

# Chapter 9: Role of Mucosal Human Papillomavirus in Nongenital Cancers

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**Established associations between human papillomavirus (HPV) and lower genital tract cancers provide a framework from which to evaluate a possible pathogenic role for the virus in cancers at nongenital sites. Proposed associations must fit coherently within the context of our current knowledge of the epidemiology and biology of HPV. In this article, insights obtained from studies of the etiologic link between mucosal-type HPV infection and four specific human cancers are described briefly. Specific characteristics, shared among cancers caused by HPV, are then used by extrapolation to discuss possible associations between certain other nongenital cancers and mucosal HPV infections in a manner intended to supplement, and in no way to supplant, the classic Hill criteria for causal inference. [J Natl Cancer Inst Monogr 2003;31:57–65]**

## INSIGHTS FROM ESTABLISHED ASSOCIATIONS

### Squamous Cell Carcinoma of the Cervix

A paradigm for human papillomavirus (HPV)-mediated tumorigenesis has emerged from studies of the association of HPV and cervical cancer. For this cancer, the evidence from all lines of inquiry came together in a mutually reinforcing manner. Cervical cancer had long been known to have the risk factors of a sexually transmitted disease. In 1982, Skegg et al. (1) proposed that cervical cancer incidence in different geographic areas of the world could be deduced from information on three factors: 1) the sexual practices of the women, 2) the sexual practices of their male partners, and 3) the standard of health care (a proxy for an effective Pap smear screening program).

HPV type 16 (HPV16) was first isolated from a cervical cancer specimen in 1983 (2). Subsequently, HPV viral genomes were identified in virtually all cervical cancer cases and in most of the cancer precursor lesions of cervical intraepithelial neoplasia (CIN) (3). The HPV genotypes that were recovered from invasive cancers were the same as those found to have a high ability to transform human cells experimentally and are, therefore, considered to be high risk. These include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83. The viruses come armed with two virus-specific oncoproteins, E6 and E7, which are invariably expressed in naturally occurring cancers. These oncoproteins form a complex with and inactivate two cellular tumor suppressor proteins, p53 and pRB, and are, therefore, able to distort the cell cycle and produce the genomic instability that, in a subset of infected individuals, eventually leads to the development of cancer. Several additional mechanisms by which these oncoproteins alter cellular homeostasis have been reported (4,5). High titers of antibodies to HPV16 E6 and E7 oncoproteins in patients with HPV16-positive cervical cancer provide further evidence for expression of the viral genome. Seroreactivity to these virus-specific oncoproteins was found to be a highly specific biomarker for the presence of an

invasive cervical cancer (6). Individuals with impaired cell-mediated immunity, whether due to human immunodeficiency virus (HIV) infection or bone marrow or solid organ transplantation, have been found to be at increased risk of developing a CIN as a consequence of HPV infection (7–12).

The etiologic link between HPVs and cervical cancers is very strong, both in the areas of high and low incidence of cervical cancer. HPV infection seems to account for all cases of cervical cancer worldwide. The strong and consistent association between HPV exposure and cervical cancer risk, the stepwise clinical–pathologic progression from HPV infection to invasive cervical cancer, and the genomic instability induced by HPV E6 and E7 oncoprotein expression all support the conclusion that HPV is necessary for cervical cancer development. As such, HPV has the largest attributable fraction among all known human carcinogens. The prevention of HPV infection would virtually eliminate cervical cancer.

### Squamous Cell Carcinoma of the Vulva

Although infection of the vulva by low-risk HPVs was known to occur and to cause benign genital condylomata, the investigation of the relationship between HPV infection and cancer of the vulva initially presented a paradox. HPV genomic sequences were recovered readily from tissues that exhibit high-grade vulvar intraepithelial neoplasia (VIN), lesions analogous to CIN. However, in studies of invasive vulvar cancers, HPV prevalence varied over a wide range. The paradox was resolved when it was recognized that vulvar carcinomas are etiologically heterogeneous (13–15). One subset of vulvar cancers occurs in young women and has basaloid histopathology, adjacent VIN, and risk factors related to sexual practices; these cancers are HPV associated (13). High-risk HPV types 16, 31, and 33 are most frequently isolated from both vulvar carcinomas and their precursor lesions, VIN (16). Women with the HPV-associated subset of vulvar carcinoma have antibodies to E6 and E7 proteins (17). The more common keratinizing vulvar carcinoma in older women does not have adjacent VIN, sexual history risk factors, seroreactivity to E6 or E7 greater than the control subjects, or the HPV genome. Analogous to cervical cancer, both immunosuppressed individuals and individuals with a previous HPV-associated malignancy are at increased risk of VIN lesions and invasive vulvar carcinomas (8,18,19). Vulvar carcinomas provide an example where HPV infection is not necessary for the development of all cancers at a particular anatomic site but

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appears to be responsible for a well-defined subset of the cancers at that site.

