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Dennis J. McCance, Editor

Human Papilloma Viruses

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Human Papillomaviruses

Editor

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Human Papillomaviruses

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Preface

Human papillomaviruses are the cause of benign, premalignant and malignant lesions of stratified epithelia. While benign warts were a recognized clinical lesion in Roman times, it was not until the 1970s that papillomavirus infection was associated with certain cytological properties of premalignant disease of the cervix. In the last two decades the evidence that papillomaviruses cause various epithelial cancers has been building, and it is now clear these viruses are the causative agent. However, while papillomaviruses cause benign and premalignant lesions, there are unknown factors that are necessary in combination with the virus for progression to malignant disease.

This volume looks at the epidemiological evidence for association of HPV infection and disease, at the biology of viral proteins and the immune response to infection, all of which contribute to the pathogenesis. The effort is concentrated on those viruses which cause premalignant and malignant disease, as most of our knowledge of the interactions of the virus with host cells comes from the study of the oncogenic viruses. For all papillomaviruses to replicate in the stratified epithelium they must stimulate keratinocytes, which are programmed for terminal differentiation, to re-enter the cell cycle and progress through G1 into the S-phase of the cell cycle. The S-phase is necessary so that the replicative machinery of the cell will be available for the virus to utilize. The early proteins of HPV, E6, E7 and E5, are involved in the G1 to S-phase progression and their role and functions are discussed. Two other early proteins, E1 and E2, are involved in the replication process itself and possibly in the control of transcription of the early genes. New functions of another, rather enigmatic early protein, E4, is also discussed. The pathogenesis of papillomaviruses is limited by the host's immune response and this is discussed with particular emphasis on the potential vaccines, which are being tested at present. The composition of a protective immune response is unknown, but there is evidence that both a T- and B-cell response is required with the latter being directed against conformational epitopes on the L1 and 2 capsid proteins. This volume will be of benefit to scientists, clinicians and students who want an up-to-date account of the progress in human papillomavirus biology.

Contents

Human Papillomaviruses
D.J. McCance (editor)

Clinical aspects and epidemiology of HPV infections

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Overview

Human papillomaviruses (HPVs) are a ubiquitous group of viruses that infect the epithelium and are associated with a broad range of clinical manifestations. Numerous HPVs cause no apparent disease whereas others are involved with the development of benign conditions such as common hand and foot warts, rare disorders such as epidermodysplasia veruciformis (EV) and invasive cancers of various anatomic sites. The recent intense focus on HPVs is founded in the establishment of their causal relationship with invasive cervical cancer.

Infection by HPVs most often occurs in cells of the epithelium including the genitalia and elsewhere. In addition to extragenital HPV infections of the skin, infections of the mouth, esophagus, larynx, trachea and conjunctiva have been reported. Table 1 lists the different types of HPV [39] for which genomes have been cloned and their more common clinical manifestations.

The majority of HPV infections remain clinically inapparent; however microfoci of infection obviously contribute to their spread within populations. The specific modes of HPV transmission are not well understood. Infection of actively proliferating basal epithelium through microlesions is the presumed point of viral entry. The number of proliferating cells exposed and the infecting dose of virus may affect the outcome of the HPV infection, but again, little is known in this regard. Expression of early viral genes occurs within the proliferative and differentiating part of the infected epithelium whereas the late-gene expression is limited to the outer differentiated layers. Thus the full virus life-cycle and production of virion is tightly linked to and requires cell differentiation. Until recently, this requirement had prevented for many years the successful production of infectious HPV particles under culture conditions.

HPV infection of host cells can result in both permissive and persistent infections. Permissive infections are characterized by a complete virus life-cycle including virion production. Persistence of productive HPV infections appears to be common for durations of at least months although in some instances viral persistence may continue for years or decades. The extent to which long-term persistent HPV infections are productive has not been defined. It is, however, presumed that persistent infections of these longer durations can increase the risks for cancer outcomes. This risk association can be easily rationalized given the fact that like most small

