Pathol. Oncol. Res. (2008) 14:345–354
DOI 10.1007/s12253-008-9056-6

REVIEW PAPER

Human Papilloma Virus (HPV) and Host Cellular Interactions

Ioannis N. Mammas · George Sourvinos · Athena Giannoudis · Demetrios A. Spandidos

Received: 5 February 2008 / Accepted: 17 April 2008 / Published online: 21 May 2008
© Arányi Lajos Foundation 2008

Abstract Viral-induced carcinogenesis has been attributed to the ability of viral oncoproteins to target and interact with the host cellular proteins. It is generally accepted that Human papilloma virus (HPV) E6 and E7 function as the dominant oncoproteins of 'high-risk' HPVs by altering the function of critical cellular proteins. Initially it was shown that HPV E6 enhances the degradation of p53, while HPV E7 inactivates the function of the retinoblastoma tumor suppressor protein Rb. However, recent studies during the last decade have identified a number of additional host cellular targets of both HPV E6 and E7 that may also play an important role in malignant cellular transformation. In this review we present the interactions of HPV E6 and E7 with the host cellular target proteins. We also present the role of DNA integration in the malignant transformation of the epithelial cell.

Keywords Human papilloma virus · HPV · E6 · E7 · Host cellular proteins · DNA integration

Abbreviations

HPV	human papilloma virus
Rb	retinoblastoma protein
E6-AP	E6-associated protein
E6-BP	E6-binding protein
hDlg	human *Drosophila* discs large protein
hScrib	human Scribble tumor suppressor protein
MAGI	membrane-associated guanylate kinases
IRF-1 and -3	interferon regulatory factor 1 and 3
MMP-7	multicopy maintenance protein 7
hTERT	human telomerase reverse transcriptase
hE6TP1	human E6-targeted protein 1
Gps2	G-protein pathway suppressor 2
IFNAR1	interferon-alpha receptor 1
MUPP1	multi-PDZ-domain protein 1
CDK	cyclin-dependent kinases
TBP	TATA box-binding protein
CKI and II	casein kinase I and II
S4	subunit 4
M2-PK	M2 pyruvate kinase
ISGF3	interferon-stimulated gene factor 3
IFN	interferon

I. N. Mammas · G. Sourvinos · D. A. Spandidos (✉)
Department of Virology, School of Medicine, University of Crete,
Heraklion 71100, Crete, Greece
e-mail: spandidos@spandidos.gr

A. Giannoudis
Faculty of Medicine, University of Liverpool,
Liverpool, UK

Introduction

The ability of viral oncoproteins to induce single-step cellular transformation has provided an excellent paradigm to delineate the various biochemical pathways that control normal cell growth and differentiation. Indeed, recent studies have provided ample evidence that viral oncoproteins target cellular pathways whose aberrations are also critical in the development of human cancer [1, 2]. The ability of E6 and E7 oncoproteins of 'high-risk' HPVs to efficiently immortalize human epithelial cells has led to considerable interest in identifying their cellular targets. 'High-risk' HPVs, such as HPV 16 and HPV 18 have been strongly implicated in the pathogenesis of cervical cancer, a leading cause of death due to cancer among women worldwide [1–3]. 'Low-risk' HPVs have been associated

with benign warts of oral and urogenital epithelium in adults as well as in children and they are only rarely found in malignant tumours. Different HPV types vary in tissue distribution, oncogenic potential and association with anatomically and histologically distinct diseases.

The functions of the viral oncogenes E6 and E7 are essential for carcinogenesis and for support of the viral life cycle by interacting with a number of cellular proteins. Expression of the E6 and E7 proteins, as a consequence of viral integration is paramount to the establishment and maintenance of the tumorigenic state. In addition, expression of E6 and E7 increases genomic instability of the host cell thus accelerating malignant progression [4]. E6 and E7 target important cellular growth regulatory circuits among them the p53 and retinoblastoma tumor suppressor protein Rb, respectively. Comparisons between the E6 and E7 proteins of 'high-risk' and 'low-risk' HPVs have indicated functional distinctions based on differential affinities for cellular target proteins. HPV E6 has been shown to interact with and enhance the degradation of p53, which plays an important role in cell cycle control and apoptosis in response to DNA damage, while HPV E7 disables the function of the retinoblastoma tumor suppressor protein Rb.

However, during the last decade, it has been well demonstrated that both HPV E6 and E7 interact with host cell targeting a plethora of key host cellular proteins that are involved in apoptosis and malignant cellular transformation. In this review we present the interactions of HPV E6 and E7 with the host cellular target proteins. We also present the role of DNA integration in the malignant transformation of the epithelial cell. Integration of the HPV genome into a host cell chromosome is frequently a prequel to malignant progression and results in the consistent but deregulated expression of the HPV E6 and E7 oncoproteins. However, little information is currently available concerning changes in host cellular proteins that are associated with integration of HPV.

HPV E6 Interactions

HPV E6 protein has been shown to have multiple properties and interacts with a plethora of cellular proteins. These include (a) E6-AP, (b) E6-BP, a calcium-binding protein that may play a role in epithelial differentiation, (c) Bak, a regulator of the apoptosis pathway, (d) the focal adhesion protein paxillin, (e) the human homologue of the *Drosophila* DPZ domain containing proteins hDlg and hScrib (f) the membrane-associated guanylate kinase homologues MAGI-1, -2 and -3, (g) IRF-3, a transcriptional activator possibly involved in antiviral cellular responses, (h) MMP-7, (i) the transcriptional coactivator CBP/p300, (j) telomerase, (k) the Rap GTPase-activating protein homologue E6TP1, (l) PKN a Rho-regulated serine threonine kinase, (m) Gps2 and (n) c-myc.

P53 and E6-AP (associated protein) HPV 16 E6 down-regulates p53 activity by promoting its degradation through the ubiquitin-dependent proteolytic pathway followed by proteasome-mediated degradation [5–8]. HPV E6 binds to E6-associated protein E6-AP, a cellular protein of 100 kd that acts as a ubiquitin ligase. The E6-E6–AP complex specifically interacts with p53, resulting in the rapid ubiquitin-dependent degradation of p53 [9]. The ability to form an E6–E6AP–p53 complex appears to be limited to high-risk E6 proteins [5]. The 'high-risk' HPV E6 proteins bind to E6AP within its amino-terminal substrate recognition domain, and formation of a stable E6–E6AP complex precedes association with p53 [5, 6]. HPV16 E6 degradation has been correlated to cellular immortalization [10]. However, it has been demonstrated that E6-induced immortalization can proceed in the absence of p53 inactivation indicating that targets other than may play a critical role in the E6-induced cellular transformation [11, 12].

