AIMS Molecular Science, 8(3): 184–192.
DOI: 10.3934/molsci.2021014
Received: 25 March 2021
Accepted: 22 June 2021
Published: 23 July 2021

http://www.aimspress.com/journal/Molecular

Review

Viral load and interaction of HPV oncoprotein E6 and E7 with host cellular markers in the progression of cervical cancer

Bilal Ahmad Mir[1], P. F. Rahaman[2] and Arif Ahmad[1,*]

[1] Department of Zoology, School of Sciences, Maulana Azad National Urdu University, Hyderabad, India
[2] HRDC, Maulana Azad National Urdu University, Hyderabad, India

*** Correspondence:** Email, arifamu@gmail.com.

Abstract: Cervical cancer is the sequel of a multi-factorial, long-term unresolved disease that includes genetic, epigenetic, and viral components responsible for its development and progression. It is the second most common cancer of females in India. Human papillomavirus (HPV) is considered the primary causative agent of pre-neoplastic and cancerous lesions and 90% of all cervical carcinomas are linked to high-risk HPV type 16 and type 18. Although most HR-HPV infections are asymptomatic, transient, and self-limiting, the persistent infection with a high risk (HR-HPV) may cause precancerous lesions that can progress to cervical cancer. HPV type 16 is the most common HPV in India associated with more than 75% of cervical cancer, followed by HPV type 18 and other high-risk types. Infection with HPV alone is not sufficient for the development of cervical cancer but there is the involvement of some host genetic factors also that are responsible for the development and progression of cervical cancer. This article briefly reviews molecular pathogenesis, viral load, and the interaction of HPV oncoprotein E6 and E7 with host cellular markers in the progression of cervical cancer.

Keywords: cervical cancer; human papillomavirus; HPV-E6; E7 protein; p53; pRb

1. Introduction

Globally, cervical cancer is the 4th most common cancer with an estimated 570,000 cervical cancer cases in 2018 (WHO's 2018 report) contributing considerably to the cancer burden. Over half

a million new cervical cancer cases are diagnosed worldwide annually and their incidence in developing countries is significantly higher than in developed countries. In India, cervical cancer is the most prevalent cancer among women. Every year 122,844 women are diagnosed with cervical cancer every year and 76,478 die from the cervical cancer (HPV related cancer fact sheets 2017). Over half a million new cervical cancer cases are diagnosed worldwide annually and their incidence in developing countries is significantly higher than in developing countries [1].

The sexually transmitted Human Papillomavirus, a member of papovoviridae family is an oncogenic DNA virus and is the causative agent of cervical cancers and precancerous lesions. Infection with HPV has been shown to play a vital role in the etiology of cervical cancer. Several forms of HPV have been reported, out of which 24 most common types are classified into three groups based on severity and high-risk type. HPV 16 is The most frequently detected human papillomavirus (HPV) followed by HPV18 [2,3]. HPV18 type is closely linked to cervical adenocarcinoma. While studying the association between HPV16, HPV18, and other HR-HPV viral load and histological classification, HPV 16 has been confirmed the most prevalent in the development and progression of cervical cancer lesions by various authors [4]. More than 70% of cervical cancer cases have been reported to have HPV types 16 and 18. The prevalence of HPV-type16 in India is also recorded as very high compared to other types [5]. HPV-type18 occurrence ranges from 5–25% followed by HPV-33, 35, 45, 52, 58 and 59 respectively [6,7].