### Anal Squamous Cell Carcinomas

Heterogeneity in etiology has also been reported in anal squamous cell carcinomas in men. Whereas several studies (20,21) have detected HPV-DNA in a variable proportion of anal squamous cell carcinomas, the clinical and pathologic correlates of HPV presence have been reported in a single, large study of 331 cases of anal carcinoma. These results remain to be confirmed. In this study (22), squamous cell carcinomas of the anal region that are positive for genital HPV (types 16, 18, 31, and 33) occur in young men, are related to homosexual behavior, occur in the anal canal, have poorly differentiated basaloid histopathology, and have associated anal intraepithelial neoplasia (AIN) (22). By contrast, HPV-negative anal carcinomas occur in older men, arise from the perianal skin, are well keratinized, and are not associated with AIN. Rectal adenocarcinomas are similarly negative for genital HPVs. The relationship between perianal skin cancers and HPV types normally found on the skin has yet to be investigated. In women, HPV-positive anal squamous cell carcinomas are more likely to be located in the anal canal and exhibit associated AIN and basaloid histology as compared with HPV-negative cancers (22). High-risk sexual behaviors, immunosuppression (due to HIV infection or solid organ transplantation), and a history of an HPV-associated malignancy are all associated with the risk of anal carcinoma (8,20,23).

Notably, a heterogeneity of etiologies for penile cancer is now also being described as analogous to that observed for vulvar and anal squamous cell carcinomas (24–30).

### Oropharyngeal Cancers

Among extragenital malignancies proposed to be associated with HPV infection, the data for oropharyngeal cancer are the most compelling. Low-risk HPV types 6 and 11 were known to infect the upper aerodigestive tract and to cause benign respiratory papillomas. The early studies (31) in which head and neck cancers (cancers of the oral cavity, pharynx, and larynx) were examined for HPV sequences gave widely varying results, both with respect to HPV prevalence and HPV type distribution. Recently, several studies (see chapter 7) have consistently identified a distinct subset of these cancers that appears to be etiologically linked to HPV infections. These HPV-associated cancers are distinguished from head and neck cancers not associated with HPV by the former cases' location predominantly in the oropharynx (especially tonsils and base of tongue), more frequent basaloid pathology, less frequent p53 mutations, and a better prognosis (a finding unique to this HPV-associated malignancy) (32,33). High-risk HPV (predominantly types 16, 31, and 33) are present in the majority of these tumors, and the viral genome is specifically localized to the tumor cells and is transcriptionally active (34–36).

A case-control study of oral cancers (37) determined that the total number of lifetime sexual partners, young age at first intercourse, and a history of genital warts are each associated with the risk of oral cancer after adjustment for alcohol consumption and tobacco exposure. Although measures of sexual behavior have been associated with the risk of oral cancer in another study in North America (38), no associations were found in the case-control studies performed in Cuba (39) or Italy (44). Their lack of association with sexual behavior may be due to a low pro-

portion of HPV-associated cancers in the study population. An ongoing analysis (41) of these samples for the HPV genome as part of an international study of oral cancer and HPV should address this possibility.

Individuals with head and neck cancer have a higher prevalence of HPV types 16 and 18 E6 and E7 antibodies when compared with age- and sex-matched control subjects (41,42). HIV-infected individuals and individuals with a history of an HPV-associated anogenital malignancy are at increased risk of tonsillar carcinoma (19,43). HPV-associated head and neck squamous cell carcinoma appears to be a distinct clinical-pathologic subset of aerodigestive tract tumors. Case-control and cohort studies will be required to characterize both behavioral risk factors for this subset of cancers (and their interaction with known risk exposures, such as alcohol and tobacco) and the natural history of oral HPV infection.

### EXPECTED FEATURES OF HPV-CAUSED CANCERS

Our knowledge of HPV biology and characteristics of cancers etiologically linked to mucosal HPVs suggests by extrapolation that other suspected HPV-associated cancers would have the following features (Table 1).

