Human Papillomaviruses: Some Genital-Mucosal Types*

Known to be a human carcinogen
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Carcinogenicity

Some human papillomaviruses (HPVs) of the genital-mucosal type are *known to be human carcinogens* based on sufficient evidence of carcinogenicity in humans. In epidemiological research, numerous case-control studies have consistently reported strong associations between cervical cancer and infection with HPV-16, HPV-18, or "high-risk" HPVs as a class (discussed under "Properties," below). Moreover, several recent case-control studies have provided strong evidence of positive associations between cervical cancer and other individual HPVs, including HPV types 31, 33, 35, 39, 45, 51, 52, 58, and 59 (Muñoz 2000). Cohort studies have demonstrated that infection with HPV-16 or with high-risk HPVs as a class occurs before the development of high-grade cervical intraepithelial neoplasia (CIN), which is thought to be a precursor of invasive cancer. The evidence from cohort studies is weaker for individual high-risk viruses possibly because they are less common; among these, the evidence for an association with cervical cancer appears to be strongest for HPV-18 (NTP 2003). It is highly unlikely that the association between HPV infection and cervical cancer is due to confounding by other factors that could increase the risk of cancer, because many studies have adjusted for most potential confounders, and because of the large magnitude of the ORs estimated in the case-control studies. Thus, these studies demonstrate that some genital-mucosal HPVs cause cervical cancer. In addition, there is strong evidence that HPV-16 infection is associated with other anogenital cancers, especially cancer of the vulva (NTP 2003). Evidence also suggests associations between HPV infection and some cancers of the head and neck and, especially, the oropharynx (the soft palate, tonsils, and back of the tongue and throat) (NTP 2003).

In 1995, the International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of HPVs and concluded that HPV types 16 and 18 were known human carcinogens (Group 1), HPV types 31 and 33 were probably carcinogenic to humans (Group 2A), and some HPV types other than 16, 18, 31, or 33 were possibly carcinogenic to humans (2B). IARC also stated that there was some evidence suggesting that HPV types 6 and 11 did not cause cervical cancer (IARC 1995). At the time of the IARC review, few studies had evaluated the carcinogenicity of HPV types other than 16 and 18 because of the lower prevalence of these viruses. The conclusions above concerning these other HPV types are based mainly on the findings of numerous studies published since the 1995 IARC review.

Based on testing of tissue specimens from more than 1,000 invasive cervical cancers in women from 22 countries (collected for the International Biological Study of Cervical Cancer), it was estimated that HPV is present in 99.7% of all cervical carcinomas, suggesting that HPV infection may be necessary for development of cervical cancer (Walboomers *et al.* 1999). Nonetheless, not all individuals infected with HPV develop cervical cancer. Most HPV infections (about 70%) are transient, clearing within 1 to 2 years, and thus confer little risk of cancer. The specific risk factor for cervical cancer appears to be persistent infection with HPV-16 or other high-risk HPVs. Whether HPV infections persist probably depends both on viral characteristics, such as greater persistence of specific HPV types or variants, and on characteristics of the patient, such as sex hormone levels, smoking behavior, or immune-system status.

Because HPV infections are specific to humans, laboratory animals cannot be experimentally infected with them. Many studies have investigated the carcinogenicity of various animal papillomaviruses both in their natural host species and in other species. Studies in monkeys, cattle, rabbits, and sheep have shown that animal papillomaviruses cause cancer in their natural hosts. Studies in transgenic mice carrying HPV genes have demonstrated that HPV proteins play a role in the development of dysplasia (abnormal tissue growth) and progression to tumor formation. Transgenic mice expressing some HPV type 16 or 18 genes and producing the corresponding viral proteins develop tumors of the cervix and other tissues (Arbeit *et al.* 1994, Comerford *et al.* 1995).

Additional Information Relevant to Carcinogenicity

Infection with high-risk HPVs is associated with genetic instability (chromosomal aberrations) including abnormal centrosome numbers, chromosomal imbalances at specific chromosomal regions (e.g., extra copies of the region in one member of a pair of chromosomes), and changes in chromosome number, including tetrasomy (the presence of two extra chromosomes of one type) and other types of aneuploidy.