Table 1

HPV types with cloned genomes and common clinical manifestations

HPV type	Clinical manifestations	Subfamily/ Genus	Accession #	Ref.
HPV 1	Plantar warts	E1	V01116	35
HPV 2	Common warts	A4	X55964	130)
HPV 3	Flat and juvenile warts	A2	X74462	95
HPV 4	Palmar and plantar warts	B2	X70827	72
HPV 5	EV lesions and carcinomas	B1	M17463	191
HPV 6	Genital warts, CIN, VIN	A10	X00203	40
HPV 7	Butcher warts	A8	X74463	131
HPV 8	EV lesions and carcinomas	B1	M12737	139
HPV 9	EV lesions	B1	X74464	96
HPV 10	Flat warts	A2	X74465	95
HPV 11	Genital warts, CIN, laryngeal, nasal and conjunctival papillomas	A10	M14119	61
HPV 12	EV lesions	B1	X74466	95
HPV 13	Oral hyperplasia	A10	X62843	138
HPV 14	EV lesions and carcinomas	B1	X74467	94
HPV 15	EV lesions	B1	X74468	94
HPV 16	Warts, CIN, VaIN, carcinomas of cervix, penis, bronchus	A9	K02718	43
HPV 17	EV lesions and carcinomas	B1	X74469	94
HPV 18	Warts, CIN, VaIN, carcinomas of cervix and penis	A7	X05015	21
HPV 19	EV lesions	B1	X74470	94
HPV 20*	EV lesions and carcinomas	B1	U31778	59
HPV 21	EV lesions	B1	U31779	94
HPV 22	EV lesions	B1	U31780	94
HPV 23	EV lesions	B1	U31781	94
HPV 24	EV lesions	B1	U31782	94
HPV 25	EV lesions	B1	U74471	59
HPV 26	Cutaneous warts, CIN	A5	X74472	133
HPV 27	Cutaneous warts	A4	X73373	132
HPV 28	Cutaneous warts	A2	U31783	54
HPV 29	Cutaneous warts	A2	U31784	50
HPV 30	Laryngeal carcinoma, CIN	A6	X74474	41
HPV 31	CIN, carcinoma of cervix	A9	J04353	106
HPV 32	Oral hyperplasia	A1	X74475	13
HPV 33	CIN, carcinoma of cervix	A9	M12732	11
HPV 34*	CIN, Bowen's disease of skin	A11	X74476	86
HPV 35	CIN, carcinoma of cervix	A9	M74117	108
HPV 36	EV lesions, actinic keratosis	B1	U31785	85
HPV 37	Keratoacanthoma	B1	U31786	150
HPV 38	Malignant melanoma	B1	U31787	150
HPV 39	CIN, PIN, carcinoma of cervix	A7	M62849	12

continued

Table 1 (continuation)

HPV type	Clinical manifestations	Subfamily/ Genus	Accession #	Ref.
HPV 40	CIN, PIN	A8	X74478	37
HPV 41	Cutaneous warts	NA	X56147	69
HPV 42	Genital warts, CIN	A1	M73236	12
HPV 43	Genital warts, CIN	A8	M27022	107
HPV 44	Genital warts, CIN	A10	U31788	107
HPV 45	CIN and carcinoma of cervix	A7	X74479	124
HPV 47	EV lesions	B1	M32305	1
HPV 48	Squamous carcinoma of skin	B2	U31789	122
HPV 49	Cutaneous warts	B1	X74480	53
HPV 50	EV lesions	B2	U31790	52
HPV 51	CIN, carcinoma of cervix	A5	M62877	126
HPV 52	CIN, carcinoma of cervix	A9	X74481	159
HPV 53	Normal cervix, CIN	A6	X74482	58
HPV 54	Genital warts	A7	U37488	51
HPV 55	Bowenoid papulosis	A10	U31791	51
HPV 56	CIN, carcinoma of cervix	A6	X74483	41
HPV 57	CIN, cutaneous and nasal warts	A4	X55965	37
HPV 58	CIN, carcinoma of cervix	A9	D90400	116
HPV 59	CIN, VIN, carcinoma of cervix	A7	X77858	142
HPV 60	Cutaneous warts, epidermoid cyst	B2	U31792	115
HPV 61	Normal cervix, CIN, VaIN	A3	U31793	117
HPV 62	Normal cervix and CIN, VaIN	A3	U12499	117
HPV 63	Myrmecia wart	E1	X70828	44
HPV 65	Pigmented wart	B2	X70829	44
HPV 66	CIN and carcinoma of cervix	A6	U31794	170
HPV 67	CIN, VaIN, and carcinoma of cervix	A9	D21208	117
HPV 68	CIN and carcinoma of cervix	A7	X67161	104
HPV 69	CIN, VaIN, and carcinoma of cervix	A5	AB027020	164
HPV 70	Vulvar wart and CIN	A7	U21941	104
HPV 71	VaIN	A15	AB040456	164
HPV 72	CIN, oral warts	A3	X94164	176
HPV 73	CIN, oral warts and carcinoma	A11	X94165	176
HPV 74	VaIN	A10	U40822	105
HPV 75	Cutaenous warts	B1	Y15173	42
HPV 76	Cutaneous warts	B1	Y15174	42
HPV 77	Cutaneous warts, carcinoma of skin	A2	Y15175	42
HPV 78	NA (cutaneous)	A2	NA	NA
HPV 79	NA (genital)	A8	NA	NA
HPV 80	Normal skin	B1	Y15176	42
HPV 81	NA (genital)	A3	NA	NA
HPV 82	VaIN	A5	AB027021	88
HPV 83	Normal cervix	A3	AF151983	26
HPV 84	Normal cervix	A3	AF293960	171