HPVs are non enveloped, double-stranded DNA viruses. Their genome is approximately 8 kb in size and divided mainly into eight open reading frames, six early (E1, E2, E4, E5, E6, and E7) and two late (L1 and L2) genes, coding for “early” (E) or “late” (L) proteins. The E6 and E7 have to transform properties to transform normal cells into cancerous cells. In the development of cervical cancer, the human papillomavirus binds to and enters the host cell by cellular receptor binding followed by integration of viral DNA into host cell DNA. Upon entry into the host cell, the E1 and E2 genes express and encode proteins necessary for viral DNA replication. The E1 and E2 protein complex binds to the origin of replication and recruits host DNA polymerase for replication. The E2 protein negatively regulates transcription of the viral E6 and E7 oncogenes through its specific binding to DNA recognition sites located within the promoter sequences. The viral E6 and E7 oncoproteins act mainly through protein-protein interactions to alter major pathways of the cell cycle and are therefore necessary for malignant conversion [8]. E6 targets host cellular p53 tumor suppressor protein and E7 protein binds to and inactivates retinoblastoma (Rb) pocket proteins to overcome the p53/pRb cell cycle checkpoints and stimulate cellular immortalization and growth [9].

Although the majority of the HPV infections are transient, asymptomatic, and self-limiting, persistent infections with a high-risk HPV may cause precancerous lesions that can progress to cancer [10]. The relationship between HPV exposure and disease progression varies with the HPV type, viral integration, and viral load [11]. Infection with HR-HPV is linked to cervical dysplasia or squamous intraepithelial lesion (SIL), and the cancer of the cervix is supposed to occur from these lesions after long-term persistent infection with high-risk HPV [12]. CIN I (mild dysplasia) and CIN II (moderate dysplasia) lesions display comparatively low levels of expression of E6 and E7 genes and the viral genome replicate episomally, while in the case of CIN III and invasive cancerous lesion, the viral genome integrates into the host genome and E6 and E7 genes show high expression and usually leads to neoplastic transformation [13].

2. HPV viral load

An important factor associated with HPV infection is viral load. The productivity of viral genome copies in the life cycle of HPV reflects the viral load. Its value can be used to determine the progression of the disease. The HPV viral Load test identifies mRNA of E6 and E7 transforming genes Instead of identification of different HPV serotypes. The assay of viral load thus determines if the HPV genes that cause malignant changes are present and active. Increased persistence of HPV infection and increased risk of developing CIN II/CIN III or cancer is associated with high viral load. The viral load of human papillomavirus type 16 has been shown as a determinant for the development of cervical carcinoma in situ. It has been shown one of the risk factors in invasive for high-grade CIN and cervical cancer [14]. The Co-relation between HPV DNA load and the risk of persistent infection and CIN II and CIN III were to be found positive in various reports [15]. The contribution of HPV 16/18 viral load and the presence of infection with multiple HPV types might be used as a marker for persistent infection [16]. An increased cumulative prevalence of cancerous lesions of the cervix has also been found associated with increased viral load in persistently infected women [17]. The more the HPV viral load, the worse the survival of the patient would be as revealed by some reports [18] while studying the association of viral load with HPV16 positive cervical cancer pathogenesis. Their result showed a significantly higher median viral load among cases compared to control. HPV18 viral load is found to be low in precancerous cases, but going up in cancer. The in vivo semi-quantitative viral load measurement may be a useful method in clinical practice while studying the prognostic value of HPV viral load in correlation with various therapeutic modalities in cervical cancer patients [19].

HPV16 Viral load has been found higher in women with CIN III compared to early stages of CIN. Increasing viral load possibly enhances the frequency of integration of the virus into the host genome which ultimately increases the disease severity [20]. among women infected with HPV type16, those with higher viral load are more likely to progress to high-grade SIL compared to those with low viral load [21]. Although the initial enthusiasm of clinical value of HPV viral load testing is not evident, reliable type-specific viral load measurements in the samples where the integration status is established may provide useful insight into the natural history and relationship of HPV infections.