#### Viral Characteristics

In mucosal HPV-associated tumors, high-risk viral DNA occurs in the tumor cell at a minimum of one viral copy per cell genome, is transcriptionally active, and is often, although not always, integrated. Even in cervical cancers, a proportion of the cancers have only episomal HPV-DNA, and in skin cancers associated with epidermodysplasia verruciformis, the HPV genome remains episomal. In very rare instances, such as squamous cell carcinomas in individuals with recurrent respiratory papillomatosis, low-risk HPV types 6 and 11 may be found in tumors. Thus, in cancers hypothesized to be associated with mucosal HPV, one would expect the genome of high-risk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 73, 82, and 83) to be localized in tumor cell nuclei and to express viral oncogenes. The presence of the viral genome in the full spectrum of disease, from premalignant lesions to carcinoma *in situ*, invasive disease, and metastatic cells, would argue for the importance of the virus in the initial promotion and maintenance of the malignant phenotype.

The one-to-one tumor cell-virus relationship may not hold for all papillomavirus-associated tumors. For instance, in bovine

**Table 1.** Expected characteristics of malignancies associated with mucosal human papillomaviruses (HPVs)

- 1) The genome of high-risk HPV types (e.g., 16, 18, 33, 45, 51, 58, and 59) would be localized to the tumor cell nuclei and express viral oncogenes.
- 2) HPV-associated cancers would be predominantly squamous cell in origin and may have basaloid pathology.
- 3) HPV-associated malignancies would occur at anatomic sites of exposure by direct contact.
- 4) HPV-associated malignancies would very likely arise from anatomic sites where HPV is known to cause benign lesions.
- 5) Patients with HPV-associated invasive cancers would develop serum antibodies to HPV E6 and E7 proteins.
- 6) Cancers caused by mucosal HPVs would have risk factors related to sexual practices.
- 7) An HPV-associated malignancy would occur at greater than expected rates in immunosuppressed individuals and in individuals with a previous HPV-associated malignancy.

alimentary tract cancers associated with bracken exposure and bovine papillomavirus type 4 (BPV-4) infection, the viral genome is lost from the tumor cells but is present in the benign papillomas from which they arise. Although there is no evidence for such a hit-and-run role for genital HPV types in human tumors, it has been hypothesized for skin cancers (*see* chapter 8). Investigating this possible mechanism in humans would be difficult, since it would require an exhaustive search for the virus in premalignant lesions that are often subclinical. Such a hit-and-run role for the virus would be suggested by a strong association (after adjustment for possible confounding factors) between HPV L1 seroreactivity and risk in the absence of detectable HPV-DNA in tumors. There are as yet no consistent reports of such an association in the literature.

Detection of the genomic DNA by highly sensitive polymerase chain reaction (PCR) techniques alone is insufficient evidence for the presence of pathogenically significant virus. However, a detailed study of a single tumor can provide compelling preliminary evidence for the HPV etiology of that tumor type, as, for example, when a tumor of the oral cavity and a cell line derived from the tumor had identical patterns of HPV16 integration and of other genetic changes (45).

### Pathology of the Lesions

HPVs are strictly epitheliotropic and preferentially infect squamous epithelia. The HPV-associated tumors at anatomic sites with etiologic heterogeneity (the vulva, anus, penis, and oropharynx) frequently have a basaloid squamous histopathology. Therefore, one would expect that HPV-associated cancers would be predominantly squamous cell in origin and may have basaloid pathology. This histology could represent either a predilection for the virus to infect a particular cell type or possibly a tumor differentiation induced by the virus in the process of transformation. Tumors of other histologies (e.g., adenocarcinomas) are less likely to be HPV related. However, given the important exception of cervical adenocarcinomas clearly etiologically associated with HPV, there may be less common circumstances in which *adenocarcinomas at sites of known HPV exposure* are HPV related.

### HPV Exposure

There is no viremic phase in the pathogenesis of HPV infection in humans, so the infection is not widely disseminated in the body. Thus, one would expect that HPV-associated malignancies would occur at anatomic sites of exposure by direct contact. HPV-caused primary tumors would occur only at sites where the virus enters the body (e.g., genital tract, skin, and aerodigestive tract). Tumors of internal organs, such as the brain, the kidney, and the liver, are unlikely to be HPV related.

