Human Papillomavirus in Head and Neck Cancer: Its Role in Pathogenesis and Clinical Implications

Christine H. Chung[1,2] and Maura L. Gillison[3]

Abstract Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with an annual incidence of approximately 400,000 worldwide. Although the principal risk factors for head and neck cancer remain tobacco and alcohol use, human papillomavirus (HPV) has recently been found to be etiologically associated with 20 to 25% of HNSCC, mostly in the oropharynx. HPV causes human cancers by expressing two viral oncoproteins, E6 and E7. These oncoproteins degrade and destabilize two major tumor suppressor proteins, p53 and pRb, through ubiquitination. Additional studies have shown that E6 and E7 can directly bind to multiple host proteins other than p53 and pRb (e.g., Bak and $p21^{Cip1}$), further contributing to genetic instability. However, expression of E6 and E7 alone is not sufficient for cellular transformation, and the additional genetic alterations necessary for malignant progression in the setting of virus-induced genomic instability are unknown. In addition to the etiological differences, HPV-positive cancers are clinically distinct when compared with HPV-negative cancers with regard to treatment response and survival outcome, with tumor HPV-positivity being a favorable prognostic biomarker. Further understanding of carcinogenesis and clinical behavior of HPV-positive cancers will improve disease prevention, patient care, and surveillance strategies for HNSCC patients. (Clin Cancer Res 2009;15(22):6758–62)

Background

Human papillomavirus (HPV) is a circular, double-stranded DNA virus. The viral genome, consisting of approximately 8,000 base pairs in size, encodes two regulatory proteins (from "early" genes E1 and E2), three oncoproteins (E5, E6, and E7), and two structural capsid proteins (from "late" genes L1 and L2; reviewed in ref. 1). More than 100 unique HPV types are known, but these different types are generally divided into those with a predilection to infect the skin versus mucosal surfaces (2). Mucosal HPV infections are well known to associate with a spectrum of human diseases from benign papillomas (or warts) to invasive carcinomas including cervical, vulvar, vaginal, anal, penile, and more recently head and neck squamous cell carcinoma (HNSCC; reviewed in refs. 1, 3, 4). Because carcinomas of the cervix and anogenital region have been extensively reviewed in the past, we will focus on the recent data in HNSCC in this review.

HNSCC is the sixth most common cancer with an annual incidence of approximately 400,000 worldwide (5). These cancers arise from five major anatomic sites: oral cavity, oropharynx, nasopharynx, hypopharynx, and larynx. Although Epstein-Barr virus is a long-established cause of nasopharyngeal cancer (6), a causal association between HPV and oropharyngeal cancer has only recently been established (7, 8). Compared with the association of HPV-negative tumors with heavy tobacco and alcohol use, HPV-positive tumors are strongly associated with sexual behavior, which is consistent with the known predominant means of HPV transmission via sexual contact (9). The incidence of HPV-related cancers has been increasing since the early 1990s in the United States and Western Europe, but the underlying reasons for this rapid increase are unclear (10, 11).

The HPV life cycle is complex. During early infection, viral DNA is present as a nuclear episome at low copy number in the basal cell layer of the stratified epithelium. HPV DNA is amplified and encapsidated to progeny virions only in terminally differentiated epithelial cells (reviewed in ref. 4). Recent data indicate that establishment of the initial infection is tightly linked to cell cycle progression through the mitotic phase (12). When human kidney cells (293T) or immortalized keratinocytes (HaCaT) are exposed to HPV in the presence of approximately 5,000 bioactive compounds via high-throughput screening, a subset of cell cycle inhibitors (including etoposide, aphidiocolin, and 5-fluorouracil) blocks HPV infection. This effect is observed even at low concentrations that do not affect

Authors' Affiliations: [1]Division of Hematology/Oncology, Department of Medicine and [2]Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, Tennessee and [3]The Ohio State University Comprehensive Cancer Center, Columbus, Ohio

Received 8/5/09; revised 8/18/09; accepted 8/19/09; published OnlineFirst 10/27/09.

