

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

Christine H. Chung^{1,2} and Maura L. Gillison³

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 ano-

genital 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: ¹Division of Hematology/Oncology, Department of Medicine and ²Department of Cancer Biology, Vanderbilt University School of Medicine, Nashville, Tennessee and ³The 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.

© 2009 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-09-0784

The diagram illustrates the CCR Molecular Pathways, showing the interplay between the Cytoplasm and Nucleus in the context of cancer progression. The pathways are divided into several sections labeled A through F, and a central red starburst indicates 'Malignant progression'.

- Section A (Nucleus):** Shows the ABL/DP-1 complex and the HDAC/E2F-1 complex. A red arrow indicates inhibition of the HDAC/E2F-1 complex by the ABL/DP-1 complex. A red arrow also points from the HDAC/E2F-1 complex to the RB-P complex.
- Section B (Cytoplasm):** Shows the E7/P27 complex and the CDK2/Cyclin E complex. A red arrow indicates inhibition of the CDK2/Cyclin E complex by the E7/P27 complex.
- Section C (Cytoplasm):** Shows the E7/P21 complex and the P21 protein. A red arrow indicates inhibition of the P21 protein by the E7/P27 complex.
- Section D (Cytoplasm):** Shows the P53/E6AP complex and the P21 protein. A red arrow indicates inhibition of the P21 protein by the P53/E6AP complex. A red arrow points from the P21 protein to the 'Apoptosis resistance' box.
- Section E (Cytoplasm):** Shows the P16 protein and the CDK4/CDK6/Cyclin D complex. A red arrow indicates inhibition of the CDK4/CDK6/Cyclin D complex by the P16 protein.
- Section F (Nucleus):** Shows the NF1/E6AP complex and the hTERT protein. A red arrow indicates inhibition of the hTERT protein by the NF1/E6AP complex. A red arrow points from the hTERT protein to the 'Telomere erosion' box.
- Central Pathway:** A red starburst labeled 'Malignant progression' is the central outcome. It is influenced by 'Genomic instability' (which receives input from 'Apoptosis resistance' and 'Telomere erosion'), 'Additional genetic alterations (?)', and 'Deregulated cell cycle progression' (which receives input from 'S phase gene expressions').
- Other Labels:** 'Deregulation of DNA damage repair and cellular senescence' is linked to the P53/E6AP complex. 'Cellular immortalization' is linked to the hTERT protein. 'S phase gene expressions' is linked to the DP-1/E2F-1 complex.

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 uncon-

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. Cur-

rently, 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

- McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. *Virus Res* 2009;143:195-208.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004;324:17-27.
- Parkin DM, Bray F. Chapter 2: The burden of HPV-related cancers. *Vaccine* 2006;24 Suppl 3: S3/11-25.
- zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002;2:342-50.
- Parkin DM, Bray F, Ferlay J, Pisani P. Global Cancer Statistics, 2002. *CA Cancer J Clin* 2005; 55:74-108.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF. Establishment of human papillomavirus infection requires cell cycle progression. *PLoS Pathog* 2009;5:e1000318.
- 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.
- 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.
- 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.
- 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.
- 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.
- Schwarz E, Freese UK, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 1985;314:111-4.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Strati K, Lambert PF. Role of Rb-dependent and Rb-independent functions of papillomavirus E7 oncoprotein in head and neck cancer. *Cancer Res* 2007;67:11585-93.
- 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.
- 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.
- 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.
- 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.
- Thomas M, Banks L. Inhibition of Bak-induced apoptosis by HPV-18 E6. *Oncogene* 1998;17: 2943-54.
- 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.
- 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.
- 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.
- 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. Zerbass-Thome K, Zwierschke 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. NF1–123 increases hTERT expression and telomerase activity posttranscriptionally in human papillomavirus type 16 E6 keratinocytes. *J Virol* 2009;83:6446–56.