### Associated Benign Lesions

The presence of benign lesions associated with any HPV type provides evidence that the virus can infect the epithelium in question. One would expect that HPV-associated malignancies would arise from anatomic sites where HPV is known to cause benign lesions. Benign lesions, which may or may not serve as precursor lesions to malignancy, are found at the anatomic sites where HPV is known to play a role in the pathogenesis of human cancers. Knowledge of the cellular receptor for HPV and the mechanism of cellular uptake may allow identification of human cells that may be susceptible to HPV infections.

### Presence of HPV Antibodies

Individuals who have been exposed to HPV with or without an associated cancer may have serum antibodies to the viral capsid proteins. Whereas anticapsid antibodies are found in many normal individuals because HPV infections are so common, antibodies to the viral E6 and E7 proteins, by contrast, are strongly associated with invasive cancer and are rarely found in individuals without cancer. High levels of E6 and E7 serum antibodies have a greater than 95%–99% specificity and about a 50% sensitivity for invasive cervical cancer (6,17,46). Therefore, patients with hypothesized HPV-associated invasive cancers would develop serum antibodies to HPV E6 and E7 proteins.

Antibodies to E6 and E7 are not measures of risk of cervical cancer (47) and do not play a role in the pathogenesis of disease, but rather they provide specific immunologic evidence for transcription of the viral genome in patients. The specificity of these antibodies to individuals with invasive cancer is likely due to presentation of the oncoproteins to the immune system during or after the process of microinvasion. Given the low sensitivity of E6 and E7 antibodies, seroreactivity may have limited utility as a diagnostic tool for an individual patient. However, in the case of etiologic heterogeneity at a site (e.g., vulvar and oropharyngeal cancers), higher prevalence rates of antibodies to E6 and E7 among a distinct subgroup of affected individuals would provide immunologic evidence that the distinct histopathologic subtype is HPV associated.

### Behavioral Risk Factors

One would expect that cancers caused by genital tract HPVs would have risk factors related to sexual practices. Sexual behaviors associated with risk of cervical cancer are, in large part, surrogate measures for HPV exposure. It is likely that the transmission of viral infection to nongenital sites occurs as a consequence of certain sexual behaviors, such as oral–genital contact, or by autoinoculation from a genital infection and, therefore, will be captured by the use of standard sexual behavior measures. Sexual behaviors known to be associated with HPV exposure include a high number of lifetime sexual partners, young age at first intercourse, and a history of sexually transmitted disease. Although other mechanisms, such as intrapartum transmission and transmission through nonsexual or noncoital sexual contact (such as deep kissing), are possible, they will likely be less common mechanisms (48). Interactions between sexual behaviors and other known risk factors for cancers at a particular site must be considered. For instance, in the case of oropharyngeal cancers, alcohol consumption and tobacco use may confound analysis of sexual behavior.

### Individuals at Risk

One would postulate that an HPV-associated malignancy would occur at greater than expected rates in immunosuppressed individuals and in individuals with a previous HPV-associated malignancy. Malignancies that are causally associated with viral infections would be increased in individuals with immunosuppression. This increase is presumably due to antigen-specific hyporesponsiveness in patients with disorders of cell-mediated immunity. All of the tumors with established associations with HPV are known to occur at greater than expected rates in individuals who are immunosuppressed as a consequence of HIV

infection (19). Cervical, vulvar, and anal squamous cell carcinomas have also been reported to occur at greater than expected rates in individuals on immunosuppressants after solid organ transplantation and in individuals after allogeneic bone marrow transplantation (7–10). Second cancers in individuals with a history of an HPV-associated malignancy suggest a common etiology (43). It is important to consider confounding by tobacco exposure when evaluating data on the risk of second primary tumors (49). Rare syndromes in which individuals appear to be at increased risk of HPV-associated malignancies may provide further insight into HPV-mediated pathogenesis (i.e., epidermodysplasia verruciformis and, perhaps, Fanconi anemia) (50–52).

## RELATIONSHIP OF HPV TO NONGENITAL TUMORS

Genomic sequences of genital HPVs have been reported from a wide variety of human cancers. The possible significance of these reports for some human cancers is discussed below. The relationship of these cancers to the characteristics of HPV-associated malignancies is displayed in Table 2.