Requests for reprints: Christine H. Chung, Division of Hematology/Oncology, Department of Medicine, Vanderbilt University School of Medicine, 2220 Pierce Avenue, 777 Preston Research Building, Nashville, TN 37232-6307. Phone: 615-322-4967; Fax: 615-343-7602; E-mail: Christine.Chung@Vanderbilt.edu.

doi:10.1158/1078-0432.CCR-09-0784

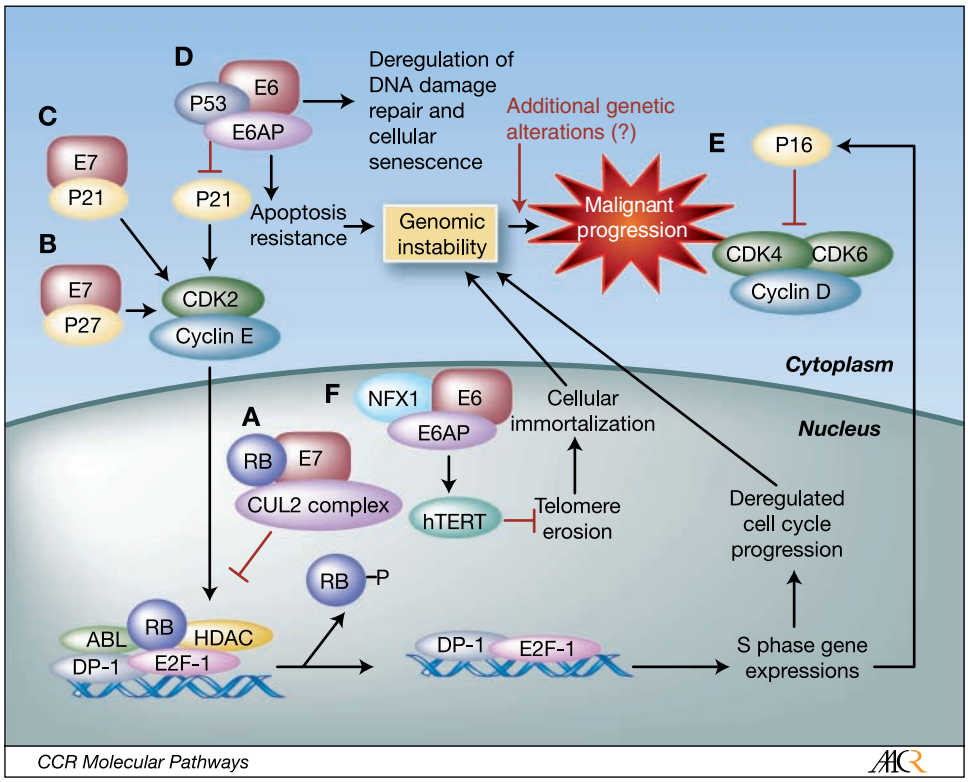

Fig. 1. Diagram of malignant transformation in keratinocytes caused by the HPV oncoproteins, E6 and E7; clockwise from *A*, ubiquitination by E7 and the cullin 2 ubiquitin ligase complex leading to pRb degradation (23, 25, 56, 57); *B*, interaction between E7 and $p27^{Kip1}$ resulting in inhibition of cell cycle arrest contributing to carcinogenesis (58); *C*, interaction between E7 and $p21^{Cip1}$ resulting in inhibition of cell cycle arrest contributing to carcinogenesis (31, 59); *D*, ubiquitination by E6 and ubiquitin ligase E6AP leading to p53 degradation (19–21); *E*, increased expression of $p16^{INK4A}$ by a consequent of feedback loops from the absence of pRb function (42); and *F*, degradation of NFX1, a transcriptional repressor of hTERT, by association with E6/E6AP resulting in hTERT activation and cellular immortalization (60).

cell viability (12, 13). On further examination, mitosis phase progression is found to be required for HPV infection and early gene expression. More specifically, the early prophase segment of mitosis is the critical phase for the infection, as determined by arresting the cell cycle at the G2/M phase using the cyclin-dependent kinase (CDK) 1 inhibitor, purvalanol A (12, 14).

