Review

Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis

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Human papillomavirus (HPV) is small, double-stranded DNA virus that infects mucosal and cutaneous epithelial tissue. HPV is sexually transmitted and the viral DNA replicates extrachromosomally. The virus is non-enveloped and has an icosahedral capsid. There are approximately 118 types of HPV, which are characterized as high-risk or low-risk types. High-risk HPVs cause malignant transformation while the low-risk ones cause benign warts and lesions. The expression of E6 and E7 is normally controlled during the normal viral life cycle when viral DNA replicates extrachromosomally. HPV E6 and E7 oncoproteins are overexpressed when the viral genome integrates into the host DNA. Deregulated overexpression of E6 and E7 oncoproteins can cause several changes in cellular pathways and functions leading to malignant transformation of cells and tumorigenesis. In this review, we focus on several cellular mechanisms and pathways that are altered in the presence of E6 and E7, the target proteins of E6 and E7 inside the host cell and how they contribute to the development of the transformed phenotype..

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1. Introduction

Human papillomavirus (HPV) is a double-stranded DNA virus that is non-enveloped and has an icosahedral capsid (Longworth and Laimins 2004a). HPV causes malignant transformation as well as genital warts and lesions. HPV is transmitted sexually and the integrin alpha 6 has been identified as a receptor for the entry of HPV into the epithelial cells (Evander *et al* 1997). The virus replicates as an extrachromosomal DNA inside the nucleus of the host cell. At present, roughly 118 different types of HPV have been characterized (Jo and Kim 2005). These can be classified into cutaneous or mucosal, depending on whether they infect cutaneous or mucosal epithelial cells. Depending on the risk of malignancy, HPVs are further grouped as high-risk or low-risk types. The high-risk ones such as HPV-8, -16, -18 and -31 cause malignant progression of lesions (Motoyama *et al* 2004), while the low-risk ones such

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Abbreviations used: AAK1, adaptor protein 2 associated kinase 1; ADA3, adenosine deaminase 3; BRCA1, breast cancer-associated protein 1; CAL, cystic fibrosis receptor-associated ligand; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; CBP, CREB-binding protein; Dlg, disc large; E6AP, E6-associated protein; E6TP1, E6 targeted protein 1; ERBB3, epidermal growth factor receptor-related protein tyrosine kinase B3; EZH2, enhancer of zeste homologue 2; FADD, fas-associated death domain; GIPC, GAIP-interacting protein c-terminus; HDAC, histone deacetylase; HPV, human papillomavirus; hTERT, human telomerase reverse transcriptase; IAP2, inhibitor of apoptosis 2; IFN, interferon; IL, interleukin; Jak, janus kinase; LCR, long control region; PDZ, PSD95/Dlg/ZO1; IRF, interferon regulatory factor; MAGI, membrane-associated guanylate kinase; MCM7, minichromosome maintenance 7; MDM2, mediator of DNA damage 2; MMP, matrix metalloprotease; MUPP1, multiple PDZ domain-containing protein 1; NF-κB, nuclear factor kappa B; OSCC, oesophageal squamous cell carcinoma; PATJ, PALS-1 associated tight junction protein; PI3K, phosphatidylinsotol-3-OH kinase; PP2A, protein phosphatase 2A; PSD95, post-synaptic density protein 95; PTPN1, protein tyrosine phosphatase N1; Rb, retinoblastoma; Scrib, scribble; STAT1, signal transducer and activator of transcription; Tyk2, tyrosine kinase 2; USF, upstream sequence factor; TNFR1, tumour necrosis factor receptor 1; TSSK2, testis-specific serine/threonine kinase 2

as HPV-6, -11 cause benign warts and lesions that do not become malignant (Li *et al* 2005; Sur and Cooper 1998). The so-called 'low-risk' types, especially HPV-6 and -11, however, have been identified in penile cancer (Dianzani *et al* 1998), laryngeal and bronchogenic carcinomas (Reidy *et al* 2004) and oesophageal squamous cell carcinoma (OSCC) (Cooper *et al* 1995; Matsha *et al* 2002; Chen *et al* 1994; Dreilich *et al* 2006). HPV is associated with several cancers; the best-studied one is the cervical cancer. HPV is a causative agent in at least 99% of cases of cervical cancers (Longworth and Laimins 2004a). Several reports have shown an aetiological role for HPV in other cancers such as breast cancer (zur Hausen 1999, 2002; deVilliers *et al* 2004; Khan *et al* 2008), head and neck squamous cell carcinoma (Ferris *et al* 2005; Fakhry and Gillison 2006) and anogenital cancer (Finzer *et al* 2002).