E6-BP (binding protein) or ERC-55 E6-BP interacts with 'high-risk' HPV E6 and with bovine papillomavirus type 1 (BPV-1) E6 [13]. The transforming activity of BPV-1 E6 mutants have been correlated with their E6-BP-binding ability. E6-BP is identical to a putative calcium-binding protein, ERC-55, that appears to be localized in the endoplasmic reticulum and may play a role in epithelial differentiation. However, into primary human keratinocytes, it seems that there is no absolute correlation between the ability of E6 mutant proteins to bind E6BP indicating additional cellular targets for E6 which mediate this alteration in cellular differentiation [14].

Bak E6 proteins of HPV 18, HPV 16 and HPV 11 can all bind in vitro to the Bak protein and stimulate its degradation in vivo, reducing Bak-induced apoptosis [15, 16]. The non-oncogenic HPV 11 E6 is less effective than the oncogenic E6 proteins, indicating that the ability of HPV to circumvent the apoptosis induced by Bak may contribute to the oncogenic potential of the virus [16]. It has been suggested that E6-induced degradation of Bak occurs thought its interaction with E6 AP [15]. Bak is a pro-apoptotic member of the Bcl-2 family, a group of proteins involved at a critical point in the control of apoptosis [17, 18]. The interaction between E6 and Bak indicates that HPV inhibit apoptosis in both a p53-dependent and p53-independent manner involving the degradation of Bak.

Paxillin Paxillin is a multi-domain protein that localizes to sites of cell adhesion to extracellular matrix, called focal

adhesions [19]. Focal adhesions form a structural link between the extracellular matrix and the actin cytoskeleton and are important sites of signal transduction. Paxillin has been shown to bind to β-integrin, oncoproteins such as v-Src, v-Crk, $p210^{BCR/ABL}$, $p125^{FAK}$, vinculin, and talin and is involved in effecting changes in the organization of the actin cytoskeleton. HPV E6 interacts with paxillin [20, 21]. In vitro experiments have shown that paxillin binds to the E6 proteins of 'high-risk' HPV 16 but not of the 'low-risk' types 6 and 11 indicating a correlation between the ability of E6 to complex with paxillin and its transformation function [20]. E6 binds paxillin through small protein interaction motifs called LD motifs that have been identified as important in regulating association of paxillin with vinculin and focal adhesion kinase [21]. The LD motifs of paxillin that bind BE6 share homology with the E6 binding site of E6-AP. Paxillin binding to E6 excludes simultaneous binding to E6-AP. E6 binding to paxillin has been implicated in the disruption of the actin cytoskeleton' which is critical for many aspects of cell function. Disruption of the actin cytoskeleton is also a characteristic of many transformed cells.

hDlg and hScrib 'High risk' HPV E6 interacts and induces the degradation of the human homologues of two Drosophila PDZ domain-containing proteins, hDlg and hScrib [7, 8, 22–25]. hDlg, the human homologue of the *Drosophila* discs large protein, is a tumor-suppressor protein in *Drosophila* and hScrib is the human homologue of the Drosophila Scribble tumor suppressor protein. Both hDlg and hScrib contain PDZ domains that have subsequently been identified in a diverse set of proteins that are typically associated with cell junctions. E6 binds to the second PDZ domain of the hDLG through their C-terminal XS/TXV/L motif [22]. E6 mutants losing the ability to bind to hDLG are no longer able to induce E6-dependent transformation of rodent cells. hScrib is also targeted for ubiquitination by the E6–E6AP complex in vitro and that in vivo expression of E6 induces degradation of hScrib [24]. Characterization of the E6AP–E6–hScrib complex has indicated that hScrib binds directly to E6 and that this binding is mediated by the PDZ domains of hScrib and a carboxyl-terminal epitope conserved among the high-risk HPV E6 proteins. The fact that Scrib and Dlg appear to cooperate in a pathway that controls Drosophila epithelial cell growth suggests that the combined targeting of hScrib and hDlg is an important component of the biologic activity of high-risk HPV E6 proteins.

MAGI-1, -2 and -3 'High-risk' HPV E6 proteins bind to the MAGI-1 protein as well as the related MAGI-2 and MAGI-3 proteins for degradation [26–28]. This interaction results in the degradation of the targeted MAGI proteins. MAGI-1, MAGI-2 and MAGI-3 proteins are membrane-associated guanylate kinase homologues (MAGUKs) that contain a number of protein interaction domains, allowing them to act as molecular scaffolds in the formation of multimolecular complexes including several PDZ domains. These proteins are found particularly at areas of cell–cell contact, such as synaptic junctions in neurons and at the tight junctions in epithelial cells and are thought to act in signalling pathways. The PDZ1 domain of MAGI-1 and -3 is the specific site bound by the oncogenic HPV E6 [27–29]. It has also been demonstrated that co-expression of this domain can protect each of the full-length MAGI proteins from E6-mediated degradation. HPV-18 E6 binds more strongly to, and induces degradation of MAGI-1, -2 and -3 more efficiently than HPV-16 E6 both in vitro and in vivo [26, 29]. This is caused by HPV-18 E6 having a perfect PDZ-binding motif, while that of HPV-16 is suboptimal.

IRF-3 HPV 16 E6 binds to interferon regulatory factor 3 (IRF-3), a component of the virus-activated transcription factor complex [30, 31]. IRF-3 is activated by the presence of double-stranded RNA or by virus infection to form a stable complex with other transcriptional regulators that bind to the regulatory elements of the IFN beta promoter. The interaction of E6 with IRF-3 can inhibit IRF-3's activity as a potent transcriptional activator. The binding of HPV16 E6 to IRF-3 does not result in its ubiquitination or degradation.

MMP 7 The multicopy maintenance protein 7, MMP 7, is a subunit of the replication licensing factor M [32]. MMP 7 has been identified as a substrate of the E3-ubiquitin ligase/E6-AP by its interaction with human papillomavirus-18E6 [32]. MMP 7 is ubiquitinated in vivo in both an E6-AP-dependent and -independent manner.

CBP/p300 It has been demonstrated that HPV-16 E6 interacts with the transcriptional coactivator CBP/p300 [33]. E6 targets CBP/p300 in an interaction involving the C-terminal zinc finger of E6 and CBP residues 1808 to 1826. Through the interaction with specific transcription factors, CBP/p300 regulates a variety of signal-modulated events, activates p53-dependent transcription and plays an important role in the inhibition of cell cycle progression and cellular differentiation [34–37]. The interaction between E6 and CBP/p300 is limited to E6 proteins of high-risk HPVs suggesting that CBP/p300 is an important target for the transformation process [33]. The ability to bind to CBP/p300 correlates with the down-regulation of p53 transcriptional activity in a manner similar to that of the Ad E1A protein. Recently, it has been demonstrated that E7 also interacts with CBP/p300 by direct binding between E7 and p300 and may contribute to the regulation of E2 transcriptional activity [38].

