I, immunofluorescence was performed. At first the cells were washed and fixed in fixing solution which contains PBS, formaldehyde and sucrose, then the cells were incubated in permeabilising solution made by PBS, NP40 and sucrose. Next the cells were incubated with previously mentioned antibodies at 4°C for 1 h in dark. After 3 final washes with PBS the slides were mounted with glycerol/PBS solution and analyzed under fluorescence confocal microscope. The results of their total experiment were: (1) HPV-16 E5 and HLA I HC interact physically. E5 prevents the transport of HLA I to the cell surface by retaining the complex in Golgi apparatus^[27]. The result of western blotting concluded that E5 and HLA I exist in a physical complex in E5-expressing HaCaT cells; (2) E5 interacts with the HC of different HLA I haplotypes to prove this they performed *in-vitro* coprecipitation experiments. After this experiments they concluded that the interaction between HC and E5 is likely to take part with most of the alleles of HLA I such as A1, A2, A3, B8 etc. The four deletion mutations shows different characters of E5 mutants, here mutant R79 lacks the last 5C-terminal amino acids, A54 lacks the complete third hydrophobic domain, V36 and R30 lack the 2nd and 3rd hydrophobic domains and Del 1 lacks the 1st hydrophobic domain (Figure 3); (3) The first hydrophobic domain of E5 is responsible for surface HLA I downregulation. They proved it by using flow cytometry, where the level of total (surface+intracellular) HLA I in HaCaT cells, stable expressed the wild type E5 and its mutants. The clones expressing the E5 mutants containing the first hydrophobic domain (R79, A54, V36, and R30) had reduced level of surface HLA I to the same extent as E5 wt. In contrast, expression of E5 Del1, lacking the first hydrophobic domain, did not have any effect on the levels of surface HLA I. Finally these results

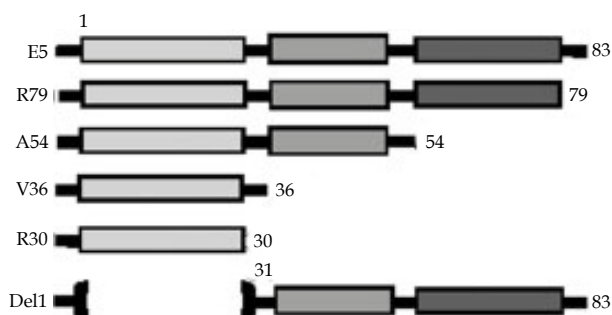


Figure 3 Diagrammatic representation of E5 wt, showing the 3 hydrophobic domains, and E5 mutants, showing the missing domains of E5 (Ashrafi *et al* 2006).

conclusively showed that E5 both physically interacts with HC and downregulates surface HLA I via its first hydrophobic domain.

In 2007 Gruener *et al* reported that Calnexin, a molecular chaperone involved in HLA-I transport to the cell surface, also physically interacts with this domain of HPV 16 E5 and is required for intracellular retention of HLA-I^[41]. In 2010 Campo *et al* reported that MHC class I is arrested in the GA by the alkalisation of the endomembrane compartments which is induced by HPV 16 E5, and the direct interaction of E5 with the heavy chain of the MHC class I complex. These conclusions have been reached in cultured HaCaT cells and W12 cell line. The functional effect of the decreased expression of HLA is a reduction of the recognition by CD8+ T cells *in vitro*^[42].

Interaction with Bax protein

The role of E6 and E7 has been extensively studied but the role of the E5 protein in cervical carcinogenesis and apoptosis is not clearly recognized^[29]. E5 acts as an oncogene primarily and increased the tumorigenicity by enhancing the activation of the epidermal growth factor receptor in a ligand-dependent manner^[30]. Apoptosis is a physiological cellular response and one type of programmed cell death. It plays an important role in homeostasis and development^[31]. Many viruses, including HPV, have developed various strategies to regulate and block apoptosis and used it as a hallmark of cancer cells^[32]. It was already reported that HPV E5 regulate Fas and TRAIL mediated apoptosis and also reported to protect human keratinocytes from UV radiation induced apoptosis by enhancing the phosphatidylinositol 3-kinase-Akt pathway and other signaling pathways^[33].