A study in a high-risk area in Southern China showed that 60% of oesophageal cancer biopsies contained HPV (Chang *et al* 1994, 1997). Out of the HPV-positive tumours, 50% contained the low-risk HPV-6 DNA and 8% showed HPV-16 DNA. Another study showed that HPV-11 is the predominant type (48%) present in HPV-positive OSCC patients (Matsha *et al* 2007) in the Transkei region of South Africa. Therefore, the role of low-risk HPV types in tumour development is not very clear. It has been shown that viral oncoprotein E6 is involved in the transformation of host cells. Malignant cells show deregulated overexpression of E6 and E7 oncoproteins, which ultimately leads to the development of cancer (Snijders *et al* 2006). The overexpression of E6 and E7 requires integration of viral DNA into the host genome. It has been shown that HPV E6 expression alone can lead to cellular transformation *in vitro* (Sedman *et al* 1991, 1992; Gao *et al* 1997; Kiyono *et al* 1997). This review focuses on the expression of E6 and E7 during the viral life cycle, mechanisms of cellular transformation by E6 and E7, and cellular targets for E6 and E7 proteins.

2. HPV genome and life cycle

The HPV genome is approximately 8 kb in length and is divided into three regions, the non-coding long control region (LCR, ~1 kb), and the protein coding early (E, ~4 kb) and late (L, ~3 kb) regions. The viral genome encodes six early (E1, E2, E4, E5, E6 and E7) and two late (L1 and L2) proteins (figure 1). The transcription of early and late genes is controlled by the LCR. The viral proteins are translated from polycistronic mRNAs containing overlapping reading frames (Jo and Kim 2005). Upon entry into the host cell, the *E1* and *E2* genes are expressed first and encode proteins required for viral DNA replication (Motoyama *et al* 2004). E1 and E2 form a complex that binds to the viral origin of replication and recruits host polymerases necessary for the replication. E1 also exhibits helicase activity, unwinding the DNA ahead of the replication complex. E2 also regulates transcription of early genes from the viral promoter; when the level of E2 is low; it binds to the promoter and induces transcription, while at high levels, it represses transcription of the early genes including *E6* and *E7* by blocking the binding of host factors to viral promoters (Longworth and Laimins 2004a). HPV E4 is expressed only at the later stage of the viral life cycle when virus particles are being assembled. HPV E5 is frequently deleted in cervical carcinoma cells,

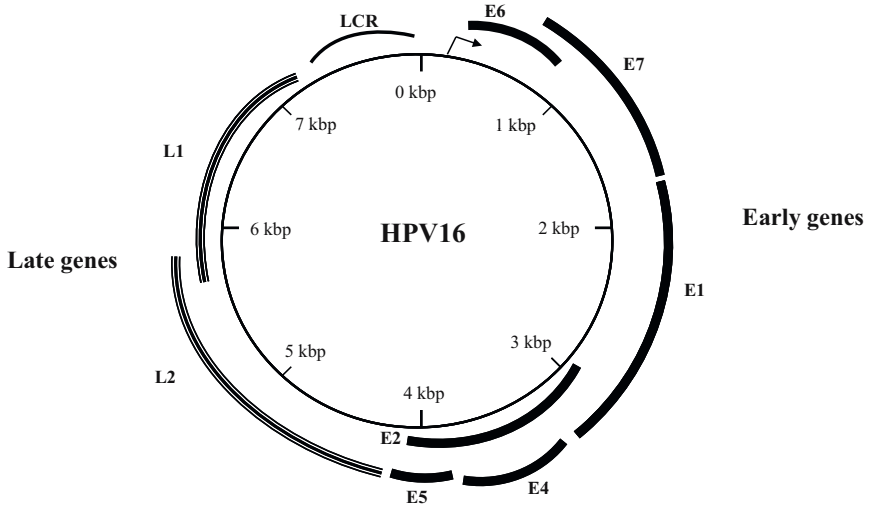

Figure 1. The double-stranded circular genome of HPV-16 is roughly 8 kb in size and grouped as having early (E) or late (L) genes. The early genes encode six proteins E1, E2, E4, E5, E6 and E7, and the late genes encode two structural proteins L1 and L2. Viral transcription and DNA replication is controlled by the long control region (LCR). The proteins are encoded by a polycistronic mRNA with overlapping reading frames. The viral DNA replicates extrachromosomally as an episome.

which suggests that it may not be important for maintaining the transformed state. HPV *E6* and *E7* genes encode oncoproteins that cause transformation of the host cell. Also, E6 and E7 are involved in maintenance of the HPV genome extrachromosomally. The two late genes *L1* and *L2* encode structural proteins that form the viral icosahedral capsid consisting of 72 capsomers (Jo and Kim 2005).