At least 15 high-risk or oncogenic HPV types have been identified on the basis of associations with cervical cancer. HPV16 is the most common type found in 87 to 90% of HPV-positive oropharyngeal cancers (7, 15, 16). The role of high-risk HPV oncoproteins (E5, E6, and E7), in cellular transformation and maintenance of the malignant phenotype was first described in cervical cancers. The HPV E5 protein plays an important role during the early course of infection by binding to epidermal growth factor receptor (EGFR), platelet derived growth factor β receptor, and colony stimulating factor 1 receptor to promote cell proliferation (17). However, the E5 coding sequence is frequently deleted from the episomal viral DNA during integration to host genome; therefore, E5 is thought not to be required for the late stage of carcinogenesis (18). The significant role for carcinogenesis and maintenance of malignant phenotype is played by E6 and E7.

The HPV E6 protein forms a complex with an E3 ubiquitin ligase, E6-associated protein (E6AP), and ubiquitinates the p53 tumor suppressor protein (Fig. 1D; refs. 19–21). The ubiquitination causes rapid degradation of p53, which results in deregulation of both the G1/S and G2/M cell cycle checkpoints upon DNA damage and other cellular stress leading to genomic instability (22). The HPV E7 protein binds to the cullin 2 ubiquitin ligase complex and ubiquitinates the retinoblastoma (pRb) tumor suppressor protein (Fig. 1A; refs. 23–25). Again, the ubiquitination induces degradation of pRb resulting in uncontrolled G1/S phase of the cell cycle (26). In the absence of the pRb function, the E2F family of transcription factors is released and transcription of S-phase genes occurs, leading to cell proliferation. When E6 and E7 proteins are silenced in HPV-positive oropharyngeal cancer cell lines using shRNAs, the expression of p53 and pRb is restored, as are p53-dependent up-regulation of *CDKN1A* (*p21Cip1*) and *FAS* expression, and pRb-dependent down-regulation of *DEK* and *B-MYB*. Furthermore, inhibition of E6 and E7 induces apoptosis and decreases cell viability, indicating that expression of the E6 and E7 oncoproteins is required for tumor maintenance in oropharyngeal cancers, as is seen in cervical cancers (27). In addition, E7 is found to be the major transforming oncogene during the early stages of carcinogenesis in HNSCC when compared with E6, which seems to function at later stage (28). When *E6*, *E7*, and both *E6/E7* are expressed under *keratin 14* (K14) promoter regulation in transgenic mice, mice develop tumors in their tongue and esophagus after treatment with 4-nitroquinolin-*N*-oxide (4-NQO) in their drinking water. The *K14E6* mice developed tumors at a much lower rate than the *K14E7* mice (22% versus 95%), although the nontransgenic mice had a tumor formation rate at 16%. The *K14E6E7* bi-transgenic mice had a similar rate of tumor formation as the *K14E7* mice, but they had a higher rate of multiple invasive lesions with high-grade carcinoma. This characteristic also indicates that E6 and E7 function in concert to form invasive carcinoma in HNSCC.

Recent studies indicate that E6 and E7 have multiple binding partners that exert oncogenic effects beyond degradation of p53 and pRb, and have complementary effects in transforming activity. When *Rb* is deleted *in vivo*, loss of pRb recapitulates some of the phenotypes of E7 expression, but not entirely, indicating that E7 may have a Rb-independent function contributing to