HPV integration is categorized into two types. A single genome is integrated into the host cellular DNA in type I, and in type II, several head-to-tail genomes repeats are integrated into the host cellular DNA. Integration is observed at only one chromosomal site in the tumor cells in nearly all cervical cancers studied, consistent with the idea that cervical cancer is a clonal disease [22]. Integration occurs at some specific integration target sequences (regions of microhomology) in the human and viral genome. A FRAB3 analysis was carried out by [23] to demonstrate specifically the coincidence between viral integration sites and fragile sites. They proposed that over-representation of fragile sites is attributed to their increased susceptibility to integration-induced chromosomal alterations, whereas others are of the view that this is merely a reflection of their greater accessibility [24]. The association between HPV16 viral load and cervical cancer progression now becomes apparent. Cross-section analysis indicates that the viral load of HPV16 increases with increasing disease severity [15].

3. Host proteins p53 associated with HPV E6 oncoprotein

E6 oncoprotein, with two zinc fingers, has about 150 amino acids. It consists of two pairs of CXXC motifs and interferes with the cell cycle pathways [25]. Both E6 and E7 viral oncoproteins are crucial because they interfere with the cell cycle regulation and apoptosis to instigate and maintain the cellular transformation. The crucial part of the cellular transformation is thought to be Genomic instability. When introduced into the cell, E6 and E7 together have been shown to induce polyploidy. This results in the deregulation of Plk1 due to the degradation of p53 through E6 and inactivation of pRb family members by E7 viral oncoprotein [26]. Integration of HR HPV DNA which occurs downstream of the early genes E6 and E7, mostly in the E1 or E2 region into the host genome usually results in the specific disruption of the E2 viral gene. By binding to its specific DNA recognition sites found within the promoter sequence, the E2 protein negatively controls the transcription of E6 and E7 viral oncogenes. By hijacking a cellular E3 ubiquitin ligase UBE3A/E6AP (E6 associated protein), binding through E6's LXXLL motif, E6 protein targets p53 protein for proteasomal degradation [10]. Although E6 induced loss of p53 is an essential element of E6 induced cellular transformation, some additional cellular targets of E6 have been identified by recent studies such as cell immortalization of epithelial cells by maintaining telomere Length [27] anti-apoptotic effect [28], chromosomal destabilization, Activation of telomerase [29] and activation of Bak protein. An interesting study by [30] showed the expression of proteins in response to transfection by E6. According to their study, E6 oncoprotein targets the p53 for degradation by proteasome which consequently abrogates the p53 transcriptional pathway. Apart from targeting cellular p53, E6 oncoprotein is also known to target other pro-apoptotic factors like BAK and BAX.

High-risk HPV E6 proteins show interaction with certain cellular proteins containing a PSD95/Dlg/ZO-1 (PDZ) domain along with several PDZ domain-containing proteins that are involved in cell signaling such as PALS-1 associated tight junction protein (PATJ), GAIP-interacting protein c-terminus (GIPC), and protein tyrosine phosphatase N1 (PTPN1) through complex formation with E6AP and targets them for degradation by the proteasome [31]. Unlike low-risk types, High-risk E6 mRNAs undergo alternative splicing due to the presence of essential dinucleotides (GT) of splice consensus sequences. Such essential dinucleotides are present in high-risk HPV types only; the low-risk types do not show alternative splicing because they lack such dinucleotides [32]. The alternatively spliced E6* mRNA is found at much higher levels compared with the full-length E6 transcripts [33]. A study by [34] also showed that E6* can bind in vitro to full-length E6 protein as well as E6AP protein, which inhibits E6 from binding to E6AP and degrading p53.