HPVs are mostly sexually transmitted and infect the genital region; they are limited to the genital tract as ~20% of oropharyngeal cancers contain high-risk HPV DNA. The viral life cycle is linked to the differentiation stage of the infected host cell, and can be divided into productive and non-productive stages. During persistent infection of basal cells, the viral genome replicates as an episome and there are 20–100 such episomes in the infected cells. When the cells undergo differentiation, shedding of new virions occurs. Deregulated expression of E6 occurs when the HPV genome integrates into the host genome, which disrupts the *E1* and *E2* genes and therefore the transcriptional repression of early genes is lost (Finzer *et al* 2002). It was also shown that integration of the viral genome results in stabilization of E6 and E7 mRNAs (Jeon and Lambert 1995). Integration occurs near the fragile sites in the human genome (Thorland *et al* 2003) and results in termination of the viral life cycle as large portions of the genome are disrupted and therefore it becomes functionally inactive.

3. HPV E6 and E7 oncoproteins and cellular transformation

The HPV E7 oncoprotein is about 100 amino acids in length (Munger and Howley 2002). The HPV E7 oncoprotein has been shown to bind to the retinoblastoma protein (pRb) and inactivate its function by preventing the binding of pRb to E2F transcription factor (Dyson *et al* 1989). The pRb protein is active in its hypophosphorylated form and binds to E2F transcription factors to prevent S-phase entry. During the normal cell cycle, pRb is phosphorylated by cyclin D1/cyclin-dependent kinase (CDK)4 and cyclin E/CDK2 complexes causing dissociation of pRb from E2F thereby allowing normal S-phase progression (Munger and Howley 2002; Jo and Kim 2005). HPV E7 binds to the hypophosphorylated pRb, preventing its interaction with E2F. Therefore, in cells overexpressing HPV E7 protein, the checkpoint control at G1/S transition is lost and the cells continually traverse the cell cycle leading to uncontrolled cellular proliferation (Dyson 1998; Jo and Kim 2005). HPV E7 has also been shown to cause degradation of pRb through the ubiquitin–proteasome mediated pathway (Boyer *et al* 1996). The binding of HPV E7 to pRb occurs through the motif LXCXE present within the E7 protein (Liu *et al* 2006). Apart from its interaction with pRb family members, E7 interacts with a wide variety of cellular proteins. One such class of proteins is the histone deacetylases (HDACs), which function as transcriptional co-repressors; binding of HPV E7 to HDACs via Mi2 allows E2F-dependent transcription (Brehm *et al* 1999; Longworth and Laimins 2004b) which promotes cell proliferation. HPV E7 also binds to CDK2/cyclin A and CDK2/cyclin E, and activates these kinases which in turn phosphorylate pRb and induce transcription of S-phase genes (Arroyo *et al* 1993; McIntyre *et al* 1996). HPV E7 was also found to bind to CDK inhibitors (CKIs) p27 and p21, which removes the cell cycle checkpoint control at the G1/S interface (Zerfass-Thome *et al* 1996; Funk *et al* 1997) and promotes uninhibited rounds of cell-cycle progression.