tumorigenesis (28). For example, E7 interacts with the pRb-related "pocket proteins" p107 and p130, and the CDK inhibitors $p21^{CIP1}$ and $p27^{KIP1}$ (29–31). Inhibition of these key regulatory proteins for cell cycle arrest contributes to uncontrolled cellular proliferation and carcinogenesis (Fig. 1B and C). E6, in concert with E6AP, induces telomerase activity through activation of hTERT via degradation of NFX1, a transcription repressor of hTERT, thus contributing to cellular immortalization (Fig. 1F; ref. 32). In addition to exerting anti-apoptotic effects by degrading p53, E6 directly binds a pro-apoptotic protein Bak with E6AP, which further contributes to anti-apoptosis (33). These transforming effects work in concert such that E6 prevents E7-induced apoptosis by exerting anti-apoptotic effects degrading p53 and Bak, and that E7 rescues E6 from $p16^{INK4A}$ inhibition by direct activation of cyclins A and E and functional inactivation of $p16^{INK4A}$ bypassing its regulation (33, 34).

However, despite having multiple binding partners with oncogenic effects as well as enabling the cells to acquire genetic alterations that cause genomic instability, expression of E6 and E7 alone is not sufficient to cause malignant progression or oncogenic transformation (reviewed in ref. 1). Currently, the additional genetic events that are required for the development of cancer are unknown. Also unclear is whether these genetic events will be common among specific HPV-associated cancers (for instance, oropharyngeal cancers) or common to all HPV-associated cancers (e.g., cervical, vulvar, vaginal, anal, penile, and oropharyngeal cancers). Compared with HPV-negative head and neck cancers, HPV-positive cancers have fewer genome-wide DNA copy number alterations, less genome-wide hypomethylation, less frequent *TP53* mutations, and lower expression of EGFR (7, 35–38). For instance, HPV-negative tumors have losses at 3p11.2-26.3, 5q11.2-35.2, and 9p21.1-24, and gains or amplifications at 11q12.1-13.4, which are absent in HPV-positive tumors. Interestingly, 18q12.1-23 is gained in HPV-positive tumors and lost in HPV-negative tumors (35). In gene expression analyses, the most prominent differences between HPV-positive and -negative tumors are found in cell cycle regulatory pathways (39, 40). HPV-positive tumors have up-regulation of cyclins E and B as well as multiple S-phase proteins that are responsive to activation by the E2F transcription factors, likely as a consequence to E7-induced pRb loss (41). As a consequence of feedback loops from pRb loss, HPV-positive tumors have up-regulation of *CDKN2A* (*p16INK4a*; Fig. 1E; ref. 42). In addition to the cell cycle-regulated genes, up-regulation of testis-specific genes (e.g., *SYCP2*, *TCAM1*, and *STAG3*) is observed in HPV-positive tumors. Although *SYCP2* and *TCAM1* expression are synergistically up-regulated by E6 and E7, *STAG3* expression increase is not an immediate effect of the viral infection; rather, it is a delayed response that is passage-dependent (40).

Further understanding of these additional genetic alterations leading to malignant progression is critical to future secondary prevention strategies as well as rationally targeted therapeutic interventions for patients with HPV-positive cancers. The prevalence of genital HPV infection among women aged 14 to 59 years in the United States is ~26.8% (43), with HPV16 prevalence being ~1.5%. Among men, initial estimates for genital infection are even higher at ~60% (44, 45). Prevalence estimates for oral HPV infection in the U.S. population are currently unknown, but it is assumed from cervical data that the majority of individuals with an oral HPV16 infection will not develop cancer. Identification of the additional factors that promote cancer among those with an oral HPV infection may result in novel screening methods or novel therapeutic targets in selected high-risk subpopulations.