In the cell cycle progression, many proteins are involved including E2F5 and CDK5, which regulate the cell cycle progression. Transformed cells expressing E6 lose the G1 checkpoint at a very early point due to degradation of p53, The increased chromosomal insatiability in E6 expressing cells over time probably caused observed attenuation of the G2 checkpoint function [35]. In a study, E6 and Myc interaction have been shown to activate telomerase reverse transcriptase promoter a repressor complex of TERT promoter, containing USF1 and USF2, is replaced by Myc in presence of E6 which correspond to higher telomerase activity [36]. Downregulation of Notch1, a p53 target gene is involved in tumor suppression and HPV E6 also down-regulates the expression of Notch1 in cervical cancer cells and contributes to tumorigenesis [37]. Studies on the Transgenic mouse model in cervical cancer have shown that E7 increases the proliferation and centrosome copy number and induce the progression of multifocal micro-invasive cervical cancers; while E6 viral oncoprotein has

been shown to elevate centrosome copy number and eliminate detectable p53 protein, but did not produce neoplasia or cancer [38]. Importantly, the combination of both E6 and E7 oncoproteins resulted in an increased centrosome number and invasive cancers.

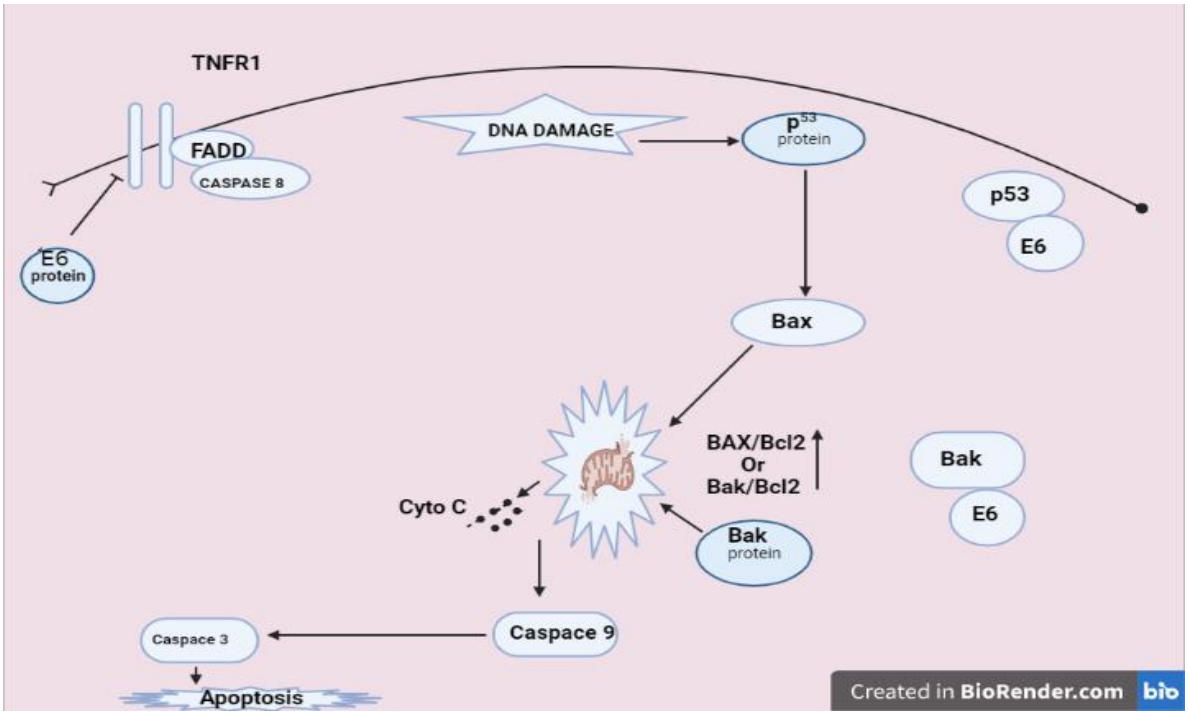

Figure 1. Role of HPV oncoprotein E6 in cell cycle deregulation. E6 and E7 are the major viral oncoproteins. E6 protein modulates cellular proteins that regulate the cell cycle. It binds to p53 tumor suppressor protein and targets it for ubiquitin-mediated degradation leading to downstream regulation of cdk inhibitors and thus activates cell cycle progression. E6 also targets proapoptotic Bak protein thus preventing an abnormal cell to undergo apoptosis.