### Squamous Cell Carcinoma of the Conjunctiva

HPV likely plays a role in the pathogenesis of a subset of conjunctival squamous cell carcinomas. The HPV genome has been detected in several benign conditions of the conjunctiva, including papillomas and pterygium, by PCR and *in situ* hybridization (53,54). Histopathologic correlates of HPV infection such as koilocytosis have been observed in these lesions. HPV has been detected in the spectrum of disease, including dysplasias, carcinoma *in situ*, and invasive carcinomas. Immunosuppressed individuals, in particular, those with HIV infection, are at an estimated 13-fold increased risk of squamous cell carcinoma of the conjunctiva (55). HPV type 16 or 18 E6 gene expression has been demonstrated by *in situ* reverse transcription-PCR in conjunctival neoplasia (56). HPV infection of the conjunctiva may occur intrapartum as the fetus travels through an infected birth canal or by autoinoculation via HPV-contaminated fingers. HPV16 has been detected in ocular swabs from women with cervical infection (57). Conjunctival tumors are likely heterogeneous, with HPV-negative conjunctival tumors

occurring in older individuals and related primarily to sun exposure. Sun exposure is likely to be a cofactor for HPV-related disease as well. A single case-control study in Uganda (58) reported an increased risk of conjunctival squamous cell carcinoma among HIV-positive individuals with a history of exchanging sex for gifts but no association with number of sexual partners, age of coitarche, or history of sexual discharge, perhaps a surrogate for certain sexually transmitted diseases. There was no association with HPV type 16, 18, or 45 L1 seropositivity and risk, and E6 and E7 serology was not performed.

### Esophageal Cancer

It is plausible that HPV could play a role in the pathogenesis of squamous cell carcinoma of the esophagus; however, there are limited conclusive data to support the hypothesis. The esophagus is lined by squamous epithelium that could be exposed to HPV in the same manner as the oral cavity and pharynx. Benign squamous papillomas of the esophagus occur but are rare, and HPV is infrequently detected in these lesions (40,59–61). Given that esophageal adenocarcinomas arise from sites of metaplasia in the distal esophagus, it is conceivable that HPV could be present in these lesions if the infection precedes the metaplasia. Esophageal carcinomas in the United States, Europe, and Japan are infrequently HPV positive by PCR analysis. However, there are regions in the world with a high incidence of esophageal cancer, in particular, China and South Africa, where some investigators have consistently detected HPV-DNA in a minority ( $\approx 20\%$ ) of squamous cell esophageal cancers by PCR and *in situ* hybridization (60–63). An HPV16 viral load greater than one viral copy per tumor cell genome, consistent with a clonal relationship, has been reported in a small proportion of tumors from high-incidence areas in China (64). Analysis of viral integration or transcription in these tumors has not been reported.

Seropositivity to HPV16 capsid proteins has been associated with an estimated 4.5-fold increased risk of esophageal cancer in a Chinese population (65), after adjustment for age and gender, whereas no association with risk was reported in a Scandinavian population (66). The difference could possibly be attributed to a

**Table 2.** Relationship of site-specific cancers to the characteristics of human papillomavirus (HPV)-associated malignancies\*

Anatomic site	HPV		Squamous cell histology (basaloid)	Direct exposure to HPV possible	HPV-positive benign lesions present at site	E6 and E7 serum antibodies present in case patients	Association with high-risk sexual behavior	High risk in	
	Genome present	E6 and E7 transcripts present						Immunosuppressed individual	Individual with history of HPV-associated malignancy
Cervix	+	+	+	+	+	+	+	+	+
Vulva	+	+	+(+)	+	+	+	+	+	+
Anal canal	+	+	+(+)	+	+	?	+	+	+
Oropharynx	+	+	+(+)	+	+	+	+	†	+
Penile cancer	+	?	+(+)	+	+	?	+	†	?
Conjunctiva	+	+	+	+	+	?	?	†	?
Esophagus	?	?	+	±	–	?	?	–	‡
Retinoblastoma	?	?	–	–	–	?	?	?	?
Prostate	–	N/A	–	+	–	?	+	–	–
Breast	–	N/A	–	–	–	?	–	–	–
Lung	?	?	±	–	+	?	?	–	‡
Bladder	–	N/A	±	+	+	?	?	–	‡
Colon	–	N/A	–	–	–	?	?	–	–

\*+ = association present; ? = not sufficiently evaluated; – = evaluated, no association present; N/A = not applicable.

†Data for human immunodeficiency virus infection only.