The HPV E6 oncoprotein is 160 amino acids in size (Munger and Howley 2002). The E6 protein from high-risk HPV is sufficient for the induction and maintenance of cellular transformation (Hawley-Nelson *et al* 1989; Thompson *et al* 1997; Duensing and Munger 2002; Munger *et al* 2004). HPV E6 protein has been reported to bind to p53 and cause proteasomal degradation of p53 by 26S proteasome (Scheffner *et al* 1990; Werness *et al* 1990; Crook *et al* 1991; Band *et al* 1993; Havre *et al* 1995; Scheffner *et al* 1993; Li and Coffino 1996). Interestingly, the levels of p53 in normal cells are very low; during overexpression of E7 protein, p53 levels are increased due to an inhibition of mediator of DNA damage 2 (MDM2)-mediated proteasomal degradation of p53 in normal cells (Eichten *et al* 2002). The degradation of p53 occurs through a trimeric complex containing E6, E6-associated protein (E6AP) and p53 (Talis *et al* 1998; Zanier *et al* 2005). E6AP acts as an E3 ubiquitin ligase that ubiquitinates p53 and targets it for degradation by proteasome. HPV E6 protein also inactivates p73, which is a homologue of p53 protein (Park *et al* 2001). High-risk HPV E6 proteins can also downregulate p53 activity by targeting CREB-binding protein (CBP) and p300 (Zimmermann *et al* 1999). However, a recent report has shown that high-risk HPV E6 can degrade p53 protein even in the absence of E6AP (Massimi *et al* 2008). The inactivation of p53 compromises the integrity of the replicated DNA and causes DNA damage (Havre *et al* 1995; Kessis *et al* 1996) and chromosomal instability (Schaeffer *et al* 2004; Thomas and Laimins 1998); these abnormalities result in cell proliferation or tumour development (Foster *et al* 1994; Cheng *et al* 2007; Hebner *et al* 2007; Cooper *et al* 2007). Low-risk HPV E6 oncoproteins can also bind to p53, but with very low affinity and do not degrade p53 (Slebos *et al* 1995). There is accumulation of p53 after DNA damage in cells expressing HPV-11 E6 protein and the cells are arrested in the G1 phase of the cell cycle (Slebos *et al* 1995). It has been further shown that low-risk HPV E6 proteins do not bind E6AP to form a trimeric complex of E6/E6AP/p53 like the high-risk E6 proteins (Zanier *et al* 2005). The high-risk HPV E6 protein HPV-16 E6 has been reported to prevent apoptosis

by a p53-independent mechanism which involves inhibition of *Bax* gene expression and degradation of Bax protein in human keratinocytes (Magal *et al* 2005). Inhibition of the pro-apoptotic protein Bax results in inhibition of apoptosis and therefore cells accumulate mutations in their DNA. High-risk E6 proteins also prevent apoptosis by binding to tumour necrosis factor receptor 1 (TNFR1) and inhibit TNFR1 apoptotic signalling (Duerksen-Hughes *et al* 1999; Filippova *et al* 2002).

High-risk E6 mRNAs undergo alternative splicing resulting in generation of smaller E6* protein (Inagaki *et al* 1988; Schneider-Gadicke *et al* 1988; Sedman *et al* 1991; Stacey *et al* 1995; Vaeteewoottacharn *et al* 2005). This phenomenon of alternative splicing has been reported in high-risk HPV types only; the low-risk types do not show alternative splicing. This is because the low-risk types lack the essential dinucleotides (GT) of splice consensus sequences that are present in the high-risk types HPV-16, -18, -31 and -33 (Schneider-Gadicke *et al* 1988). The alternatively spliced E6* mRNA is found at much higher levels compared with the full-length E6 transcripts (Smotkin and Wettstein 1986; Zheng and Baker 2006). The full-length E6 protein is roughly 18.9 kDa and the spliced E6* protein is 6.5 kDa (Schneider-Gadicke *et al* 1988). The E6* protein negatively regulates the expression of the active full-length E6 protein thereby resulting in lower E6 levels (Sedman *et al* 1991). A study also showed that E6* can bind *in vitro* to full-length E6 protein as well as E6AP protein (Pim *et al* 1997), which inhibits E6 from binding to E6AP and degrading p53.