Clinical-Translational Advances

To investigate the effects of HPV in human tumors, optimization of HPV detection methods with both high sensitivity and high specificity is crucial. Several well-established methods exist. Although detection of HPV E6 and E7 expression is the gold standard for classifying a tumor as HPV-positive, detecting viral RNA in existing clinical samples (e.g., formalin-fixed paraffin-embedded tumors or cytologic specimens from fine-needle aspiration) is impractical for cancer diagnostics at this time. Therefore, several PCR-based, as well as *in-situ* hybridization (ISH) assays, are currently used to detect HPV DNA in tumors. The $p16^{INK4A}$ immunohistochemical (IHC) staining of tumors has also been used as a surrogate marker for HPV presence (7, 8, 46, 47). Overall, the PCR-based assays have higher sensitivity with lower specificity owing to the presence of transcriptionally inactive viral DNA or cross-contamination of samples that may cause falsely positive results (48). Commercial ISH assays capable of detecting multiple high-risk HPV types have lower sensitivity but can be used to visualize the HPV genome specifically within tumor cell nuclei (49). IHC staining for $p16^{INK4A}$ is an excellent surrogate marker for HPV infection, reflecting the functional effects of E7-induced inactivation of pRb (Fig. 1D; refs. 47, 49, 50). Some investigators have proposed $p16^{INK4A}$ IHC staining as an initial screen, followed by HPV detection with more specific assays in tumors that are $p16^{INK4A}$ IHC positive (51).

When clinical outcomes are evaluated on the basis of tumor HPV status, patients with HPV-positive oropharyngeal cancers have favorable outcomes compared with patients with HPV-negative cancer. In a phase III trial, Radiation Therapy Oncology Group 0129, 64% of 323 oropharyngeal tumors analyzed for HPV were HPV positive using ISH capable of detecting the most common 13 high-risk HPV types. These patients with HPV-positive tumors were younger, had less extensive tobacco exposure, better performance status, and smaller primary tumors compared with HPV-negative patients (49). The clinical outcomes after treatment with cisplatin and radiation therapy were significantly better in patients with HPV-positive compared with HPV-negative tumors (2-year overall survival 87.9% versus 65.8%, P value < 0.001; progression-free survival 71.8% versus 50.4%, P value < 0.001; and local-regional failure 13.6% versus 24.8%, P value 0.004). Favorable outcomes with HPV-positive patients have been observed independent of the treatment modalities used including chemotherapy, radiation, and/or surgery (52–54). However, a subset of the HPV-positive patients experience worse outcomes when compared with the average HPV-positive patient, resembling more of the clinical course in HPV-negative patients. This subset of patients has more extensive smoking histories, *TP53* mutations, and higher EGFR and Bcl-xL expressions suggesting that the HPV status alone is not an adequate prognostic marker to perfectly segregate patients (38, 49, 55).

Because of the distinct biology and clinical behavior within subgroups of oropharyngeal cancer patients, clinician-scientists now propose to do clinical trials separately or at least stratify based on HPV status. For example, when a phase III clinical trial is done to compare drug X versus drug Y, the drug X arm may falsely show survival benefit if there are disproportionately more HPV-positive patients in the drug X arm compared with the drug Y arm. The apparent survival benefit of drug X may be entirely driven by a disproportionate number of patients with good prognosis, which may lead to the erroneous conclusion that drug X is better than drug Y. In addition, the distinct molecular mechanisms underlying HPV-positive and -negative tumors may lead to interaction effects whereby responses to drug X and/or drug Y are influenced by HPV status, which may also give the appearance that drug X and drug Y have different treatment responses. Therefore, the investigators have to ensure that the same number of HPV-positive patients is included in comparison groups by using the recent data for stratification. Currently, many of the completed clinical trial data that enrolled unselected patients are undergoing retrospective analyses in order to see the effect of HPV status versus treatment variables with respect to their survival outcomes. Furthermore, investigators are reevaluating current approaches for intensification of treatment by stratifying risk groups in order to avoid unnecessary toxicities among patients with favorable prognoses. How best to treat these patients is the subject of ongoing clinical trial design discussions and will best be determined through the cooperative groups. Additional correlative studies are required to further delineate the subset of patients with HPV-positive tumors that have a worse prognosis than would be expected from their HPV status. The role of the currently available HPV vaccines should be also investigated for cancer prevention.