Telomerase Telomerase has been demonstrated to be a target of HPV E6 and this interaction stimulates telomerase activity [11, 39–41]. E6 mutants that increase the telomerase activity are able to induce cellular immortalization, whereas E6 mutants that do not induce telomerase activity were defective in immortalization. Notably, it has been shown that certain immortalization-competent E6 mutants that do not target p53 for degradation were capable of increasing telomerase activity. Constitutive activation of the telomerase is a key step in the cellular malignant transformation and the development of human cancer. Telomerase enzyme is responsible for replicating telomeres, the DNA elements located at the ends of chromosomes [42, 43]. Telomerase is composed of an RNA subunit, which acts as a template for replication, and the catalytic subunit hTERT, which functions as a reverse transcriptase. Telomerase activity has been found to be low in most of the normal tissues in vivo but is known to be elevated during tumorigenesis. However, there is no direct evidence that E6-induced telomerase activity is responsible for E6-induced immortalization. In human keratinocytes, E6 oncoprotein regulates hTERT transcription [41]. Induction of both hTERT expression and telomerase activity by HPV-16 E6 has been associated with acetylation of histone H3 at the hTERT promoter and is dependent on the E6-AP as well as E-box or X-box elements in the hTERT promoter [40].

E6TP1 E6TP1, E6-targeted protein 1, is a Rap GTPase-activating protein homologue [44]. E6TP1 acts as a negative regulator of the mitogenic signaling pathways mediated by Rap family proteins. Rap are Ras-like small G proteins involved in regulating cell proliferation and malignant cellular transformation [45]. HPV E6 binds directly to E6TP1 and this results in its degradation both in vitro and in vivo [45–48]. E6 targets E6TP1 for ubiquitination through E6AP and subsequent degradation by the proteasome pathway [49]. It has been demonstrated that E6TP1 degradation is strongly related to cellular immortalization suggesting a critical role of functional inactivation of E6TP1 in E6-induced cellular immortalization [45]. The interaction with E6TP1 has been observed with cancer-associated high-risk HPV E6 protein but not with benign lesion-associated low-risk HPV E6 protein.

PKN (a Rho-regulated serine threonine kinase) PKN is a fatty acid- and Rho small G protein-activated serine/threonine kinase with a catalytic domain homologous to that of the protein kinase C [50]. A small GTP-binding protein Rho binds to the amino-terminal region of PKN in a GTP-dependent manner, suggesting that PKN functions downstream of Rho in the Rho signal transduction pathway. Rho family GTPases have emerged as critical regulators of actin cytoskeleton remodeling that accompanies cellular activation by extracellular stimulation. HPV16 E6 interacts with PKN by direct binding both in vitro and in vivo. It has been shown that only E6 of 'high-risk' HPVs are able to bind PKN. E6 binding to PKN may be required, but is not sufficient for immortalizing normal mammary epithelial cells.

Gps2 (G-protein pathway suppressor 2) Gps2 interacts with E6 proteins from HPVs of high and low oncogenic risk [51]. It has been demonstrated that the E6 induce the degradation of Gps2 in vivo but not in vitro. Gps2 exhibits transcriptional activation activity and 'high-risk' E6 suppresses this activity [51]. Gps2 is ubiquitously expressed in different tissues and cell types, which is consistent with its ability to interact with both mucosal and cutaneous HPV E6 proteins. The regions that are necessary for efficient binding of Gps2 have significant homology with p300, another Gps2-binding protein and are highly conserved between mucosal and cutaneous HPV types.

c-myc The 'high-risk' human papillomavirus oncoprotein E6-16 stimulates conjugation and subsequent degradation of the myc proteins in vitro [52]. Expression of E6-16 in cells results in significant shortening of the t1/2 of the myc proteins with subsequent decrease in their cellular level. In the presence of E6-16, a third pair, E2-F1 and E6-AP mediate conjugation of myc by means of a mechanism that appears to be similar to that involved in the targeting of p53, formation of a myc–E6–E6-AP targeting complex. It is possible that in certain cells E6-mediated targeting of myc prevents myc-induced apoptosis and thus ensures maintenance of viral infection. However, it is not clear whether in the cell myc is degraded as an integral part of the complex or whether it has to dissociate first to be recognized by the ubiquitin conjugating machinery. The myc family of proteins is a group of structurally related transcriptional factors involved in a variety of cellular regulatory processes. The c-myc oncogene has been implicated in control of normal cell proliferation. It is rapidly induced in resting cells following mitogenic stimulation, suggesting that it plays an important role in the transition from quiescence to proliferation. In tumor cells, elevated or deregulated expression of c-myc has been observed frequently, suggesting its important role in malignant transformation.

E7 also interacts with c-myc and several binding sites have been located to the carboxyl-terminal and the amino acids regions [53]. The interaction of the high-risk type HPV E7 with c-myc can augment c-myc transactivation activity but this does not occur with low-risk type HPV E7. The myc-E6 interaction of the oncogenic human papillomaviruses (HPVs), in combination with the E7 protein, seems essential for the efficient immortalization of human

keratinocytes and modulation of cellular proliferation and differentiation [53–55].

Other proteins HPV E6 physically interacts with the protein-tyrosine kinase Tyk2 [56]. This interaction takes place preferably with HPV-18 E6 and to a lesser extent with HPV-11 E6. The E6/Tyk2 interaction requires the JH6-JH7 domains of Tyk2, which are important for Tyk2 binding to the cytoplasmic portion of IFN-alpha receptor 1 (IFNAR1). HPV E6 interaction with MUPP1 (multi-PDZ-domain protein 1) has also been reported by Lee et al. [57]. An E6-induced association with inactivation of both p16 (INK4a) and pRb with immortalization of human cells including fibroblasts and epithelial cells has been demonstrated [58]. The possible role of ras/raf signaling interaction with “high-risk” HPV 16/18 and HPV infection in cervical cancer development has also been proposed [59]. Both in vitro and in vivo experiments have demonstrated that H-ras cooperates with HPV 16 E6/E7 in transformation of cells [60]. However, these interactions are not mediated via direct binding of the proteins to the E6.

HPV E7 Interactions

HPV E7 interacts with (a) retinoblastoma protein Rb, (b) the Rb-related p107 and p130 proteins, (c) cyclins A and E, which regulates the cellular cycle, (d) CDK2, (e) histone H1 kinase, (f) the TATA box-binding protein, (g) the S4 adenosine triphosphatase, (h) c-jun, (i) hTid-1, (j) Mi2 pyruvate kinase, (k) p48, (l) the cycle-dependent kinase inhibitors $p21^{CIP1}$ and $p27^{KIP1}$ and (m) IRF-1.