High-risk HPV E6 proteins are capable of immortalizing the host cell; this is achieved by preventing the shortening of telomere length. In somatic cells, with each round of cell division, there is a shortening of telomere length which corresponds to cell ageing. In human cancer cells, a significantly high level of telomerase activity has been found which suggests a role in tumour development (Pendino *et al* 2006). High-risk HPV E6 proteins prevent telomere shortening by increasing the expression of the catalytic subunit of human telomerase reverse transcriptase (hTERT) by forming a complex with E6AP and directly binding to the hTERT promoter (Klingelhultz *et al* 1996; Oh *et al* 2001; Veldman *et al* 2001; Blasco and Hahn 2003; Veldman *et al* 2003; Gewin *et al* 2004; Seo *et al* 2004) and thereby immortalizing the host cell. E6 forms a complex with c-Myc displacing the repressor proteins upstream sequence factors (USF1 and USF2) from the hTERT promoter and activating transcription (Veldman *et al* 2003; McMurray and McCance 2003). However, one report has shown that activation of hTERT by binding of E6 to the E-box does not require c-Myc (Gewin and Galloway 2001). Furthermore, the E6/E6AP complex targets NFX1-91 (a newly identified repressor for hTERT) for degradation by ubiquitination (Gewin *et al* 2004). Other cellular targets include Bak, Fas-associated death domain-containing protein (FADD) and procaspase 8, which is degraded by E6/E6AP causing inhibition of apoptosis (Thomas and Banks 1999; Garnett *et al* 2006). HPV E6 also downregulates the expression of Notch1, a p53 target gene involved in tumour suppression (Yugawa *et al* 2007; Talora *et al* 2002). Downregulation of Notch1 is observed in cervical cancer cells and contributes to tumorigenesis. E6 binds to and inhibits tyrosine kinase 2/janus kinase–signal transducer and activator of transcription (Tyk2/Jak-STAT) activation and interferon regulatory factor 3 (IRF3) transcriptional activities, resulting in inhibition of the interferon signalling pathway (Li *et al* 1999; Ronco *et al* 1998). E6 has also been reported to bind to the tumour suppressor protein breast cancer-associated protein 1 (BRCA1) and release the inhibition of transcription in response to oestrogen (Zhang *et al* 2005). Another study has shown that HPV-16 E6 activates nuclear factor kappa B (NF-κB) leading to enhanced expression of inhibitor of apoptosis 2 (IAP-2), which prevents apoptosis of epithelial cells (Nees *et al* 2001; Yuan *et al* 2005; James *et al* 2006). A multistep model for HPV E6-mediated tumorigenesis is shown in figure 2.

4. E6 targets PDZ domain-containing proteins

High-risk HPV E6 proteins interact with certain cellular proteins containing a PSD95/Dlg/ZO-1 (PDZ) domain. High-risk E6 interacts with several PDZ domain-containing proteins such as human homologues of *Drosophila melanogaster* disc large and scribble tumour suppressors (hDlg and hScrib), post-synaptic density protein 95 (PSD95), multiple PDZ domain-containing protein 1 (MUPP1), membrane-associated guanylate kinase (MAGI-1, -2, -3), GAIP-interacting protein c-terminus (GIPC), PALS-1 associated tight junction protein (PATJ) and protein tyrosine phosphatase N1 (PTPN1) through complex formation with E6AP and targets them for degradation by proteasome (Kiyono *et al* 1997; Lee *et al* 1997; Gardiol *et al* 1999; Glaunsinger *et al* 2000; Lee *et al* 2000; Thomas *et al* 2001, 2002; Nguyen *et al* 2003; Massimi *et al* 2004; Favre-Bonvin *et al* 2005; Handa *et al* 2007; Jing *et al* 2007; Spanos *et al* 2008). Low-risk HPV E6 does not contain the PDZ-binding motif and therefore cannot target these proteins. Degradation of PDZ domain-containing proteins results in cellular transformation due to loss of cell–cell contact and loss of cell polarity (Watson *et al* 2003; Thomas *et al* 2005; Storrs and Silverstein 2007) and also because many PDZ domain-containing proteins are involved in cell signalling (Jelen *et al* 2003). One study has shown that the degradation of phosphatase PTPN13 by E6 results in anchorage-independent growth and a Ras-dependent invasive phenotype (Spanos *et al* 2008). The PDZ-binding