‡Likely confounded by tobacco exposure.

lack of adjustment in the Chinese study for other established risk factors, such as alcohol consumption, tobacco use, and possibly dietary sources of nitrosamines and micronutrients (67,68). Initial estimates of a six- to 14-fold increase in risk of esophageal cancer associated with seroreactivity to HPV16 capsid proteins in nested case-control studies in Scandinavian populations were no longer observed after adjustment for alcohol consumption and tobacco exposure in a case-control study performed in a similar population by the same investigators (66,69,70). An association between alcohol consumption and tobacco use and seroreactivity to HPV capsid proteins has been reported in some populations.

Several investigators have suggested that the role of HPV in the pathogenesis of esophageal cancer could differ by geographic regions. However, HPV infection is common and widespread throughout the world. There are no studies that have pinpointed specific cofactors that would explain why HPV would infect or transform the esophagus more readily in particular geographic regions. In some areas with a high risk of esophageal cancer in China and Latin America, the consumption of very hot beverages (hot mate or boiling water) and the consumption of fermented fish have been identified as probably carcinogenic for the esophagus (71-74). Chronic thermal injury, esophagitis, and dietary carcinogens may be predisposing factors for HPV-related carcinogenesis. A recent decline in esophageal cancer incidence rates coincident with changes in diet and food-preservation methods may underscore the importance of dietary factors in high-incidence areas of China (75). Esophageal cancer has not been associated with sexual behavior, immunosuppression, or a previous diagnosis of an HPV-associated malignancy (after adjustment for the effects of tobacco exposure).

The inability to detect HPV in esophageal cancers has led some to consider a hit-and-run mechanism for HPV at this anatomic site. By analogy, BPV-4 causes diffuse alimentary tract papillomatosis in cattle. This infection is usually self-limited, unless the cattle are ingesting bracken fern. Bracken fern contains both immunosuppressants and a DNA-damaging agent called quercetin. The immunosuppressed cattle are unable to clear the BPV-4. Viral oncoproteins expressed in the BPV-4-infected cell abrogate the G<sub>1</sub> arrest induced by the DNA-damaging agent quercetin and thereby promote tumor progression (76). Despite the presence of BPV in benign papillomas, the virus is rarely found in tumors, suggesting its importance in tumor initiation and promotion but not maintenance of the malignant phenotype. Although both the absence of HPV in benign esophageal lesions and the lack of an association between HPV L1 seroreactivity and esophageal cancer risk argue against this possibility in Western cultures, further case series and case-control studies in high-incidence areas, such as Africa and China, are warranted. These studies should include detailed evaluations of dietary or medicinal exposures that may be analogous to bracken fern in cattle.

### Retinoblastoma

A single report (77) has linked HPV types 16 and 18 with retinoblastoma, the most common ocular tumor of childhood. HPV type 16 or 18 DNA was found by PCR in 14 of 39 tumor specimens from sporadic retinoblastoma cases in Mexico City. Clinical characteristics of children with tumors classified as HPV-positive by PCR were not distinct from HPV-negative case patients (including age or proportion with bilateral disease), with

the exception of low birth weight. The presence of HPV-DNA was associated with the presence of pRB by immunohistochemistry, although this association did not reach statistical significance. No analysis of Rb mutations was performed in tumors or peripheral blood lymphocytes.

Retinoblastomas arise from fetal or infantile retinal cells that have loss of function of both allelic copies of the tumor suppressor gene Rb through either germline or somatic mutation (or rarely by promoter hypermethylation). However, approximately 10% of the tumors have neither (78). Given that the viral oncoprotein E7 of high-risk HPV types binds to and inactivates pRB, it is biologically plausible that HPV infection could be functionally equivalent to the biallelic loss of Rb. Transgenic mice expressing high-risk HPV16 E6 and E7 proteins develop retinoblastoma (79). The relevance of this finding to human disease is unclear, however, since there is no evidence that HPV has the capacity to infect retinal cells. There are no associations of HPV with benign retinal disease.

Presumably, exposure to HPV would occur peripartum from genital infection of the mother. Exposure *in utero* has been hypothesized, but there is little objective evidence to support it (80). Although HPV-DNA has been detected in vaginal secretions and amniotic fluid at delivery, several studies (80,81) have indicated that peripartum infection of neonates by high-risk HPV is a rare event. None of these studies specifically investigated ocular infection, however. Risk of retinoblastoma has not been linked to genital HPV infection or sexual behavior in the mother. There is no known increase of HPV-associated malignancies in individuals with a history of retinoblastoma. Although the incidence of retinoblastoma (as well as Burkitt's lymphoma and Kaposi's sarcoma) has increased in regions of Africa since the beginning of the HIV epidemic, suggesting an association with an infectious agent, there is no evidence that this can be attributed to HPV (82).