4. Interaction of host pRb with HPV E7 oncoprotein

E7 Oncoprotein has 98 amino acids that has Zinc- binding domain on C-terminus. The structural integrity of this zinc-binding domain is very critical for E7 activity in promoting cellular proliferation. It is a small nuclear phosphoprotein with three conserved regions denoted as CR1, CR2, and CR3 in the same way as Adenovirus E1A [39]. E7 like that of E6 oncoprotein act mainly through protein-protein interaction to alter the major pathways of cell proliferation. E7 oncoprotein binds to and inactivates the retinoblastoma (Rb) pocket protein and its family members, p107 and p130, through a binding motif LXCXE (where X represents any amino acid) conserved in its CR2 region thus resulting in the release of active E2F and activation of E2F target genes to promote cell proliferation [40]. While working on the effect of E7 on HaCaT keratinocytes, reported downregulation of cytoplasmic actin and the leukocytes elastase inhibitor (LEI) by E7 oncoprotein suggesting that E7 participate in elastase modulation activity. E7 also interferes with the activity of most endogenous molecules including CDK inhibitors $P21^{cipi}$ and $P27^{kipi}$ and other transcription factors from the AP-1 family [41] suggesting its active role in HPV-mediated malignant progression.

When pRb is inactivated by HPV E7 oncoprotein, one cyclin-dependent kinase inhibitor, $p16^{INK4a}$ which inhibits the phosphorylation of pRb family members, is overexpressed [42]. Thus $p16^{INK4a}$ overexpression may be a potential biomarker for determining HPV pathogenic activity in cervical cancer. In addition to the inactivation of pRb family members, E7 has been reported to interfere with a variety of cellular transcription factors hence perform numerous functions such as activation of cyclin A and E [43] induction of apoptosis [44] cell immortalization [45], and degradation of Blk tyrosine kinase [46]. In transformed keratinocytes, HPV-16 E7 has been shown to down-regulate the expression of the cell adhesion molecule E-cadherin [47].

5. Conclusions

The viral oncoproteins E6 and E7 of the Human papillomavirus are essential components for cellular immortalization, cellular transformation, and carcinogenesis induced by the HPV. The viral load of HPV-16 is associated with an increased risk for high-grade SIL or carcinoma and has been suggested as a type-dependent risk marker. Compared with cytology-based approaches, HPV-based tests provide a more sensitive way to classify women with high-grade cervical disease. The ability of high-risk papillomavirus to transform a cell is due to a change in the host p53 and Rb pathways caused by E6 and E7 viral oncoproteins. This suggested that interactions of viral proteins with host cellular proteins are involved in the activation or repression of cell cycle progression in cervical carcinogenesis. The time lag of HPV infection and cancer diagnosis, however, suggests that multiple steps, as well as various factors, may be crucial for the development of cervical cancer. Evaluating the risk of cervical cancer progression by establishing the threshold values of E6/E7 and pRb/p53 protein concentrations along with the viral load may be a better parameter to predict the early cancer stage.

Conflict of interest

Authors declare no conflict of interest in this manuscript.

References

1. Singh G (2012) Global inequalities in cervical cancer incidence and mortality are linked to deprivation, low socioeconomic status, and human development. *Int J MCH AIDS* 1: 17–30.
2. Mohammad A, Moheman A, El-desoky GE (2012) Amino acid and vitamin determinations by TLC/HPTLC : review of the current state. *Cent Eur J Chem* 10: 731–750.
3. Kim HJ, Kim HJ (2017) Current status and future prospects for human papillomavirus vaccines. *Arch Pharm Res* 40: 1050–1063.
4. Muñoz N, Bosch FX, de Sanjosé S, et al. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 348: 518–527.
5. Das BC, Hussain S, Nasare V, et al. (2008) Prospects and prejudices of human papillomavirus vaccines in India. *Vaccine* 26: 2669–2679.
6. Franceschi S, Rajkumar T, Vaccarella S, et al. (2003) Human papillomavirus and risk factors for cervical cancer in Chennai, India: A case-control study. *Int J Cancer* 107: 127–133.