Disclosure of Potential Conflicts of Interest

M. Gillison and C.H. Chung, consultants, Merck.

References

1. McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. Virus Res 2009;143:195–208.
2. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004;324:17–27.
3. Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. Vaccine 2006;24 Suppl 3: S3/11–25.
4. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342–50.
5. Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. CA Cancer J Clin 2005; 55:74–108.
6. Pathmanathan R, Prasad U, Sadler R, Flynn K, Raab-Traub N. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med 1995;333:693–8.
7. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709–20.
8. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356: 1944–56.
9. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008;100:407–20.
10. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol 2008;26:612–9.
11. Nasman A, Attner P, Hammarstedt L, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer 2009;125:362–6.
12. Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF. Establishment of human papillomavirus infection requires cell cycle progression. PLoS Pathog 2009;5:e1000318.
13. Pyeon D, Lambert PF, Ahlquist P. Production of infectious human papillomavirus independently of viral replication and epithelial cell differentiation. Proc Natl Acad Sci U S A 2005;102:9311–6.
14. Gray NS, Wodicka L, Thunnissen AM, et al. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. Science 1998;281:533–8.
15. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
16. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467–75.
17. Hwang ES, Nottoli T, Dimaio D. The HPV16 E5 protein: expression, detection, and stable complex formation with transmembrane proteins in COS cells. Virology 1995;211:227–33.
18. Schwarz E, Freese UK, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature 1985;314:111–4.
19. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 1990; 63:1129–36.
20. Huibregtse JM, Scheffner M, Howley PM. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. EMBO J 1991;10:4129–35.
21. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 1993;75:495–505.
22. Kessis TD, Slebos RJ, Nelson WG, et al. Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage. Proc Natl Acad Sci U S A 1993;90: 3988–92.
23. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 1989;243:934–7.
24. Munger K, Werness BA, Dyson N, Phelps WC, Harlow E, Howley PM. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J 1989;8:4099–105.
25. Huh K, Zhou X, Hayakawa H, et al. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. J Virol 2007;81:9737–47.
26. Slebos RJ, Lee MH, Plunkett BS, et al. p53-dependent G1 arrest involves pRB-related proteins and is disrupted by the human papillomavirus 16 E7 oncoprotein. Proc Natl Acad Sci U S A 1994;91:5320–4.
27. Rampias T, Sasaki C, Weinberger P, Psyrri A. E6 and e7 gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. J Natl Cancer Inst 2009;101:412–23.
28. Strati K, Lambert PF. Role of Rb-dependent and Rb-independent functions of papillomavirus E7 oncogene in head and neck cancer. Cancer Res 2007;67:11585–93.
29. Davies R, Hicks R, Crook T, Morris J, Vousden K. Human papillomavirus type 16 E7 associates with a histone H1 kinase and with p107 through sequences necessary for transformation. J Virol 1993;67:2521–8.
30. Helt AM, Funk JO, Galloway DA. Inactivation of both the retinoblastoma tumor suppressor and p21 by the human papillomavirus type 16 E7 oncoprotein is necessary to inhibit cell cycle arrest in human epithelial cells. J Virol 2002;76: 10559–68.
31. Shin MK, Balsitis S, Brake T, Lambert PF. Human papillomavirus E7 oncoprotein overrides the tumor suppressor activity of p21Cip1 in cervical carcinogenesis. Cancer Res 2009;69: 5656–63.
32. Gewin L, Myers H, Kiyono T, Galloway DA. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. Genes Dev 2004;18: 2269–82.
33. Thomas M, Banks L. Inhibition of Bak-induced apoptosis by HPV-18 E6. Oncogene 1998;17: 2943–54.
34. Zerfass K, Schulze A, Spitkovsky D, Friedman V, Henglein B, Jansen-Durr P. Sequential activation of cyclin E and cyclin A gene expression by human papillomavirus type 16 E7 through sequences necessary for transformation. J Virol 1995;69:6389–99.
35. Smeets SJ, Braakhuis BJ, Abbas S, et al. Genome-wide DNA copy number alterations in head and neck squamous cell carcinomas with or without oncogene-expressing human papillomavirus. Oncogene 2006;25:2558–64.
36. Richards KL, Zhang B, Baggerly KA, et al. Genome-wide hypomethylation in head and neck cancer is more pronounced in HPV-negative tumors and is associated with genomic instability. PLoS One 2009;4:e4941.
37. Westra WH, Taube JM, Poeta ML, Begum S, Sidransky D, Koch WM. Inverse relationship between human papillomavirus-16 infection and