HPV can integrate into the DNA of the host cell and can immortalize and transform cells, enabling them to proliferate and form tumors. Most studies on the mechanisms of HPV carcinogenesis have investigated HPV-16 and HPV-18. HPV types 16, 18, 31, and 33 have been shown to transform cells, types 16, 18, and 31 to immortalize cells, and types 16 and 18 to produce proteins that bind to regulatory proteins of the host cell. The HPV proteins E2 and E5 and the long control region of the HPV genome (discussed under "Properties," below) play a role in HPV-induced cell transformation. However, the HPV proteins primarily responsible for immortalization and transformation are E6 and E7, as shown in studies with human and rodent cell cultures. Studies with transgenic mice expressing the E6 or E7 gene further support the notion that the E6 and E7 proteins are important in HPV-associated neoplasia. Both the E6 and E7 proteins alter the pathways that regulate tissue growth, by interfering with growth receptors or growth factors; production of cytokines has been shown to be altered in cells infected with HPV-16. The E6 protein increases degradation of the p53 tumor-suppressor protein, thereby interfering with apoptosis (programmed cell death). The E7 protein disrupts complexes of the transcription factor E2F with the tumor-suppressor protein pRb and related proteins involved in control of the cell cycle and causes their degradation, altering control of transcription and progression of the cell cycle. The E7 protein has been shown to cause abnormal synthesis and duplication of centrosomes, resulting in abnormal mitotic division.

Properties

HPVs of the genital-mucosal type are DNA viruses that infect the genital skin and genital and non-genital mucosa, sometimes causing genital warts or cervical abnormalities. They are members of the *Papillomaviridae* family, which consists of species-specific non-enveloped viruses that infect the squamous epithelium of the skin and mucosal membranes of animals. More than 100 different HPVs have been identified to date (2004) and consist of viruses that cause skin warts as well as the genital-mucosal type viruses (Howley and Lowy 2001). The over 40 genital-mucosal HPVs have been classified in the literature as either "high risk" or "low risk;" high risk viruses have been associated with cervical cancer in human epidemiological studies, whereas low-risk viruses have been associated with genital warts or low-grade CIN (abnormal tissue growth in the cervical epithelium that is unlikely to progress to cancer). Most studies to date (2004) have considered HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 to be high-risk viruses; some studies also include other HPVs, most notably HPV-66. Classification of HPVs is based also on phylogenetic and mechanistic considerations. Most high-risk viruses have DNA sequences highly similar to those of either HPV-16 or HPV-18, suggesting that they are closely related to these types.

Studies on the mechanisms of carcinogenesis have shown that high-risk but not low-risk viruses immortalize human keratinocytes (skin cells), interact with the tumor-suppressor proteins pRb and p53, and induce changes in chromosome aberrations. However, most mechanistic studies have evaluated only a few HPVs, the majority focusing on HPV-16 or HPV-18 and a few on HPV-31 or HPV-33.

HPVs are small (about 52 to 55 nm in diameter), consisting of approximately 8,000 base pairs of covalently closed, double-stranded DNA. The viral genome consists of a series of open reading frames, each of which is a DNA sequence that codes for an HPV protein, and a long control region, which contains elements that regulate DNA replication and protein synthesis. Productive infection of cells (leading to replication of the virus) is linked to their stages of differentiation. Viral replication can be divided into early and late stages, which occur in cells at different stages of differentiation. Early stages of replication (attachment of the virus to the cell, entry and uncoating, early gene expression, protein production, and DNA replication) occur in basal cells (the youngest, least differentiated cells, in the lower layers of the epithelium) because these are the only dividing cells in the squamous epithelium. Late stages of viral replication include the events leading to production of viral particles (late gene expression, production of capsid proteins, vegetative viral DNA replication, virus assembly, and release) occur in the terminally differentiating squamous epithelial cells (the oldest, most differentiated cells, in the upper layers of the epithelium). The genes expressed in the early stages of viral replication, designated E1 through E8, are associated with regulation of transcription (e.g., E2) and cellular proliferation (e.g., E6 and E7). The genes expressed in the late stages, designated L1 and L2, encode the two proteins that make up the viral capsid (the coat surrounding the DNA) (Howley and Lowy 2001).

Infection, Prevention, and Treatment

Genital-mucosal HPVs infect the cervix, causing lesions of varying severity, including genital warts, low- and high-grade CIN, and invasive cervical cancer (Einstein and Burk 2001).