continued

Table 1 (continuation)

HPV type	Clinical manifestations	Subfamily/ Genus	Accession #	Ref.
CandHPV 85	Genital wart	A7	AF131950	31
CandHPV 86	Genital, CIN	A3	NA	NA
CandHPV 87	Genital, CIN	A3	NA	NA

*Denotes an HPV type that has two separate types assigned to its identity but only one is listed here. HPV 46, although assigned, is considered the same as HPV 20. Similarly, HPV 64, although assigned, is considered the same as HPV 34. Ref. denotes the published article describing the cloning of the HPV type if available. When available, accession numbers (#) are provided for GenBank access to HPV genome or fragment sequence information. Cand = candidate HPV type from PCR, CIN = cervical intraepithelial neoplasia, EV = epidermodysplasia verruciformis, PIN = penile intraepithelial neoplasia, VaIN = vaginal intraepithelial neoplasia, VIN, vulvar intraepithelial neoplasia, NA = no available information or limited information. (Ref. [39], E.-M. de Villiers, personal communication

DNA viruses, HPVs disrupt the host cell regulatory machinery by harnessing it to propagate themselves. Thus, a simplistic view of long-term HPV persistence would support an increased probability of a malignant event within a disrupted cell environment. Because HPV-associated cancer outcomes are uncommon in comparison to the observed widespread nature of HPV infections, host and viral cofactors associated with HPV persistence are an important area of investigation that will be discussed later in the context of epidemiological findings.

It appears that HPVs along with other human pathogenic viruses such as herpesviruses, adenoviruses, and polyomaviruses developed prior to the emergence of humans. Papillomaviruses have been found in birds, reptiles and many mammals. Currently over 85 distinct HPV genotypes have been identified where complete genomic sequence is available. Accumulating data based on subgenomic sequences suggests that more than one hundred additional HPV genotypes exist. This extensive genomic heterogeneity is truly unique among DNA viruses and data support a remarkable ancient history of virus adaptations rather than a rapid acquisition of genome modifications. Epidemiological studies have demonstrated that gene sequences from various HPVs isolated from discreet parts of the world are remarkably conserved. This is true for both common and rare HPV types. HPV genotypes are now defined as having less than 90% identity in DNA sequence to any other reference genome and subtypes and variants of genotypes are defined as having greater than 90% and 98% identity, respectively. This identity can usually be defined by sequence comparisons limited to the L1 open reading frame (ORF) that encodes the major HPV capsid protein.

Methods for detection of HPV genomes have progressively developed over the past two decades. Initially cloned HPV genomes were used as probes in hybridization techniques such as Southern blot and dot blot hybridization. These methods for detecting HPV genomes yielded relatively specific results but were also insensitive. The advent of polymerase chain reaction (PCR) methods provided increased sensitivity and genotype-specific oligonucleotide probes enhanced the specificity of

HPV type-specific identification. Currently there are numerous broad-spectrum and type-specific PCR-based HPV detection methods that have been applied in elaborating the epidemiology of HPV infections. Extensive characterizations of populations using PCR-based HPV detection methods and more recently serologic assays have been primarily limited to studies of female populations and genital infections although these methods have facilitated a few studies of HPV infections in males and at extragenital sites. This review will, however, focus mainly on the epidemiology of genital HPV infections.

Epidemiology of HPV infections

Detection of genital HPV

An important aspect of elaborating the epidemiology of genital HPV infections has been the evolution of HPV sampling and detection methods. Estimates of HPV infection are very dependent on the populations sampled, the specimen collection methods and devices employed [63,137], the type of sample (i.e., fresh vs. archival samples) and laboratory approaches used for HPV DNA detection [22,65,153]. Sensitivity and specificity of the laboratory methods can vary when applying the same overall method such as PCR. If different primers, probes and protocols are used for the PCR, then the estimates are likely to differ. Furthermore, even the use of the same primers and probes with a protocol that varies slightly can result in differences in estimates. Because of this, direct comparison of HPV prevalence reported in various studies is difficult.