### Prostate Cancer

HPV is unlikely to play a direct role in the pathogenesis of adenocarcinoma of the prostate. The direct physical continuity between the prostate and the urethra, the latter being a known reservoir for infection by HPV, may possibly explain why HPV-DNA is frequently reported from prostatic tissues. HPV-infected cells shed from the urethra into seminal fluid likely explain why HPV-DNA has been detected in normal prostate as well as in benign prostatic hyperplasia and prostate cancer in some series. Contamination of specimens by sampling through the urethra may also occur. There are few case reports in which HPV is detected in prostate cancer by methods other than extremely sensitive PCR (i.e., *in situ* hybridization and Southern blot). A single quantitative PCR-based study (83) detected HPV in quantities insufficient for a clonal (one-to-one) relationship to tumor. Seropositivity to HPV type 18 (HPV18) capsid proteins, the type most strongly associated with cervical adenocarcinoma, was associated with increased risk of subsequent development of prostate cancer, after adjustment for tobacco use, body mass index, and seroreactivity to *Chlamydia trachomatis* and HPV type 33 (84). However, a case-control study of prostate cancer patients (85) found no HPV-DNA in cancers or benign prostatic tissues and no difference in seropositivity to HPV type 16 or 11 in case patients and control subjects. Some epidemiologic studies (86,87) have identified a history of a sexually transmitted disease as a risk factor for prostate cancer but have not shown any

association with a particular sexually transmitted agent. It is possible that the association of sexually transmitted disease with the risk of prostate cancer, in the absence of a consistent association with any particular agent, may be explained by the role of chronic inflammation in causing genomic oxidative damage to prostate epithelium, as per a current model for prostate cancer pathogenesis (88).

### Breast Cancer

HPV is unlikely to play a role in the development of breast cancer. The hypothesis that HPV might cause adenocarcinoma of the breast originated from an experimental system in which a human mammary cell line was immortalized after transfection with the full-length HPV type 16 or 18 genome (89). However, there is no evidence that HPV can infect human mammary tissue *in vivo*. Several investigators have analyzed breast tumor specimens for HPV genomic sequences by PCR; the majority of the studies have been negative. Those studies that documented the viral genome by sensitive PCR methods have not been able to confirm the presence of the virus by more specific methods such as *in situ* hybridization. Low-risk HPV has rarely been identified in benign squamous papillomas of the mammary nipple (90), and HPV has not been found in Paget's disease of the breast (91). Individuals with breast cancer do not have an increased risk of HPV-associated malignancies and, moreover, have a seroprevalence for HPV capsid proteins that is equivalent to that in control subjects (43,92). There is no evidence that either sexual behavior or immunosuppression is related to risk of breast cancer.

### Lung Cancer

Data available to date do not make a compelling case that HPV infections contribute to the development of squamous cell carcinoma of the lung. HPV infection of the lung does occur in the few instances when children with recurrent laryngeal papillomas secondarily develop pulmonary papillomas, probably as a result of the seeding of areas of squamous metaplasia in the lung with infected cells from the laryngeal papillomas. Pulmonary involvement generally follows repeated surgical procedures to remove the laryngeal papillomas. Several studies have examined lung cancers for HPV genomic sequences and have reported HPV prevalences over a wide range, from 0% to almost 80%. Investigators who report positive tumors have not been able to ascertain the physical state of the viral genome (integrated or not) or the copy numbers of the viral genome in tumor cells. In a study in Taiwan, HPV-DNA prevalence in lung cancers, for both HPV16 and HPV18, was statistically significantly greater in females and in nonsmokers. This finding was considered noteworthy because in Taiwan, although only 10% of the women smoke, about one-third of the lung cancer deaths occur in women. Thus, it was suggested that HPV infections may contribute to the development of lung cancer in women in some areas of Taiwan (93). Although provocative, these data are inconsistent with the majority of negative studies in the literature in which HPV genomic sequences are not detected. In the Taiwanese study, HPV sequences were recovered from as many as 15% of the noncancerous lungs from control subjects. This would suggest that HPV was commonly transmitted by aerosol, but such a mode of transmission is most unlikely for HPV.