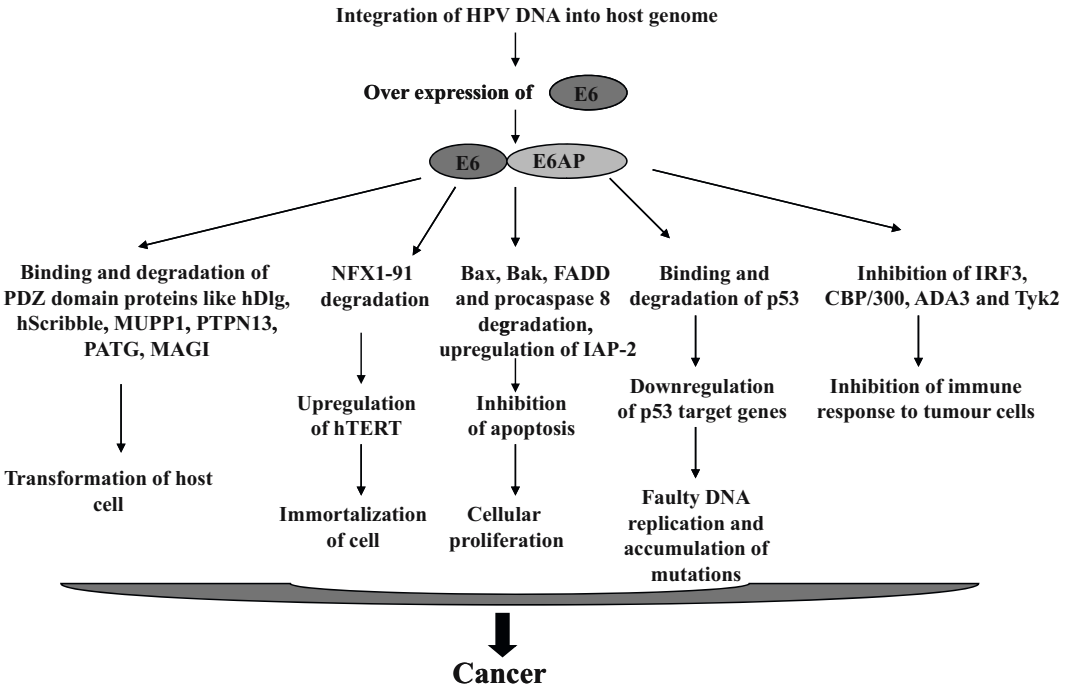

Figure 2. Multiple effects of high-risk HPV E6 protein on different cellular targets leading to malignant transformation. Integration of HPV DNA into the host genome results in deregulated overexpression of E6 which, in association with E6AP ubiquitin ligase, targets different proteins involved in several pathways. The cumulative effect of these changes in cellular programming causes tumorigenesis.

motif of high-risk HPV E6 is present at the extreme C-terminus of the protein and consists of a four amino acid stretch ETQV/L (Thomas *et al* 2001), while low-risk HPV E6 lacks the PDZ-binding motif (figure 3). Mutations in these residues result in a loss of the PDZ-binding ability of E6. Conversely, introduction of the PDZ-binding motif from a high-risk E6 into the C-terminus of a low-risk E6 protein results in the latter's ability to target and degrade PDZ domain proteins (Gardiol *et al* 2002; Pim *et al* 2002). It has recently been shown that an arginine residue at position 154 and just outside the PDZ-binding motif of E6 plays a role in binding to the PDZ domains of Dlg and MAGI (Zhang *et al* 2007; Thomas *et al* 2008). Mutations in the conserved arginine-154 severely abolished the PDZ domain-binding ability of E6 compared with wild-type E6.

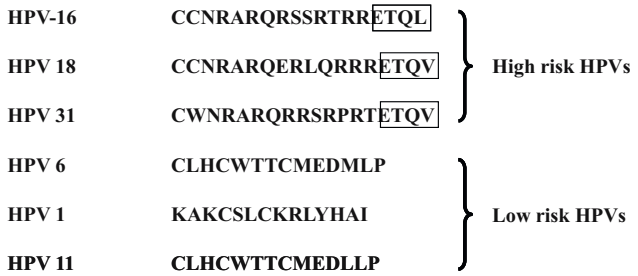

Figure 3. The sequence of E6 proteins from high-risk and low-risk HPVs at the C-terminus. High-risk E6 proteins from HPV-16, -18 and -31 have a four amino acid residue at their C-terminus (shown in box) known as the PDZ-binding motif, which is involved in binding to PDZ domain-containing proteins. Low-risk HPV E6 from HPV-1, -6 and -11 lack the PDZ-binding motif and therefore cannot target PDZ domain-containing proteins.