In 2010 Oh *et al* started to investigate the effect of HPV 16 E5 on apoptosis of cervical cancer cells. They found that the E5 protein inhibits hydrogen peroxide-induced apoptosis by stimulating ubiquitin-mediated degradation of Bax through a cyclooxygenase-2 (COX2) (Figure 4)^[40], prostaglandin E2 (PGE₂), EP2, EP4 and cyclic adenosine monophosphate-dependent protein kinase-dependent pathway in C-33A human cervical cancer cells. They analyzed the effect of HPV 16 E5 on H₂O₂ induced apoptosis using the C-33A human cervical cancer cells^[34]. They confirmed the expression of E5 mRNA and protein by RT-PCR and western blot analysis. They performed a double immunofluorescence analysis using a confocal microscope to examine the cellular location of FLAG-E5 protein and found the E5 protein was colocalized with a 58K cellular protein. The effect of E5 on hydrogen peroxide-induced apoptosis was analyzed by caspase-3 cleavage and cytochrome c release using western blot analysis. E5 expression decreased the cleavage of caspase-3 from 10.1±1.2 fold and the cytochrome c released using western blot analysis. They also found that HPV16 E5 decreases the expression of pro-apoptotic Bax and Bak but increases the expression of anti-apoptotic Bcl-2 by western blotting. They exposed the Bax protein for a long time in the blot to film and the expression of Bax protein was decreased significantly where the level of Bcl protein was not changed by E5 expression. They further examined the role of Bax in the anti-apoptotic action of E5, the effect of Bax expression on the hydrogen peroxide-induced apoptosis of C-33A cells and they observed the level of Bax expression was decreased where anti-apoptotic Bcl-2 level was increased slightly in C-33a cells. These results suggest that E5 may exert an anti-apoptotic effect by changing the expression of Bax, Bak and Bcl-2 proteins in cervical cancer cells and E5 inhibits hydrogen peroxide-induced apoptosis partly by decreasing Bax expression in cervical cancer cells. They simultaneously studied the Bax mRNA level by RT-PCR

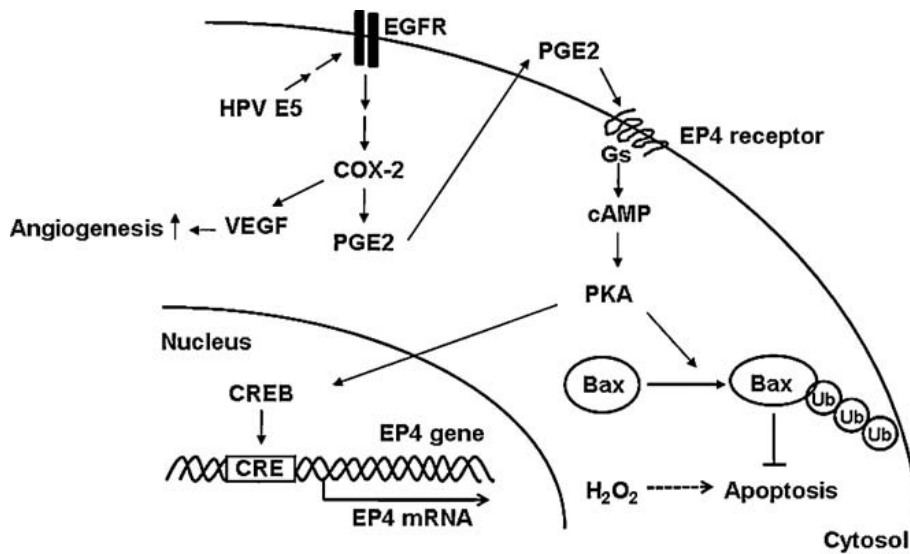


Figure 4 A schematic diagram of COX-2-PGE₂ pathway induced by HPV 16 E5. E5 induces COX-2 expression through EGFR activation^[40].

and efficiency of Bax promoter by Bax-luciferase activity but the Bax mRNA level was not decreased significantly in E5-expressing C-33A cells and also the Bax promoter activity was not decreased. This result indicates that E5 does not inhibit the transcription of Bax but the inhibition and regulation starts during post-translational modification and change the Bax expression at protein level. Next they examined the effect of PGE₂- signaling pathway on Bax-degradation by E5 protein. Treatment with PGE₂ decreased hydrogen peroxide-induced apoptosis of E5-transfected C-33A cells and also decreased the Bax expression. This result suggests that E5 inhibits hydrogen peroxide induced apoptosis by stimulating PGE₂-signaling pathways to downregulate Bax expression in C-33A cells. From this study they concluded that HPV E5 protein inhibits H₂O₂-induced apoptosis mediated by COX-2, PGE₂ which degraded the Bax protein in cervical cancer cells.