Rb and Rb-related ‘pocket-proteins’ The protein products of the Rb family genes, pRb, p107 and pRb2/p130, are also known as ‘pocket-proteins’, because they share a ‘pocket’ domain responsible for most of the functional interactions characterizing the activity of this family of cellular factors [61]. The ‘pocket-proteins’ down-regulate cell cycle processes and are able to slow down or abrogate neoplastic growth. They negatively regulate, via direct association, the activity of several transcription factors, including members of the E2F family [62]. Several of the biological activities of HPV16 E7 are mediated by inactivation of the members of the pocket protein family, Rb, p107 and p130 including cellular malignant transformation [63].

The retinoblastoma susceptibility gene product, Rb, the most investigated tumor suppressor protein, is generally believed to be an important regulator in the control of cell proliferation or differentiation. Several transcription factors have been identified as targets of modulation by Rb, which may be directly involved in modification of chromatin structure and results in regulation of a set of genes required for controlling cell growth. Transcription regulation is a control mechanism that is critical for fundamental biological processes, such as cell growth and differentiation. ‘High-risk’ HPV E7 target retinoblastoma tumor suppressor protein Rb [64]. It has been demonstrated that Rb degradation is a direct activity of E7 [65]. An amino-terminal domain of E7 that does not directly contribute to Rb binding but is required for transformation is also necessary for E7-mediated Rb degradation.

‘High-risk’ HPV E7 proteins interact with Rb at a higher efficiency than do low-risk HPV E7 proteins [66]. Interaction of E7 with Rb causes the disruption of growth-suppressive Rb–E2F complexes promoting the G_1-S cell cycle transition. E7-mediated cellular transformation correlates with Rb binding, however there are mutations in E7 that impair cellular transformation and immortalization without affecting Rb binding [66–69]. These findings indicate that Rb/E7 binding alone is not sufficient because mutations in the carboxyl-terminal sequences of E7, outside the Rb-binding site, also severely impair the transformation function.

E7 interacts with the p107 protein both in vitro and in vivo and binds both p107 and E2F complexes [70, 71]. HPV-16 E7 can target the Rb family members p107 and p130 for destabilization [72–74]. p107 can be specifically targeted for degradation by E7 and contribute to cellular immortalization and differentiation [74]. Loss of E7 Rb or p107 binding results in the loss of transforming activity.

Both p107 and p130 function as efficient growth suppressors and target E2F transcription factor complexes, but they have not been conclusively connected with tumor suppressor activity. However, it has been shown that inactivation of Rb as well as p107 and p130 is necessary for SV40 T-antigen-mediated transformation [75]. Furthermore, $p16^{INK4a}$-induced cell cycle arrest requires p107 and/or p130, even in the presence of functional Rb [76]. p107-associated proteins include cyclin A, cyclin E, and cdk2 [77]. In addition, p107 associates with 62- to 65- and 50-kDa phosphoproteins that have many of the properties of the transcription factor E2F. It has been demonstrated that E7 associate with Rb and p107 with different efficiencies [63]. However, it has been found that this comparative analysis of the different E7 proteins demonstrates that the oncogenicity of a HPV type is not determined by the ability of E7 to associate with the pocket proteins.

E2F/cyclin A complex The transcription factor E2F has been shown to be involved in the expression of several cell cycle-regulated genes, and the activity of this factor is controlled by cellular proteins such as Rb and p107 [78]. E2F is a target of the DNA virus oncoproteins HPV E7. E7 dissociate an inactive complex between E2F and Rb, and

this dissociation of the E2F–Rb complex correlates with a stimulation of the E2F-dependent transcription. In the S phase of the cell cycle, E2F forms a complex with p107, cyclin A, and the cdk2 kinase (E2F–cyclin A complex). HPV-16 E7 protein associates very efficiently with the E2F–cyclin A complex [78]. This association is dependent on the sequences that are also necessary for the transforming activity of E7. Moreover, E7 of a low-risk HPVs is much less efficient in binding to the E2F–cyclin A complex compared with that of the high-risk type. The association of E7 with cyclin A appears to be direct, not involving Rb [79].

Cyclins E and A HPV 16 and 18 E7 associates with cyclin E which controls transit through the cell cycle [80–82]. E7 complexes with a single form of cyclin E, and the binding is mediated through p107. E7-dependent activation of cyclin E gene expression can be uncoupled from activation of the cyclin A gene, since the latter requires additional protein synthesis [83]. E7 activate both the cyclin E and cyclin A promoter in the absence of growth factors [63]. This activity also does not correlate with the E7-efficiency of binding the ‘pocket-proteins’.

CDK2 Cell cycle progression is regulated by the cyclin-dependent kinase (CDK) family [84]. Formation of CDK2 complexes with cyclins E and A. CDK activity is tightly controlled throughout the cell cycle by phosphorylation, by association with cyclins, and by association with inhibitory proteins. CDK2 substrates include Rb and related pocket proteins. E7 promotes HPV replication by directly altering CDK2 activity and substrate specificity [64]. HPV E7 CDK2/cyclin A or CDK2/cyclin E upregulates histone H1 kinase activity. The CDK2 stimulatory activity is equivalent in high-risk and low-risk HPVs E7. Direct binding of E7 to the CDK2 complex is an attractive mechanism by which E7 promotes S-phase entry for various reasons, including repeated demonstrations of association of E7 with the CDK2 complex, lack of conservation of known functional activities, such as Rb binding, among high- and low-risk forms of E7, weak association of E7 Rb-binding activity with its cell cycle-promoting activity, and lack of a requirement for Rb binding for wart formation by cottontail rabbit papillomavirus. Recently, it has been demonstrated that cyclin/CDK2 activity is critically involved in abnormal centrosome duplication induced by HPV-16 E7 oncoprotein expression [85].

Histone H1 kinase E7 binds and associates with a Histone H1 kinase at the G2/M phase of the cell cycle [71, 79]. The region of E7 identified previously as important for pRb binding was found to be involved in the association with the kinase. Association with the kinase activity correlate with the ability to bind Rb. However, since kinase-binding-deficient E7 mutants are also transformation defective, this may represent an independent function of E7 which plays a role in the G2/M phase of the cell cycle.

TATA box-binding protein (TBP) HPV-16 E7 can also complex with the core component of the TATA box-binding protein, TBP [86, 87]. This interaction is partly dependent upon phosphorylation of the E7 protein by cellular casein kinase II (CKII), since phosphorylation of E7 by CKII increases the affinity with which E7 binds TBP. This interaction, which takes place through residues in the carboxy terminal half of E7, inhibits TBP binding to DNA and may provide an explanation for the transcriptional inhibitory effects of E7 [87].