7. Sowjanaya AP, Jain M, Poli UR, et al. (2005) Prevalence and distribution of high-risk human papillomavirus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. *BMC Infect Dis* 5: 116.
8. Walboomers JM, Jacobs MV, Manos MM, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 189: 12–19.
9. Scheffner M, Munger K, Byrne JC, et al. (1991) The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. *Proc Natl Acad Sci U S A* 88: 5523–5527.
10. Scheffner M, Werness BA, Huibregtse JM, et al. (1990) The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63: 1129–1136.
11. Woodman CBJ, Collins SI, Young LS (2007) The natural history of cervical HPV infection: Unresolved issues. *Nat Rev Cancer* 7: 11–22.
12. Zur Hausen H (2002) Papillomaviruses and cancer: From basic studies to clinical application. *Nat Rev Cancer* 2: 342–350.
13. K Münger, Baldwin A, Edwards KM, et al. (2004) Mechanisms of human papillomavirus-induced oncogenesis. *J Virol* 78: 11451–11460.
14. Josefsson AM, Magnusson PK, Ylitalo N, et al. (2000) Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: A nested case-control study. *Lancet* 355: 2189–2193.
15. Swan DC, Tucker RA, Tortolero-Luna G, et al. (1999) Human papillomavirus (HPV) DNA copy number is dependent on grade of cervical disease and HPV type. *J Clin Microbiol* 37: 1030–1034.
16. Obeid DA, Almatrrouk SA, Khayat HH, et al. (2020) Human papillomavirus type 16 and 18 viral loads as predictors associated with abnormal cervical cytology among women in Saudi Arabia. *Heliyon* 6: e03473.
17. Van Duin M, Snijders PJF, Schrijnemakers HFJ, et al. (2002) Human papillomavirus 16 load in normal and abnormal cervical scrapes: An indicator of CIN II/III and viral clearance. *Int J Cancer* 98: 590–595.
18. Das D, Bhattacharjee B, Sen S, et al. (2010) Association of viral load with HPV16 positive cervical cancer pathogenesis: Causal relevance in isolates harboring intact viral E2 gene. *Virology* 402: 197–202.
19. Cao M, Shah W, Qi J, et al. (2016) Prognostic significance of human papillomavirus viral load in correlation with different therapeutic modalities in cervical cancer patients. *Pathol Res Pract* 212: 804–810.
20. Shukla S, Mahata S, Shishodia G, et al. (2014) Physical state & copy number of high-risk human papillomavirus type 16 DNA in the progression of cervical cancer. *Indian J Med Res* 139: 531–543.
21. Xi LF, Hughes JP, Castle PE, et al. (2011) Viral load in the natural history of human papillomavirus type 16 infection: A nested case-control study. *J Infect Dis* 203: 1425–1433.
22. Vinokurova S, Wentzensen N, Einenkel J, et al. (2005) Clonal history of papillomavirus-induced dysplasia in the female lower genital tract. *J Natl Cancer Inst* 97: 1816–1821.
23. Wilke CM, Hall BK, Hoge A, et al. (1996) FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: Direct evidence for the coincidence of viral integration sites and fragile sites. *Hum Mol Genet* 5: 187–195.