### Bladder Cancer

It is unlikely that HPV infections play a role in the development of bladder cancers. In the developed world, the majority of bladder cancers are transitional cell carcinomas. By contrast, in schistosome endemic countries, the majority are squamous cell cancers. Although HPV sequences have been reported from 0% to 80% of bladder tumors when analyzed by PCR, the majority of the studies have been either completely negative for HPV genomic sequences or have reported sequences in fewer than 10% of the cancer tissues, regardless of histology (94–96).

The majority of transitional cell carcinomas of the bladder result from exposure of the bladder mucosa to environmental carcinogens in the urine (e.g., tobacco and industrial carcinogens). Because HPV is not hematogenously spread, HPV infections would not be able to reach the bladder in this manner. However, in rare instances, patients with urethral condylomas may develop condyloma in the bladder by retrograde infection; therefore, exposure in this manner is plausible. The increase in the risk of bladder cancer observed with time since treatment of women with a history of invasive and not *in situ* cervical cancer would suggest that radiation therapy, rather than HPV infection, is the relevant exposure (97). Individuals with HIV infection are not at increased risk of bladder cancer (98). Sexual behavior has not been specifically linked with bladder cancer risk.

### Colon, Ovarian, and Endometrial Cancers

There is no credible evidence that HPV infections contribute to the development of colon, ovarian, or endometrial cancer. Studies of these cancers have reported both positive and negative findings for HPV genomic sequences, but the case has not been made that HPV infections contribute to their development. Of interest, low-risk HPV types 6 and 11 have been demonstrated to secondarily colonize regions of squamous metaplasia of the endometrium (99) and regions of squamous differentiation within adenocarcinomas of the endometrium (100).

### DISCUSSION

The International Agency for Research on Cancer came to the conclusion that high-risk mucosal HPVs (types 16 and 18) are carcinogenic in humans, after formal evaluation of the cause–effect relationship between HPV and cervical cancer. This analysis applied the modern criteria for causal inference attributed to Sir A. Burton Hill (101,102); these criteria include the strength, consistency, and specificity of the association, biologic plausibility, coherence and gradient (dose–effect), temporality, experimental evidence, and analogy. Hill's criteria do not establish conditions that must be met as proof of cause but rather provide a framework for the process of continual reassessment of evidence supporting a given factor's role in a causal pathway for disease. Using these criteria of Hill, a comprehensive review of the literature on the association between cervical cancer and HPV infection has been published recently (103).

When considering a possible cause–effect relationship between mucosal HPV and a nongenital cancer, one might expect that proposed associations would fit coherently within our current framework of knowledge of the epidemiology and biology of HPV infection. The shared characteristics of cancers that are well documented as HPV associated provide a basis for extrapolation to other possible HPV-associated tumors in general. This constellation of shared features is distinct from the formal evalu-

ation of a possible cause–effect relationship between an exposure and disease, as per the criteria of Hill. Although these latter criteria are applied in investigating the relationship between an hypothesized carcinogen and cancer, the set of recurrent characteristics described here can only be applied to cancers proposed to have a common etiology, i.e., mucosal HPV-associated tumors. It seems reasonable to extrapolate in this way, based on the strong and extensive evidence that has established HPV as a human carcinogen.

If HPV plays a role in the pathogenesis of other human tumors, those HPV-associated tumors would likely be more analogous to distinct subsets of vulvar and oropharyngeal cancers, i.e., as one class of such etiologically heterogeneous tumors. In this case, all evidence, including risk factors, clinical presentation and natural history, histopathology, immunology, and virology, would be used to create a coherent picture of the distinct subset of tumors that is HPV associated. Early clues in the studies of human cancers for HPV etiology have almost always come from the identification by PCR analysis of HPV genomic sequences in cancer tissues. This is true for all of the cancers discussed above. However, the PCR technology is well-known to be potentially error prone. Therefore, studies that are unconfirmed for HPV presence by other more specific methods should be viewed with considerable caution. Even if viral sequences are authentically present, such results provide only the rationale for examining their etiologic significance by other means. Important steps in this effort would include a detailed study of the tumor cell–virus relationship, moving from a case series to a case–case and case–control design, and evaluation of the HPV association in the context of other known risk factors for the cancer in question.

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