S4 adenosine triphosphatase The subunit 4 (S4) ATPase of the 26 S proteasome has been identified as an E7-binding protein [88]. E7 binds to S4 through the carboxyl-terminal zinc binding motif, and the binding is independent of E7 sequences involved in binding to Rb. E7 increases the ATPase activity of S4, while it has been shown in epithelial cells that E7 degrades Rb through the 26 S proteasome pathway. The 26 S proteasome is a large multimeric protein complex, which catalyzes ATP- and ubiquitin-dependent protein degradation [89]. The 26 S proteasome controls programmed degradation of many critical cell cycle regulatory proteins, like cyclins' maturation-promoting factor, and the inhibitor of cyclin-dependent kinase p27.

c-jun Rb binds to members of the AP-1 family of transcription factors, including c-Jun, and stimulates c-jun transcriptional activity [90, 91]. Rb and c-jun interaction has been involved in controlling cell growth and differentiation mediated by transcriptional regulation. It has been demonstrated that HPV 16 E7 protein binds to both c-Jun and Rb and inhibits the ability of Rb to activate c-Jun [90]. In addition to the transcription regulation, c-*jun* also has a functional role in cellular proliferation, differentiation, and transformation.

hTid-1 The hTid-1, a human homologue of the Drosophila tumor suppressor protein Tid56, form complexes with the HPV E7 oncoprotein [92]. The carboxyl terminal cysteine-rich metal binding domain of E7 is the major determinant for interaction with hTid-1. The carboxyl terminus of E7 is essential for the functional and structural integrity of E7 and has previously been shown to function as a multimerization domain. The hTid-1 protein is a member of the DnaJ-family of chaperones. Its mRNA is widely expressed in human tissues, including the HPV-18-positive cervical carcinoma cell line HeLa and human genital keratinocytes, the normal host cells of the HPVs. The ability of HPV E7

to interact with a cellular DnaJ protein suggests that these two viral oncoproteins may target common regulatory pathways through J-domains.

Mi2-pyruvate kinase HPV 16 binds to the glycolytic enzyme type M2 pyruvate kinase, M2-PK [93, 94]. M2-PK occurs in a tetrameric form with a high affinity to its substrate phosphoenolpyruvate and a dimeric form with a low affinity to phosphoenolpyruvate, and the transition between both conformations regulates the glycolytic flux in tumor cells. M2-PK is the key enzyme controlling the exit from the glycolytic pathway. Its activity determines the relative amount of glucose that is channelled into synthetic processes or used for glycolytic energy production and thus regulates the glycolytic flux in tumor cells. E7 shifts the equilibrium to the dimeric state despite a significant increase in the fructose 1,6-bisphosphate levels. The interaction of HPV-16 E7 with M2-PK may be linked to the transforming potential of the viral oncoprotein. The HPV-16 E7/M2-PK interaction induces E7 dimerization and restores nucleic acid synthesis as well as cell proliferation [94].

P48 HPV16 E7 correlates with the loss of formation of the interferon-stimulated gene factor 3 (ISGF3) transcription complex [95]. It has been demonstrated that E7 interacts with ISGF3 via p48. E7 targeting of p48 results in the loss of IFNalpha-mediated signal transduction and may provide a means by which HPV can avoid the innate immune system. A direct protein–protein interaction has been identified between E7 and p48 with the site of interaction within a domain that includes the binding site for the retinoblastoma protein Rb.

$p21^{WAF1/CIP1}$ and $p27^{KIP1}$ HPV E7 binds to and blocks the function of $p21^{CIP1}$ and $p27^{KIP1}$ in a p53-independent manner [96–98]. Both cell cycle-dependent kinase (CDK) inhibitors CKIs are implicated in various forms of cell cycle arrest preventing the phosphorylation of the pocket proteins through binding to G1 phase cyclin–CDK complexes. In vitro and in vivo studies indicate that the overriding of cell cycle-inhibitory signals is mediated in part by the E7 binding and inactivation of $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ [98]. $p21^{WAF1/CIP1}$ and $p27^{KIP1}$ are critical for cell cycle advancement and S-phase entry. Binding and inhibition of these proteins promote entry into the S phase by abolition of Rb effects on the transcription factor E2F. HPV-16 E7 modulates the cytoplasmic localization of $p27^{KIP1}$ and may in turn regulate tumor development and metastasis through the PI3K/Akt pathway [99]. E7 inhibits $p21^{CIP1}$ function in the context of Raf signaling by altering Raf-Akt antagonism and preventing the proper subcellular localization of $p21^{CIP1}$ [100]. $p21^{CIP1}$ is an important determinant in the cellular response to Ras/Raf activation and is independently isolated as an inducer of cellular senescence, a transcriptional target of the p53 tumor suppressor, and a direct inhibitor of CDK2. However, CKI neutralization by HPV E7 is more likely to be required for viral DNA replication rather than for malignant transformation of the host cell.

IRF-1 IRF-1 is involved in antiviral cellular responses. It has also been proposed as a potential mediator of IFN-gamma-induced attenuation of telomerase activity and human telomerase reverse transcriptase hTERT expression [31]. E7 interaction with IRF-1 have been reported [101]. E7 transgene expression inactivates the transactivation function of IRF-1 in vivo, which might be important for the elucidation of the E7-mediated immune evading mechanism that is frequently found in cervical cancer.

HPV Integration

In the normal viral life cycle, the genome replicates as episomal molecules. Eventhough the HPV genome is consistently retained in the episomal state in early dysplastic and low grade lesions, it is integrated to the host chromosome in many cases of high grade lesions and the majority of HPV associated cervical carcinomas [102–104]. Integration seems to be a direct consequence of chromosomal instability and an important molecular event in the progression from high-grade lesions to invasive cervical cancer [105]. A possible explanation for the progression of the disease towards malignancy might be the structural changes that take place after HPV genome integration, leading to deregulated expression of viral oncogenes. HPV E6 and E7, the major transforming genes, confer a much stronger transforming capacity in primary cells when they derive from integrated than episomal transcripts [106].

In addition, critical cellular genes could also be affected from viral integration [107]. Coding regions are rarely hit by HPV but gene expression and mRNA structure can be altered by insertion of the strong HPV promoter. Some of the genes disrupted by HPV integration are known to be involved in other cancers, such as myc, APM1, TP63, TNFAIP2, hTERT [108–110]. Up to know about 200 HPV DNA integration sites have been mapped in primary tumour samples and cell lines [111, 112]. From all the data analysed it has been concluded that HPV integration sites are randomly distributed over the whole genome with a clear preference for genomic fragile sites. Viral integration is a consequence of an overall destabilisation process of the chromosomal integrity in replicating epithelial cells that express the viral E6 and E7 genes. Therefore, the consequences of the structural alterations of the viral

genome and the impact of cellular sequences on its transcriptional regulation seem to be more important than any functional cellular alteration cause by HPV integration.