Low-grade CIN (CIN I) is a well-differentiated lesion in which the squamous epithelial cells show alterations characteristic of the cytopathogenic effects of a replicative viral infection, such as the presence of two nuclei or other nuclear abnormalities and koilocytosis (the presence of cells with abnormal nuclei and a hollow appearance resulting from collapse of the cell's internal structure). The alterations seen in CIN I are not usually considered to be precursors of cancer. The majority of CIN I lesions are transient and resolve spontaneously, but a small percentage may progress to high-grade CIN or invasive cancer (Jastreboff and Cymet 2002). Both high-risk and low-risk HPVs can cause low-grade CIN (IARC 1995).

High-grade CIN (CIN II or III) is characterized by the presence of undifferentiated cells above the lower third of the epithelium (extending into the upper layers) and by nuclear crowding, substantial pleomorphism, loss of tissue organization and cellular polarity, abnormal mitotic figures (i.e., abnormal appearance of chromosomes in dividing cells), and larger numbers of atypical cells than observed in low-grade CIN (IARC 1995). High-grade CIN probably results from persistent HPV infection, and it is more likely than low-grade CIN to progress to invasive cancer. (CIN III also is known as carcinoma *in situ*, or non-invasive cancer.) Microinvasive squamous-cell cervical cancer usually arises from high-grade CIN.

Treatment of HPV infection depends on the severity of the disease and may involve topical applications, interferon-related therapies, or excision of the lesion via laser methods, surgery, or cryotherapy. As of 2004, early-phase clinical trials of prophylactic vaccines using HPV-16 L1 capsid antigens were under way.

Detection

HPV infection is detected by observation of visible lesions or microscopic changes in cells, by detection of HPV DNA, or by serological tests (assays to detect antibodies to HPV antigens in the blood).

Genital warts (*condylomata acuminata*) are genital lesions visible to the naked eye; they have a fleshy red appearance and a raised surface that usually extends in fingerlike projections (papillae). Flat condylomata are flat, nonpapillary lesions; they are more difficult to detect and may be apparent only after swabbing with acetic acid and colposcopic examination, in which they appear as white, flat, shiny lesions. The Papanicolaou (Pap) smear, which involves microscopic examination of stained exfoliated genital cells, detects koilocytosis and other signs of CIN; it is used to screen for cervical cancer by detecting high-grade CIN (Trofatter 1997).

The most sensitive and specific method for detecting HPV infection is to test for HPV DNA. DNA testing can be used to detect a broad spectrum of HPV genotypes (Trofatter 1997). Detection of HPV DNA signifies present exposure or persistent infection resulting from a past exposure (Dillner 2000). The most sensitive HPV DNA tests are (1) those based on the polymerase chain reaction and (2) the hybrid capture assay, which is based on the formation of hybrids between HPV DNA and RNA probes.

The most commonly used serological tests for HPV infection measure antibodies (immunoglobulin G) against capsid antigens (most often tested as virus-like particles). Several validation studies have estimated the sensitivity of such serological tests to be approximately 50%, using detection of HPV DNA as a standard (Dillner 2000). Because of their low sensitivity, serological assays are not recommended for diagnostic use, but they are useful for comparison of groups in epidemiological studies, which also commonly use HPV DNA testing. Currently, (2004), clinical diagnosis of HPV most commonly is based on the hybrid capture II assay

Exposure

Genital-mucosal HPVs are transmitted primarily through sexual contact with infected cervical, vaginal, vulvar, penile, or anal epithelium (IARC 1995). This finding is supported by numerous epidemiological studies demonstrating that HPV infection is associated with behaviors related to sexual activity. Numerous studies of HPV in women have reported a positive association between lifetime number of sex partners and HPV seropositivity (Sun *et al.* 1999, Silins *et al.* 2000) or the presence of HPV DNA (Franco *et al.* 1995, Kjær *et al.* 1997, Lazcano-Ponce *et al.* 2001). Recent sexual activity, the number of sex partners, frequency of sexual intercourse, and presence of genital warts on sex partners are strong predictors of HPV infection, as indicated by HPV DNA testing (Franco *et al.* 1995, Ho *et al.* 1998). The role of men in carrying HPV infection from one woman to another has been demonstrated in studies showing that cervical cancer is relatively more frequent among wives whose husbands have detectable HPV DNA in their penis or whose husbands have had more extramarital partners (Bosch *et al.* 1996). Penile lesions containing the DNA of high-risk HPVs are frequent among male sex partners of women with CIN (Bleeker *et al.* 2002). There are conflicting reports as to whether HPV is transmitted at birth or perinatally. Infants exposed perinatally to HPV-11, or less commonly to HPV-6, may develop a rare benign tumor of the airway called juvenile-onset recurrent respiratory papillomatosis (Shoultz *et al.* 1997).