24. Wentzensen N, Clarke MA, Bremer R, et al. (2019) Clinical evaluation of human papillomavirus screening with p16/Ki-67 dual stain triage in a large organized cervical cancer screening program. *JAMA Intern Med* 179: 881–888.
25. Mcintyre MC, Frattini MG, Grossman SR, et al. (1993) Human papillomavirus type 18 E7 protein requires intact Cys-X-X-Cys motifs for zinc binding, dimerization, and transformation but not for Rb binding. *J Virol* 67: 3142–3150.
26. Incassati A, Patel D, McCance DJ (2006) Induction of tetraploidy through loss of p53 and upregulation of Plk1 by human papillomavirus type-16 E6. *Oncogene* 25: 2444–2451.
27. Band V, De Caprio JA, Delmolino L, et al. (1991) Loss of p53 protein in human papillomavirus type 16 E6-immortalized human mammary epithelial cells. *J Virol* 65: 6671–6676.
28. Werness BA, Levine AJ, Howley PM (1990) Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248: 76–79.
29. Klingelhutz AJ, Foster SA, McDougall JK (1996) Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* 380: 79–82.
30. Fischer M, Uxa S, Stanko C, et al. (2017) Human papilloma virus E7 oncoprotein abrogates the p53-p21-DREAM pathway. *Sci Rep* 7: 1–11.
31. 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 U S A* 94: 11612–11616.
32. Ganguly N, Parihar SP (2009) Human papillomavirus E6 and E7 oncoproteins as risk factors for tumorigenesis. *J Biosci* 34: 113–123.
33. Olmedo-Nieva L, Muñoz-Bello JO, Contreras-Paredes A, et al. (2018) The role of E6 spliced isoforms (E6*) in human papillomavirus-induced carcinogenesis. *Viruses* 10: 45.
34. Thomas M, Pim D, Banks L (1999) The role of the E6-p53 interaction in the molecular pathogenesis of HPV. *Oncogene* 18: 7690–7700.
35. Yim EK, Park JS (2005) The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. *Cancer Res Treat* 37: 319.
36. McMurray HR, McCance DJ (2003) Human papillomavirus type 16 E6 activates TERT gene transcription through induction of c-Myc and release of USF-mediated repression. *J Virol* 77: 9852–9861.
37. Yugawa T, Handa K, Narisawa-Saito M, et al. (2007) Regulation of notch1 gene expression by p53 in epithelial cells. *Mol Cell Biol* 27: 3732–3742.
38. Riley RR, Duensing S, Brake T, et al. (2003) Dissection of human papillomavirus E6 and E7 function in transgenic mouse models of cervical carcinogenesis 1. *Cancer Res* 63: 4862–4871.
39. Liu X, Marmorstein R (2007) Structure of the retinoblastoma protein bound to adenovirus E1A reveals the molecular basis for viral oncoprotein inactivation of a tumor suppressor. *Genes Dev* 21: 2711–2716.
40. Lee HG, Yu KA, Oh WK, et al. (2005) Inhibitory effect of jaceosidin isolated from Artemisia argyi on the function of E6 and E7 oncoproteins of HPV 16. *J Ethnopharmacol* 98: 339–343.
41. Wise-Draper TM, Allen HV, Thobe MN, et al. (2005) The human DEK proto-oncogene is a senescence inhibitor and an upregulated target of high-risk human papillomavirus E7. *J Virol* 79: 14309–14317.

42. Ishikawa M, Fujii T, Saito M, et al. (2006) Overexpression of p16INK4a as an indicator for human papillomavirus oncogenic activity in cervical squamous neoplasia. *Int J Gynecol Cancer* 16: 347–353.
43. Arroyo M, Bagchi S, Raychaudhuril 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.
44. Pitti RM, Marsters SA, Ruppert S (1996) Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 271: 12687–12690.
45. Zur Hausen H (2000) Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. *JNCI J Natl Cancer Inst* 92: 690–698.
46. Oda H, Kumar S, Howley PM (1999) Regulation of the Src family tyrosine kinase Blk through E6AP-mediated ubiquitination. *Proc Natl Acad Sci U S A* 96: 9557–9562.
47. Caberg JHD, Hubert PM, Begon DY, et al. (2008) Silencing of E7 oncogene restores functional E-cadherin expression in human papillomavirus 16-transformed keratinocytes. *Carcinogenesis* 29: 1441–1447.

AIMS Press