Conclusion

HPV E6 has been shown to enhance the degradation of p53, which plays an important role in cell cycle control and apoptosis in response to DNA damage, while HPV E7 binds and inactivates the function of the retinoblastoma tumor suppressor protein Rb. However during last decade it has been well demonstrated that both HPV E6 and E7 interact with host cells targeting a plethora of key host cellular proteins that are involved in apoptosis and malignant cellular transformation. The relative importance of the various E6 and E7 targets in cellular transformation and malignant progression remains to be elucidated.

References

1. zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2:342–350
2. zur Hausen H (1996) Papillomavirus infections—a major cause of human cancers. Biochim Biophys Acta 1288:F55–F78
3. Steben M, Duarte-Franco E (2007) Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol 107:S2–S5
4. Fehrmann F, Laimins LA (2003) Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene 22:5201–5207
5. Huibregtse JM, Scheffner M, Howley PM (1991) A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J 10:4129–4135
6. Huibregtse JM, Scheffner M, Howley PM (1993) Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. Mol Cell Biol 13:4918–4927
7. Kuballa P, Matentzoglu K, Scheffner M (2007) The role of the ubiquitin ligase E6-AP in human papillomavirus E6-mediated degradation of PDZ domain-containing proteins. J Biol Chem 282:65–71
8. Handa K, Yugawa T, Narisawa-Saito M et al (2007) E6AP-dependent degradation of DLG4/PSD95 by high-risk human papillomavirus type 18 E6 protein. J Virol 81:1379–1389
9. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM (1993) The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p 53. Cell 75:495–505
10. Dalal S, Gao Q, Androphy EJ, Band V (1996) Mutational analysis of human papillomavirus type 16 E6 demonstrates that p53 degradation is necessary for immortalization of mammary epithelial cells. J Virol 70:683–688
11. Kiyono T, Foster SA, Koop JI et al (1998) Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 396:84–88
12. Liu Y, Chen JJ, Gao Q et al (1999) Multiple functions of human papillomavirus type 16 E6 contribute to the immortalization of mammary epithelial cells. J Virol 73:7297–7307
13. Chen JJ, Reid CE, Band V, Androphy EJ (1995) Interaction of papillomavirus E6 oncoproteins with a putative calcium-binding protein. Science 269:529–531
14. Sherman L, Itzhaki H, Jackman A et al (2002) Inhibition of serum- and calcium-induced terminal differentiation of human keratinocytes by HPV 16 E6: study of the association with p53 degradation, inhibition of p53 transactivation, and binding to E6BP. Virology 292:309–320
15. Thomas M, Banks L (1998) Inhibition of Bak-induced apoptosis by HPV-18 E6. Oncogene 17:2943–2954
16. Thomas M, Banks L (1999) Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. J Gen Virol 80:1513–1517
17. Nagata S (1997) Apoptosis by death factor. Cell 88:355–365
18. Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26:1324–1337
19. Turner CE (2000) Paxillin interactions. J Cell Sci 113:4139–4140
20. Tong X, Howley PM (1997) The bovine papillomavirus E6 oncoprotein interacts with paxillin and disrupts the actin cytoskeleton. Proc Natl Acad Sci USA 94:4412–4417
21. Vande Pol SB, Brown MC, Turner CE (1998) Association of Bovine Papillomavirus Type 1 E6 oncoprotein with the focal adhesion protein paxillin through a conserved protein interaction motif. Oncogene 16:43–52
22. Kiyono T, Hiraiwa A, Fujita M et al (1997) Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the drosophila discs large tumor suppressor protein. Proc Natl Acad Sci USA 94:11612–11616
23. Lee SS, Weiss RS, Javier RT (1997) Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. Proc Natl Acad Sci USA 94:6670–6675
24. Nakagawa S, Huibregtse JM (2000) Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. Mol Cell Biol 20:8244–8253
25. Liu Y, Herny GD, Hegde RS, Bleja JD (2007) Solution structure of the hDlg/SAP97PDZ2 domain and its mechanism of interaction with HPV-18 papillomavirus E6 protein. Biochemistry 46:10864–10874
26. Glaunsinger BA, Lee SS, Thomas M et al (2000) Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. Oncogene 19:5270–5280
27. Thomas M, Laura R, Hepner K et al (2002) Oncogenic human papillomavirus E6 proteins target the MAGI-2 and MAGI-3 proteins for degradation. Oncogene 21:5088–5096
28. Zhang Y, Dasgupta J, Ma RZ et al (2007) Structures of a human papillomavirus (HPV) E6 polypeptide bound to MAGUK proteins: mechanisms of targeting tumor suppressors by a high-risk HPV oncoprotein. J Virol 81:3618–3626
29. Thomas M, Glaunsinger B, Pim D et al (2001) HPV E6 and MAGUK protein interactions: determination of the molecular basis for specific protein recognition and degradation. Oncogene 20:5431–5439
30. Ronco L, Karpova A, Vidal M, Howley PM (1998) Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev 12:2061–2072
31. Lee SH, Kim JW, Lee HW et al (2003) Interferon regulatory factor-1 (IRF-1) is a mediator for interferon-gamma induced attenuation of telomerase activity and human telomerase reverse transcriptase (hTERT) expression. Oncogene 22:381–391
32. Kuhne C, Banks L (1998) E3-ubiquitin ligase/E6-AP links multicopy maintenance protein 7 to the ubiquitination pathway by a novel motif, the L2G box. J Biol Chem 273:34302–34309