HPV infection is one of the most common sexually transmitted diseases. It appears that the majority of those infected have no symptoms, and it is estimated that approximately 20 million people in the United States are infected with HPV (CDC 2001). The percentage of infected individuals (prevalence) is highest among those who are young and sexually active. U.S. epidemiological studies based on HPV DNA testing indicate that between 25% and 40% of sexually

active women aged 15 to 25 are infected (Lowy and Howley 2001). Among all U.S. men and women aged 15 to 49, the estimated prevalence of HPV infection (based on HPV DNA testing) is 10% to 20%, whereas only 1% have genital warts, and 4% show cellular abnormalities associated with HPV infection (Koutsky 1997). For most populations of mixed age groups, prevalence of HPV infection has been estimated at 5% to 15%. HPV-16 appears to be the most prevalent type worldwide (Jastreboff and Cymet 2002). In a study of women aged 18 to 40 with no history of high-grade CIN, among whom the prevalence of HPV was 39%, high-risk HPVs were more common (occurring in 26.7% of women) than low-risk HPVs (occurring in 14.7%) (Peyton *et al.* 2001).

The Centers for Disease Control and Prevention estimates the number of new genital HPV cases per year (incidence) to be approximately 5.5 million (CDC 2001). In the general population of Rochester, MN, the average age- and gender-adjusted incidence of genital warts increased from 13 per 100,000 in the early 1950s to 106 per 100,000 in the late 1970s. This is a period during which the U.S. incidence of other sexually transmitted diseases increased dramatically (IARC 1995, Shoultz *et al.* 1997). Several follow-up studies have reported very high incidences of HPV infection (as detected by HPV DNA testing) among young, sexually active individuals, with three-year cumulative incidences ranging from 43% to 55% (Ho *et al.* 1998, Moscicki *et al.* 2001).

In most women infected with HPV (70%), the infection clears within 12 to 24 months (Franco *et al.* 1999, Dillner 2000). Some studies have suggested that low-risk HPV infections are more likely to regress than are high-risk HPV infections (Franco *et al.* 1999, Elfgren *et al.* 2000). The immune system plays an important role in HPV infection; immunocompromised patients are at increased risk for persistent HPV infection (Lowy and Howley 2001).

Regulations And Guidelines

No specific regulations or guidelines relevant to reduction of exposure to HPVs were identified.

*No separate CAS registry number assigned to HPV.

REFERENCES

Arbeit, J. M., K. Münger, P. M. Howley and D. Hanahan. 1994. Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J Virol 68(7): 4358-4368.

Bleeker, M. C., C. J. Hogewoning, A. J. Van Den Brule, F. J. Voorhorst, R. E. Van Andel, E. K. Risse, T. M. Starink and C. J. Meijer. 2002. Penile lesions and human papillomavirus in male sexual partners of women with cervical intraepithelial neoplasia. J Am Acad Dermatol 47(3): 351-7.

Bosch, F. X., X. Castellsague, N. Munoz, S. de Sanjose, A. M. Ghaffari, L. C. Gonzalez, *et al.* 1996. Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J Natl Cancer Inst 88(15): 1060-7.

CDC. 2001. Tracking the Hidden Epidemics 2000: Trends in STDs in the United States. Center for Disease Control. Last reviewed 7/3/01. http://www.cdc.gov/nchstp/od/news/RevBrochure1pdfcloselookhpv.htm.

Comerford, S. A., S. D. Maika, L. A. Laimins, A. Messing, H. P. Elsasser and R. E. Hammer. 1995. E6 and E7 expression from the HPV 18 LCR: development of genital hyperplasia and neoplasia in transgenic mice. Oncogene 10(3): 587-597.