disruptive p53 gene mutations in squamous cell carcinoma of the head and neck. Clin Cancer Res 2008;14:366–9.

38. Kumar B, Cordell KG, Lee JS, et al. EGFR, p16, HPV Titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. J Clin Oncol 2008;26: 3128–37.

39. Slebos RJ, Yi Y, Ely K, et al. Gene expression differences associated with human papillomavirus status in head and neck squamous cell carcinoma. Clin Cancer Res 2006;12:701–9.

40. Pyeon D, Newton MA, Lambert PF, et al. Fundamental differences in cell cycle deregulation in human papillomavirus-positive and human papillomavirus-negative head/neck and cervical cancers. Cancer Res 2007;67:4605–19.

41. Arroyo M, Bagchi S, Raychaudhuri P. Association of the human papillomavirus type 16 E7 protein with the S-phase-specific E2F-cyclin A complex. Mol Cell Biol 1993;13:6537–46.

42. Khleif SN, DeGregori J, Yee CL, et al. Inhibition of cyclin D-CDK4/CDK6 activity is associated with an E2F-mediated induction of cyclin kinase inhibitor activity. Proc Natl Acad Sci U S A 1996; 93:4350–4.

43. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813–9.

44. Partridge JM, Hughes JP, Feng Q, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. J Infect Dis 2007;196:1128–36.

45. Nielson CM, Harris RB, Dunne EF, et al. Risk factors for anogenital human papillomavirus infection in men. J Infect Dis 2007;196: 1137–45.

46. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3′ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol 1995;76:1057–62.

47. Lassen P, Eriksen JG, Hamilton-Dutoit S, Tramm T, Alsner J, Overgaard J. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009; 27:1992–8.

48. Braakhuis BJ, Snijders PJ, Keune WJ, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. J Natl Cancer Inst 2004;96: 998–1006.

49. Gillison ML, Harris J, Westra W, et al. Survival outcomes by tumor human papillomavirus (HPV) status in stage III-IV oropharyngeal cancer (OPC) in RTOG 0129. Proc Am Soc Clin Oncol 2009;27:6003.

50. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006;24:736–47.

51. Smeets SJ, Hesselink AT, Speel EJ, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 2007;121: 2465–72.

52. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100:261–9.

53. Lindquist D, Romanitan M, Hammarstedt L, et al. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol 2007;1:350–5.

54. Licitra L, Perrone F, Bossi P, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006; 24:5630–6.

55. Kong CS, Narasimhan B, Cao H, et al. The relationship between human papillomavirus status and other molecular prognostic markers in head and neck squamous cell carcinomas. Int J Radiat Oncol Biol Phys 2009;74:553–61.

56. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell 1999;98:859–69.

57. Rubin SM, Gall AL, Zheng N, Pavletich NP. Structure of the Rb C-terminal domain bound to E2F1–1: a mechanism for phosphorylation-induced E2F release. Cell 2005;123:1093–106.

58. Zerfass-Thome K, Zwerschke W, Mannhardt B, Tindle R, Botz JW, Jansen-Durr P. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. Oncogene 1996;13:2323–30.

59. Jones DL, Alani RM, Munger K. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. Genes Dev 1997; 11:2101–11.

60. Katzenellenbogen RA, Vliet-Gregg P, Xu M, Galloway DA. NFX1–123 increases hTERT expression and telomerase activity posttranscriptionally in human papillomavirus type 16 E6 keratinocytes. J Virol 2009;83:6446–56.