5. Other cellular targets of E6

Apart from the molecules mentioned above, HPV E6 oncoproteins bind to several other protein molecules inside the host cell, which may directly or indirectly cause changes in cellular programming. High-risk HPV E6 has been shown to bind to cystic fibrosis transmembrane receptor-associated ligand (CAL) and degrade it in a PDZ domain-dependent manner (Jeong *et al* 2007); E6 also downregulated the CAL transcript levels inside the cell. High-risk HPV E6 was found to inactivate the transcriptional coactivator adenosine deaminase 3 (ADA3), which functions as a coactivator for p53 transactivation (Kumar *et al* 2002). HPV E6 also binds and inactivates the transcriptional coactivators CBP and p300 (Patel *et al* 1999), which results in downregulation of transcription from the interleukin-8 promoter (Huang and McCance 2002). One report has shown that HPV E6 protein

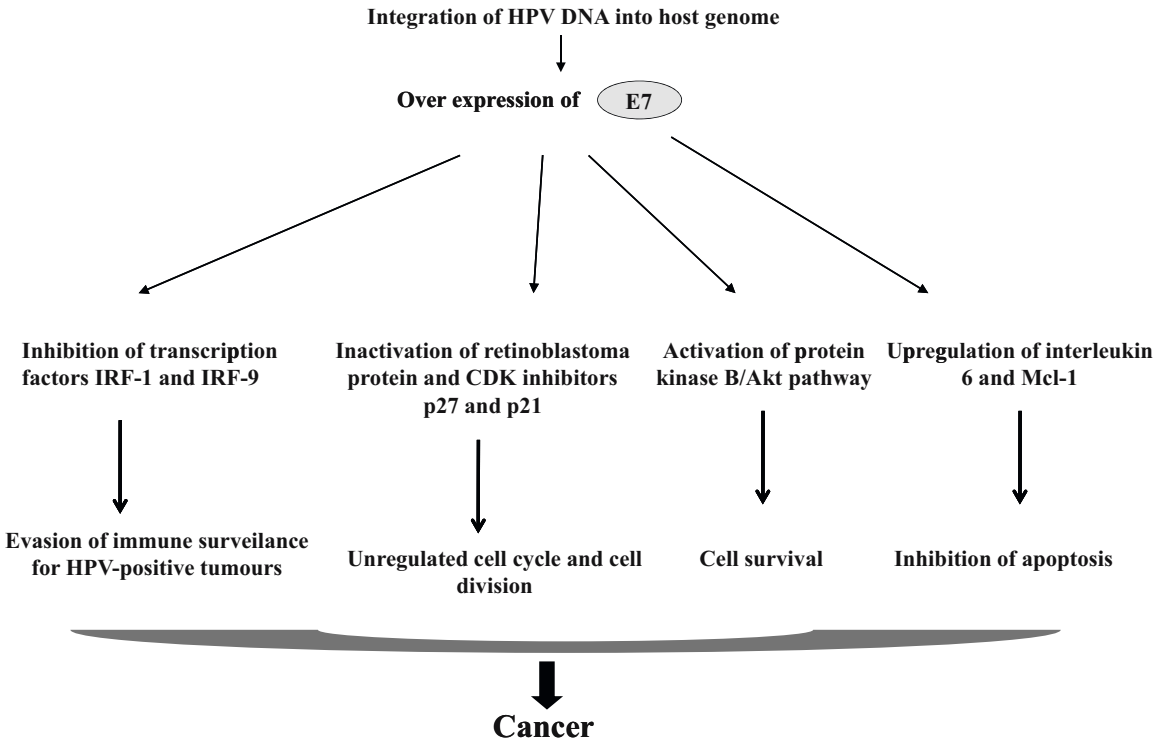

Figure 4. A multistep model showing different pathways that are commonly targeted by the HPV E7 protein. The integration of HPV DNA into the human genome causes unregulated overexpression of E7 oncoprotein. The cumulative effects of modulation of different pathways result in cancer.

binds to E6BP, which is a putative calcium-binding protein (Chen *et al* 1995; Elston *et al* 1998). High-risk HPV E6, but not low-risk ones, targets E6 targeted protein 1 (E6TP1) for degradation (Gao *et al* 1997, 1999). E6TP1 shares high homology with GTPase-activating proteins and therefore this might be a strategy by E6 to modulate G protein signalling. Another tumour suppressor tuberin has been shown to be a target of HPV-16 E6; binding of E6 to tuberin results in degradation of tuberin and enhances insulin-dependent cell proliferation (Lu *et al* 2004). It has also been shown that E6 induced minichromosome maintenance 7 (MCM7) and cyclin E in an E2F-dependent manner, which suggests that E6 causes inactivation of the pRb/p16 pathway by a different mechanism as compared with HPV E7 protein (Shai *et al* 2007).