Some recent studies on this topic said that HPV 16 E5 on COX-2 expression 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^[39].

FUTURE DIRECTIONS

In this review article I have tried to discuss the role of HPV 16 E5 protein in CaCx and some future perspective of E5 which will make this protein a major oncogene of HPV and potential biomarker of HPV infected CaCx. Oncogenic high-risk HPV types induce malignant transformation in cervical mucosal epithelia by expressing E5, E6 and E7 oncogenes and maintains its malignant phenotype by continuous expression of E6 and E7 proteins^[35]. But the overall role of E5 oncoprotein in cancer is less understood still now. So, there are some areas to work on HPV E5 protein in future and they are: (1) To analyze the random effects of E5 protein in triggering malignant changes in epithelial cells; (2) To examine the cellular levels alterations in gene and protein expression due to expression of HPV E5 in epithelial cells, identify the altered gene by microarray platforms; (3) To evaluate the effects of E5 on cellular microRNAs & study their changes in gene expression; (4) To reveal the role of E5 on cell adhesion and cell motility, epithelial to mesenchymal transitions (EMT) cellular signal transduction and transformations and finally carcinogenesis.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

- 1 John Doorbar. The papillomavirus life cycle. *Journal of Clinical Virology* 32S (2005) S7-S15
- 2 Jennifer A. Regan and Laimonis A. Laimins. Bap31 Is a Novel Target of the Human Papillomavirus E5 Protein, *JOURNAL OF VIROLOGY* 2008; **82** (20):10042-10051
- 3 John Doorbar. Molecular biology of human papillomavirus infection and cervical cancer. *Clinical Science* 2006; **10**: 525-541
- 4 Jung-Min Oh, Su-Hyeong Kim, Eun-Ah Cho, Yong-Sang Song, Woo-Ho Kim, Yong-Sung Juhn. Human papillomavirus type 16 E5 protein inhibits hydrogen peroxide-induced apoptosis by stimulating ubiquitin-proteasome-mediated degradation of Bax in human cervical cancer cells. *Carcinogenesis* 2009; **31** (3): 402-410
- 5 Koenraad Van Doorslaer, Robert D. Burk. Evolution of Human Papillomavirus Carcinogenicity. *Adv Virus Res* 2010; **77**: 41-62
- 6 Blakaj DM, Fernandez-Fuentes N, Chen Z, Hegde R, Fiser A, Burk RD, Brenowitz M. Evolutionary and biophysical relationships among the papillomavirus E2 proteins. *Front Biosci* 2009; **14**: 900-917
- 7 Liu X, Roberts J, Dakic A, Zhang Y, Schlegel. HPV E7 contributes to the telomerase activity of immortalized and tumorigenic cells and augments E6-induced hTERT promoter function. *Virology* 2008; **375**: 611-623.
- 8 Aldo Venuti, Francesca Paolini, Lubna Nasir, Annunziata Corteggio, Sante Roperto, Maria S Campo, Giuseppe Borzacchiello. Papillomavirus E5: the smallest oncoprotein with many functions, *Molecular Cancer* 2011, **10**: 140
- 9 Oetke C, Auvinen E, Pawlita M, Alonso A. *Arch* 2000; **145**: 2183-2191
- 10 Halbert CL, Galloway DA. Identification of the E5 open reading frame of human papillomavirus type 16. *J Virol* 1998; **62**: 1071-1075
- 11 Tomakidi P, Cheng H, Kohl A, Komposch G, Alonso A. Modulation of the epidermal growth factor receptor by the human papillomavirus type 16 E5 protein in raft cultures of