Dillner, J. 2000. Trends over time in the incidence of cervical neoplasia in comparison to trends over time in human papillomavirus infection. J Clin Virol 19(1-2): 7-23.

Einstein, M. H. and R. D. Burk. 2001. Persistent human papillomavirus infection: definitions and clinical implications. Papillomavirus Report 12: 119-123.

Elfgren, K., M. Kalantari, B. Moberger, B. Hagmar and J. Dillner. 2000. A population-based five-year follow-up study of cervical human papillomavirus infection. Am J Obstet Gynecol 183(3): 561-567.

Franco, E. L., L. L. Villa, A. Ruiz and M. C. Costa. 1995. Transmission of cervical human papillomavirus infection by sexual activity: differences between low and high oncogenic risk types. J Infect Dis 172(3): 756-63.

Franco, E. L., L. L. Villa, J. P. Sobrinho, J. M. Prado, M. C. Rousseau, M. Desy and T. E. Rohan. 1999. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 180(5): 1415-1423.

Ho, G. Y., R. Bierman, L. Beardsley, C. J. Chang and R. D. Burk. 1998. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 338(7): 423-8.

Howley, P. M. and D. R. Lowy. 2001. Papillomaviruses and their replication. In Fields' Virology. D. M. Knipe and P. M. Howley, eds. Philadelphia: Lippincott Williams & Wilkins. pp. 2197-2229.

IARC. 1995. Human Papillomaviruses. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 64. Lyon, France: International Agency for Research on Cancer.

Jastreboff, A. M. and T. Cymet. 2002. Role of the human papilloma virus in the development of cervical intraepithelial neoplasia and malignancy. Postgrad Med J 78(918): 225-8.

Kjær, S. K., A. J. van den Brule, J. E. Bock, P. A. Poll, G. Engholm, M. E. Sherman, J. M. Walboomers and C. J. Meijer. 1997. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev 6(10): 799-805.

Koutsky, L. 1997. Epidemiology of genital human papillomavirus infection. Am J Med 102(5A): 3-8.

Lazcano-Ponce, E., R. Herrero, N. Muñoz, A. Cruz, K. V. Shah, P. Alonso, P. Hernández, J. Salmerón and M. Hernández. 2001. Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer 91(3): 412-20.

Lowy, D. R. and P. M. Howley. 2001. Papillomaviruses. In Fields Virology. D. M. Knipe and P. M. Howley, eds. Philadelphia, PA: Lippincott Williams & Wilkins. pp. 2231-2264.

Moscicki, A. B., N. Hills, S. Shiboski, K. Powell, N. Jay, E. Hanson, *et al.* 2001. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. Jama 285(23): 2995-3002.

Muñoz, N. 2000. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 19(1-2): 1-5.

NTP. 2003. Report on Carcinogens Background Document for Human Papillomaviruses: Genital-Mucosal Types. National Toxicology Program. http://ntp-server.niehs.nih.gov/newhomeroc/roc11/HPV_RG2_Public.pdf.

Peyton, C. L., P. E. Gravitt, W. C. Hunt, R. S. Hundley, M. Zhao, R. J. Apple and C. M. Wheeler. 2001. Determinants of genital human papillomavirus detection in a US population. J Infect Dis 183(11): 1554-64.

Shoultz, D. A., L. A. Koutsky and D. A. Galloway. 1997. Epidemiology and modes of transmission. In Human Papillomavirus Infections in Dermatovenereology. G. Gross and G. von Krogh, eds. Boca Raton, Florida: CRC Press. pp. 83-97.

Silins, I., I. Kallings and J. Dillner. 2000. Correlates of the spread of human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 9(9): 953-9.

Sun, Y., J. Eluf-Neto, F. X. Bosch, N. Munoz, J. M. Walboomers, C. J. Meijer, K. V. Shah, B. Clayman and R. P. Viscidi. 1999. Serum antibodies to human papillomavirus 16 proteins in women from Brazil with invasive cervical carcinoma. Cancer Epidemiol Biomarkers Prev 8(10): 935-40.

Trofatter, K. F. 1997. Diagnosis of human papillomavirus genital tract infection. Am J Med 102(5A): 21-7.

Walboomers, J. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, *et al.* 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189(1): 12-19.