6. Other cellular targets of E7

Apart from its well-characterized interaction with pRb, HPV E7 has been found to interact with a plethora of cellular proteins involved in different pathways. High-risk HPV-16 E7 protein was found to induce transcription of enhancer of zeste homologue 2 (EZH2) via E2F transcription factors (Holland *et al* 2008). EZH2 enhances the proliferation of HPV-positive tumour cells through bypassing the G1/S checkpoint and inhibiting apoptosis. Genetic screening to identify kinases targeted by HPV-16 E7 in colorectal carcinoma cells showed five essential kinases – CDK6, epidermal growth factor receptor-related protein tyrosine kinase B3 (ERBB3), FYN, adaptor protein 2 associated kinase 1 (AAK1) and testis-specific serine/threonine kinase 2 (TSSK2) (Baldwin *et al* 2008) – which are essential for cell viability and cell proliferation. HPV-16 E7 was also found to upregulate the expression of interleukin-6 (IL-6) and anti-apoptotic Mcl-1 expression in human lung cancer cells through the phosphatidylinosotol-3-OH kinase (PI3-K) pathway which may contribute to the growth of HPV-positive tumour cells in an autocrine fashion (Cheng *et al* 2008). The transfection of H-Ras into human primary keratinocyte cells expressing HPV-18 E6/E7 resulted in abolition of senescence-like growth arrest in these cells and conferred an invasive potential (Yoshida *et al* 2008); this was due to an increased production of matrix metalloproteases-1 (MMP1) and -9 (MMP9) through the extracellular signal-regulated kinase pathway. Thus, HPV-containing tumour cells acquire invasive properties in the presence of Ras or other growth-promoting pathways. HPV-16 E7 has been found to downregulate the expression of the cell adhesion molecule E-cadherin in transformed keratinocytes (Caberg *et al* 2008); siRNA-mediated silencing of the *E7* gene

caused upregulation of E-cadherin as well as pRb. HPV E7 protein also contributes to evasion of immune surveillance of HPV-containing tumour cells by binding and inactivating interferon regulatory factor 1 (IRF1) and also inhibits the interferon-α (IFN-α) signalling pathway by inactivating IRF9/p48 (Barnard and McMillan 1999; Um *et al* 2002). The p600 protein, a member of the pRb family, has been recently found to be targeted by the E7 oncoprotein, which results in anchorage-independent cell growth and transformation (Huh *et al* 2005). HPV E7 has also been shown to activate the cell survival protein kinase B/Akt signalling pathway by inactivating protein phosphatase 2A (PP2A) which dephosphorylates PKB/Akt and terminates the signalling (Pim *et al* 2005) (figure 4).

7. Key events/mechanisms in E6- and E7-mediated transformation

Out of the diverse molecules and pathways targeted by HPV E6 and E7 oncoproteins, some pathways/molecules might play a decisive role in the development and progression of cancer. In the case of E6, targeting the PDZ domain-containing proteins such as hDlg, hScribble, and p53 for degradation constitutes a major mechanism of modulating the antitumour pathways of the host cell; this is further supplemented by induction of the catalytic subunit of hTERT, which contributes to cellular immortalization. For the E7 oncoprotein, targeting pRb and its family members such as p600, p27 and p21 for degradation constitutes a major step in tumorigenesis. This effect could be further augmented by activation of the cell-survival pathways of protein kinase B/Akt. Both E6 and E7 bind and inactivate several transcription factors involved in the immune response, e.g. IRFs. This serves to avoid immune-based destruction of HPV-containing tumours while acquiring invasive potential through modulation of other pathways. Although E6 and E7 proteins alone have shown transforming activity *in vitro*, HPV-mediated tumorigenesis *in vivo* requires the coordinated action of the two proteins in achieving cellular reprogramming.

8. Conclusion

There has been lot of research to understand the mechanisms of HPV E6 protein-mediated tumorigenesis in epithelial cells. While some mechanisms and pathways are well understood, many mechanisms are poorly understood. For example, what is the trigger for HPV to terminate its productive life cycle and integrate into the host genome? Are there any viral factor(s) that cause integration, and what is the exact role of low-risk HPV E6 proteins in the development of cancer? HPV E6 is currently being used as a vaccine candidate to protect women against HPV-induced cervical cancer. However, given the role of HPV in other types of cancer, it will be worthwhile investigating the protective efficacy of E6 protein in other HPV-associated cancers.

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