- human keratinocytes. *Eur J Cell Biol* 2000; **79**: 407-412
- 12 Crusius K, Auvinen E, Steuer B, Gaissert H, Alonso A. The human papillomavirus type 16 E5-protein modulates ligand-dependent activation of the EGF receptor family in the human epithelial cell line HaCaT. *Exp Cell Res* 1998; **241**: 76-83
 - 13 Conrad M, Bubb VJ, Schlegel R. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. *J Virol* 1993; **67**: 6170-6178
 - 14 Rodriguez MI, Finbow ME, Alonso A. Binding of human papillomavirus 16 E5 to the 16 kDa subunit c (proteolipid) of the vacuolar H⁺-ATPase can be dissociated from the E5-mediated epidermal growth factor receptor overactivation. *Oncogene* 2000, **19**: 3727-3732
 - 15 Baker C, Phelps CWC, Lindgren V, Braun MJ, Gonda MA, Howley PM. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J Virol* 1987; **61**: 962-971
 - 16 Hill CS, Marais R, John S, Wynne J, Dalton S, and Treisman R. Functional analysis of a growth factor-responsive transcription factor complex. *Cell* 1993; **73**: 395-406
 - 17 Straight S, Hinkle P, Jewers R, McCance D. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the down regulation of the epidermal growth factor receptor in keratinocytes. *J Virol* 1993; **67**: 4521-4532
 - 18 Erikson E, Tomasiewicz HG, Erikson RL. Biochemical characterization of a 34-kilodalton normal cellular substrate of pp60v-src and an associated 6-kilodalton protein. *Mol Cell Biol* 1984; **4**: 77-85
 - 19 Johnstone SA, Hubaishy I, Waisman DM. Phosphorylation of annexin II tetramer by protein kinase C inhibits aggregation of lipid vesicles by the protein. *J Biol Chem* 1992; **267**: 25976-25981
 - 20 Krawczyk E, Suprynowicz FA, Hebert JD, Kamonjoh CM, Schlegel R. The Human Papillomavirus Type 16 E5 Oncoprotein Translocates Calpactin I to the Perinuclear Region. *J Virol* 2011; **85(21)**: 10968
 - 21 Sudarshan RS, Schlegel R, Liu X. The HPV-16 E5 protein represses expression of stress pathway genes XBP-1 and COX-2 in genital keratinocytes. *Biochem Biophys Res Commun*. 2010 September 3; **399(4)**: 617-622. doi:10.1016/j.bbrc.2010.07.125
 - 22 Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. "The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases". *Cell* 1993; **75 (4)**: 805-816. doi:10.1016/0092-8674(93)90499-G. PMID 8242751.
 - 23 Rodriguez R, Meuth M. "Chk1 and p21 cooperate to prevent apoptosis during DNA replication fork stress". *Mol Biol Cell* 2006; **17 (1)**: 402-412
 - 24 Tsao YP, Li YL, Tsai CT, Chen LS. Human papillomavirus type 11 and 16 E5 represses p21 (Waf1/Sdi1/Cip1) gene expression in fibroblasts and keratinocytes. *JOURNAL OF VIROLOGY* 1996; **70**: 7535-7539
 - 25 Frazer IH, Thomas R, Zhou J, Leggatt G, Dunn L, McMillan N, Tindle RW, Filgueira L, Manders P, Barnard P, Sharkey M. Potential strategies utilised by papillomavirus to evade host immunity. *Immunol Rev* 1999; **168**:131-42.
 - 26 zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; **2**:342-50.
 - 27 Cresswell P, Bangia N, Dick T, Dietrich G. The nature of the MHC class I peptide loading complex. *Immunol Rev* 1999; **172**: 21-28
 - 28 Ashrafi GH, Haghshenas MR, Marchetti B, O'Brien PM, Campo MS. The E5 protein of human papillomavirus type 16 selectively downregulates surface HLA class. *Int J Cancer* 2005; **113**: 276-283
 - 29 Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. *Nat Rev* 2007; **7**: 11-22
 - 30 Maufort JP, Sybil M. Genter Williams, Henry C. Pitot, Paul F. Lambert. Human papillomavirus 16 E5 oncogene contributes to two stages of skin carcinogenesis. *Cancer Re* 2007; **67**: 6106-6112