33. Zimmermann H, Degenkolbe R, Bernard HU, O'Connor MJ (1999) The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. J Virol 73:6209–6219
34. Janknecht R, Hunter T (1996) Transcription. A growing coactivator network. Nature 383:22–23
35. Giles RH, Peters DJ, Breuning MH (1998) Conjunction dysfunction: CBP/p300 in human disease. Trends Genet 14:178–183
36. Gu W, Shi XL, Roeder RG (1997) Synergistic activation of transcription by CBP and p53. Nature 387:819–823
37. Avantaggiati ML, Ogryzko V, Gardner K et al (1997) Recruitment of p300/CBP in p53-dependent signal pathways. Cell 89:1175–1184
38. Bernat A, Avvakumov N, Mymryk JS, Banks L (2003) Interaction between the HPV E7 oncoprotein and the transcriptional coactivator p300. Oncogene 22:7871–7881
39. Klingelhutz AJ, Foster SA, McDougall JK (1996) Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature 380:79–82
40. James MA, Lee JH, Klingelhutz AZ (2006) HPV 16-E6 associated hTERT promoter acetylation is E6AP dependent, increased in later passage cells and enhanced by loss of p300. Int J Cancer 119:1878–1885
41. Zhang H, Jin Y, Chen X et al (2007) Papillomavirus type 16 E6/E7 and human telomerase reverse transcriptase in esophageal cell immortalization and early transformation. Cancer Lett 245:184–194
42. Greider CW (1998) Telomerase activity, cell proliferation, and cancer. Proc Natl Acad Sci USA 5:90–92
43. Holt SE, Shay JW, Wright WE (1996) Refining the telomere–telomerase hypothesis of aging and cancer. Nat Biotechnol 4:836–839
44. Altschuler DL, Ribeiro-Neto F (1998) Mitogenic and oncogenic properties of the small G protein Rap1b. Proc Natl Acad Sci USA 95:7475–7479
45. Gao Q, Singh L, Kumar A et al (2001) Human papillomavirus type 16 E6-induced degradation of E6TP1 correlates with its ability to immortalize human mammary epithelial cells. Virology 75:4459–4466
46. Gao Q, Srinivasan S, Boyer S et al (1999) The E6 oncoproteins of high-risk papillomaviruses bind to a novel putative GAP protein, E6TP1, and target it for degradation. Mol Cell Biol 19:733–744
47. Singh L, Gao Q, Kumar A et al (2003) The high-risk human papillomavirus type 16 E6 counters the GAP function of E6TP1 toward small Rap G proteins. J Virol 77:1614–1620
48. Lee C, Wooldridge TR, Laimins LA (2007) Analysis of the roles of E6 binding to E6TP1 and nuclear localization in the human papillomavirus type 31 life cycle. Virology 358:201–210
49. Gao Q, Kumar A, Singh L et al (2002) Human papillomavirus E6-induced degradation of E6TP1 is mediated by E6AP ubiquitin ligase. Cancer Res 62:3315–3321
50. Gao Q, Kumar A, Srinivasan S et al (2000) PKN binds and phosphorylates human papillomavirus E6 oncoprotein. J Biol Chem 275:14824–14830
51. Degenhardt YY, Silverstein J (2001) Gps2, a protein partner for human papillomavirus E6 proteins. J Virol 75:151–160
52. Gross-Mesilaty S, Reinstein E, Bercovich B et al (1998) Basal and human papillomavirus E6 oncoprotein-induced degradation of Myc proteins by the ubiquitin pathway. Proc Natl Acad Sci USA 95:8058–8063
53. Wang YW, Chang HS, Lin CH, Yu WC (2007) HPV-18 E7 conjugates to c-Myc and mediates its transcriptional activity. Int J Biochem Cell Biol 39:401–412
54. Liu X, Disbrow GL, Yuan H et al (2007) Myc and human papillomavirus type 16 E7 genes cooperate to immortalize human keratinocytes. J Virol 81:12689–12695
55. McMurray HR, McCance DJ (2003) Human papiloomavirus type 16 E6 activates TERT gene transcription through induction of c-Myc and release of USF-mediated repression. J Virol 77:9852–9861
56. Li S, Labrecque S, Gauzzi MC et al (1999) The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-a. Oncogene 18:5727–5737
57. Lee SS, Glaunsinger B, Mantovani F et al (2000) Multi-PDZ domain protein MUPP1 is a cellular target for both adenovirus E4-ORF1 and high-risk papillomavirus type 18 E6 oncoproteins. J Virol 74:9680–9693
58. Tsutsui T, Kumakura S, Yamamoto A et al (2002) Association of p16(INK4a) and pRb inactivation with immortalization of human cells. Carcinogenesis 23:2111–2117
59. Mammas IN, Zafiropoulos A, Sifakis S et al (2005) Human papillomavirus (HPV) typing in relation to ras oncogene mRNA expression in HPV-associated human squamous cervical neoplasia. Int J Biol Markers 20:257–263
60. Schreiber K, Cannon RE, Karrison RE et al (2004) Strong synergy between mutant ras and HPV16 E6/E7 in the development of primary tumors. Oncogene 23:3972–3979
61. Felsani A, Mileo AM, Paggi MG (2006) Retinoblastoma family proteins as key targets of the small DNA virus oncoproteins. Oncogene 25:5277–5285
62. Lavia P, Jansen-Durr P (1999) E2F target genes and cell-cycle checkpoint control. Bioessays 21:221–230
63. Caldeira S, de Villiers EM, Tommasino M (2000) Human papillomavirus E7 proteins stimulate proliferation independently of their ability to associate with retinoblastoma protein. Oncogene 19:821–826
64. Dyson N, Howley PM, Münger K, Harlow E (1989) The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243:934–937
65. Gonzalez SL, Stremlau M, He X et al (2001) Degradation of the retinoblastoma tumor suppressor by the human papillomavirus type 16 E7 oncoprotein is important for functional inactivation and is separable from proteasomal degradation of E7. J Virol 75:7583–7591
66. Heck DV, Yee CL, Howley PM, Münger K (1992) Efficiency of binding to the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. Proc Natl Acad Sci USA 89:4442–4446
67. Chellappan S, Kraus VB, Kroger B et al (1992) Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between the transcription factor E2F and the retinoblastoma gene product. Proc Natl Acad Sci USA 89:4549–4553
68. Edmonds C, Vousden KH (1989) A point mutational analysis of human papillomavirus type 16 E7 protein. J Virol 63:2650–2656
69. Strati K, Lambert PF (2007) Role of Rb-dependent and Rb-independent functions of papillomavirus E7 oncogene in head and neck cancer. Cancer Res 67:11585–11593
70. Zerfass K, Levy LM, Cremonesi C et al (1995) Cell cycle-dependent disruption of E2F-p107 complexes by human papillomavirus type 16 E7. J Gen Virol 76:1815–1820
71. Davies R, Hicks R, Crook T et al (1993) Human papillomavirus type 16 E7 associates with a histone H1 kinase and with p107 through sequences necessary for transformation. J Virol 67:2521–2528
72. Roman A (2006) The human papillomavirus E7 protein shines a spotlight on the pRB family member, p130. Cell Cycle 5:567–568

73. Zhang B, Chen W, Roman A (2006) The E7 proteins of low- and high-risk human papillomaviruses share the ability to target the pRB family member p130 for degradation. Proc Natl Acad Sci USA 103:437–442
74. Grana X, Garriga J, Mayol X (1998) Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. Oncogene 17:3365–3383
75. Stubdal H, Zalvide J, Campbell KS et al (1997) Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. Mol Cell Biol 17:4979–4990
76. Bruce JL, Hurford RK, Classon J et al (2000) Requirements for cell cycle arrest by $p16^{ink4a}$. Mol Cell 6:737–742
77. Dyson N, Dembski M, Fattaey A et al (1993) Analysis of p107-associated proteins: p107 associates with a form of E2F that differs from pRB-associated E2F-1. J Virol 67:7641–7647
78. Arroyo M, Bagchi S, Raychaudhuri P (1993) Association of the human papillomavirus type 16 E7 protein with the S-phase-specific E2F–cyclin A complex. Mol Cell Biol 13:6537–6546
79. Tommasino M, Adamczewski JP, Carlotti F et al (1993) HPV16 E7 protein associates with the protein kinase p33CDK2 and cyclin A. Oncogene 8:195–202
80. McIntyre MC, Ruesch MN, Laimins LA (1996) Human papillomavirus E7 oncoproteins bind a single form of cyclin E in a complex with cdk2 and p107. Virology 215:73–82
81. Vogt B, Zerfass-Thome K, Schulze A et al (1999) Regulation of cyclin E gene expression by the human papillomavirus type 16 E7 oncoprotein. J Gen Virol 80:2103–2113
82. Balsitis S, Dick F, Dyson N, Lambert PF (2006) Critical roles for non-pRB targets of human papillomavirus type 16 E7 in cervical carcinogenesis. Cancer Res 66:9393–9400
83. Zerfass K, Schulze A, Spitkovsky D et al (1995) Sequential activation of cyclin E and cyclin A gene expression by human papillomavirus type 16 E7 through sequences necessary for transformation. J Virol 69:6389–6399
84. He W, Staples D, Smith C, Fisher C (2003) Direct activation of cyclin-dependent kinase 2 by human papillomavirus E7. J Virol 77:10566–10574
85. Duensing S, Duensing A, Lee DC et al (2004) Cyclin-dependent kinase inhibitor indirubin-3¢-oxime selectively inhibits human papillomavirus type 16 E7-induced numerical centrosome anomalies. Oncogene 23:8206–8215
86. Massimi P, Pim D, Storey A, Banks L (1996) HPV-16 E7 and adenovirus E1a complex formation with TATA box binding protein is enhanced by casein kinase II phosphorylation. Oncogene 12:2325–2330
87. Maldonado E, Cabrejos ME, Banks L, Allende JE (2002) Human papillomavirus-16 E7 protein inhibits the DNA interaction of the TATA binding transcription factor. J Cell Biochem 85:663–669
88. Berezutskaya E, Bagchi S (1997) The human papillomavirus E7 oncoprotein functionally interacts with the S4 subunit of the 26 S proteasome. J Biol Chem 272:30135–30140
89. Coux O, Tanaka K, Goldberg AL (1996) Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 65:801–847
90. Nead MA, Baglia LA, Antimore MJ et al (1998) Rb binds c-Jun and activates transcription. EMBO J 17:2342–2352
91. Nishitani J, Nishinaka T, Cheng CH et al (1999) Recruitment of the retinoblastoma protein to c-Jun enhances transcription activity mediated through the AP-1 binding site. J Biol Chem 274:5454–5461
92. Schilling B, De-Medine T, Syken J et al (1998) A novel human DnaJ protein, hTid-1, a homolog of the Drosophila tumor suppressor protein Tid56, can interact with the human papillomavirus type 16 E7 oncoprotein. Virology 247:74–85
93. Zwerschke W, Mazurek S, Massimi P et al (1999) Modulation of type M2 pyruvate kinase activity by the human papillomavirus type 16 E7 oncoprotein. Proc Natl Acad Sci USA 96:1291–1296
94. Mazurek S, Zwerschke W, Jansen-Durr P, Eigenbrodt E (2001) Metabolic cooperation between different oncogenes during cell transformation: interaction between activated ras and HPV-16 E7. Oncogene 20:6891–6898
95. Barnard P, McMillan NA (1999) The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology 259:305–313
96. Jones DL, Alani RM, Munger K (1997) The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. Genes Dev 11:2101–2111
97. Funk JO, Waga S, Harry J et al (1997) Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. Genes Dev 11:2090–2100
98. Zehbe I, Ratsch A, Alumni-Fabbroni M et al (1999) Overriding of cyclin-dependent kinase inhibitors by high and low risk human papillomavirus types: evidence for an in vivo role in cervical lesions. Oncogene 18:2201–2211
99. Charette ST, McCance DJ (2007) The E7 protein from human papillomavirus type 16 enhances keratinocyte migration in an Akt-dependent manner. Oncogene 26:7386–7390
100. Westbrook TF, Nguyen DX, Trash BR, McCance DJ (2002) E7 abolishes raf-induced arrest via mislocalization of p21(Cip1). Mol Cell Biol 22:7041–7052
101. Um SJ, Rhyu JW, Kim EJ et al (2002) Abrogation of IRF-1 response by high-risk HPV E7 protein in vivo. Cancer Lett 179:205–212
102. Alazawi W, Pett M, Arch B et al (2002) Changes in cervical keratinocyte gene expression associated with integration of human papillomavirus 16. Cancer Res 62:6959–6965
103. Klaes R, Woerner SM, Ridder R et al (1999) Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. Cancer Res 59:6132–6136
104. Giannoudis A, van Duin M, Snijder PJF, Herrington CS (2001) Variation in the E2-binding domain of HPV 16 is associated with high-grade squamous intraepithelial lesions of the cervix. Br J Cancer 84:1058–1063
105. Pett M, Coleman N (2007) Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? J Pathol 212:356–367
106. Jeon S, Lambert PF (1995) Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: implications for cervical carcinogenesis. Proc Natl Acad Sci USA 92:1654–1658
107. Durst M, Croce CM, Gissmann L et al (1987) Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. Proc Natl Acad Sci USA 84:1070–1074
108. Ferber MJ, Thorland EC, Brink AA et al (2003) Preferential integration of human papillomavirus type 18 near the c-myc locus in cervical carcinoma. Oncogene 22:7233–7242
109. Peter M, Rosty C, Couturier J et al (2006) MYC activation associated with the integration of HPV DNA at the MYC locus in genital tumors. Oncogene 25:5985–5993
110. Ferber MJ, Montoya DP, Yu C et al (2003) Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. Oncogene 22:3813–3820
111. Ziegert C, Wentzensen N, Vinokurova S et al (2003) A comprehensive analysis of HPV integration loci in anogenital lesions combining transcript and genome-based amplification techniques. Oncogene 22:3977–3398
112. Wentzensen N, Vinokurova S, von Knebel DM (2004) Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. Cancer Res 64:3878–3884