 - 31 Matés JM, Segura JA, Alonso FJ, Márquez J. Intracellular redox status and oxidative stress: implications for cell proliferation, apoptosis, and carcinogenesis. *Arch Toxicol* 2008; **82**: 273-299
 - 32 Kabsch K, Alonso A. The human papillomavirus type 16 E5 protein impairs TRAIL- and FasL-mediated apoptosis in HaCaT cells by different mechanisms. *J Virol* 2002; **76**: 12162-12172
 - 33 Zhang B, Spandau DF, Roman A. E5 protein of human papillomavirus type 16 protects human foreskin keratinocytes from UV B-irradiation-induced apoptosis. *J Virol* 2002; **76**: 220-231
 - 34 Oh JM, Su-Hyeong Kim, Yun-Il Lee, Miran Seo, So-Young Kim, Yong-Sang Song, Woo-Ho Kim, Yong-Sung Juhn. Human papillomavirus E5 protein induces expression of the EP4 subtype of prostaglandin E2 receptor in cyclic AMP response element-dependent pathways in cervical cancer cells. *Carcinogenesis* 2009; **30**: 141-149
 - 35 Butz K, Ristriani T, Hengstermann A, Denk C, Scheffner M, Hoppe-Seyler F. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. *Oncogene* 2003; **22(38)**: 5938-5945
 - 36 Oz SZ. The Interaction between Human Papillomavirus Proteins and Cytoskeletal Filaments(2012) Human Papillomavirus and Related Diseases - From Bench to Bedside - Research aspects, Dr. Davy Vanden Broeck (Ed.), ISBN: 978-953-307-855-7, InTech.
 - 37 Gu Z, Matlashewski G. Effect of Human Papillomavirus Type 16 Oncogenes on MAP Kinase Activity. *JOURNAL OF VIROLOGY* 1995; **69(12)**: 8051-8056
 - 38 Krawczyk E, Suprynowicz FA, Sudarshan SR, Schlegel R. Membrane orientation of the human papillomavirus type 16 E5 oncoprotein. *J Virol* 2010; **84**: 1696-1703
 - 39 Kim SH, Oh JM, No JH, Bang YJ, Juhn YS, Song YS. Involvement of NF-kappaB and AP-1 in COX-2 upregulation by human papillomavirus 16 E5 oncoprotein. *Carcinogenesis* 2009; **30**: 753-757
 - 40 Mi-Kyung Kim, Hee Seung Kim, Su-Hyeong Kim, Jung-Min Oh, Jae Yong Han, Jeong Mook Lim, Yong-Sung Juhn, Yong-Sang Song. Human papillomavirus type 16 E5 oncoprotein as a new target for cervical cancer treatment. Elsevier Inc. All rights reserved. doi:10.1016/j.bcp.2010.07.013.
 - 41 Gruener M, Bravo IG, Momburg F, Alonso A, Tomakidi P. The E5 protein of the human papillomavirus type 16 down-regulates HLA-I surface expression in calnexin-expressing but not in calnexin-deficient cells. *Virol J* 2007; **4**: 116
 - 42 Campo MS, Graham SV, Cortese MS, Ashrafi GH, Araibi EH, Dornan ES, Miners K, Nunes C, Man S. HPV-16 E5 down-regulates expression of surface HLA class I and reduces recognition by CD8 T cells. *Virology* 2010; **407**: 137-142
 - 43 Kim SH, Oh JM, No JH, Bang YJ, Juhn YS, Song YS. Involvement of NFkappaB and AP-1 in COX-2 upregulation by human papillomavirus 16 E5 oncoprotein. *Carcinogenesis* 2009; **30**: 753-757
 - 44 Sarian LO, Derchain SF, Yoshida A, Vassallo J, Pignataro F, De Angelo Andrade LA. Expression of cyclooxygenase-2 (COX-2) and Ki67 as related to disease severity and HPV detection in squamous lesions of the cervix. *Gynecol Oncol* 2006; **102**: 537-541

Sur T. HPV E5 and its interacting partners in CaCx

Peer reviewers: Kiyoshi Yoshino, M.D., Ph.D, Department of Obstetrics and Gynecology, Osaka University, Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 5650871 Japan; Yutaka Ueda, Assistant Professor, Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine,

2-2 Yamadaoka, Suita, Osaka, 565-0871, Japan; Eliane Campos Coimbra, Universidade Federal de Pernambuco - UFPE, Departamento de Genética, Laboratório de Estudos Moleculares e Terapia Experimental-LEMTE, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife- Pernambuco, Brasil.