As mentioned earlier, a variety of PCR-based HPV DNA detection methods have been reported. These include type-specific HPV assays and broad-spectrum HPV typing assays such as the GP5+/6+ system [36,79], the MY09/MY11 system [9,114], a modified MY09/MY11 system designated PGMY09/PGMY11 [66] and the SPF-10 line probe assay [92]. None of these assays that amplify HPV DNA targets are approved for diagnostic use in the United States (U.S.). Currently only a signal amplified HPV DNA test, the Hybrid Capture II (HC2) assay is approved for diagnostic use. Recent data suggests that HPV testing by the HC2 assay is a viable option in the management of some low grade abnormal Pap smears designated as atypical squamous cells of unknown significance (ASCUS). The HC2 test was shown in a large randomized clinical trial to have greater sensitivity to detect cervical intraepithelial neoplasia (CIN3) or above and a sensitivity that was comparable to a single cytologic test indicating ASCUS or above [162]. In this same clinical trial, the high percentage of women (82.9%, 95% confidence interval (CI) = 79.7–85.7%) who were positive for HPV DNA by HC2 limited the potential of this test to direct the management of women with abnormal Pap smears designated as low-grade squamous intraepithelial lesions (LSIL) [172].

PCR-based data has demonstrated that sampling at a single time point results in an underestimate of HPV DNA prevalence [121,179]. This should be considered along with the fact that most reported risk factor associations have been based on

single cross sectional HPV detection measurements. HPV DNA estimates in such studies represent a component of persistent infections along with newly acquired infections. The proportions of persistent and new infections cannot be estimated accurately and thus risk factors associated with new HPV infections that have been defined in cross sectional studies are of limited value. Longitudinal cohort studies particularly in populations that are susceptible to HPV can ascertain incident infections and overcome these obvious limitations.

Transmission of genital HPV

PCR-based molecular epidemiological data accumulated for more than a decade has demonstrated that detection of HPV DNA is strongly associated with sexual behavior including lifetime and recent number of sex partners. Prior to the generation of these molecular data, clinical evidence for sexual transmission of HPV infections was provided for both genital warts and for CIN. Oriel reported that 64% of partners of individuals with genital warts developed warts [129]. Similarly Barrasso [8] and Schneider [155] have reported HPV infections in male sexual partners of women with CIN.

The sharing of HPV types among sex partners has also been evaluated at the molecular level. In the study of Schneider and coworkers [155], 87% of male partners shared specific HPV genotypes detected in their partner's cervical specimen. In studies conducted by Ho [77] and Xi [185], analysis of HPV 16 variants demonstrated concordance of specific variants between sex partners although distinct variants were found among some couples. Detection of identical variants among sex partners provides some evidence for sexual transmission however the probability of detecting any given HPV 16 variant is also a function of its prevalence in the reference population. In studies conducted in U.S. populations, a single phylogenetic branch of HPV 16 variants would be found in over 70% of HPV 16 infected individuals [181]. Thus the likelihood of detecting these particular HPV 16 variants in any individual would be extremely high. It is probably important to note that the detection of identical HPV variants among sex partners does not provide any evidence for who was initially infected.

Regarding non-sexual transmission, there is some evidence for *in utero* infection [157,188], perinatal infection [160,169], auto- and hetero-inoculation through close non-sexual contact [70,127], and potentially indirect transmission via fomites [144]. Modes of HPV transmission among children remain controversial and the frequency of perinatal infections progressing to clinical lesions is unclear. Condylomata have been detected in the first week of life [169] and HPV DNA has been detected in both nasopharyngeal aspirates in newborns [157] and amniotic fluid [188]. In addition, laryngeal papillomatosis has been reported in infants [83,161]. In children, the overall prevalence of anogenital HPV is generally considered to be low. Determinants of HPV transmission are really unknown although levels of HPV DNA may play a role [87]. A recent article by Syrjanen and Puranen [168] provides a detailed review of HPV infections in children and the role of maternal transmission.

Genital HPV prevalence, incidence and global distribution

HPV prevalence provides a measure of the percentage of persons in a population who have new, persistent, or recurring HPV infection at a particular point in time. Prevalence can vary several fold, depending on the method of HPV detection and the demographic and sexual behavior characteristics of the group under study. HPV is detected in a large number of cytologically normal individuals and in most genital neoplasias and cancers. PCR-based point prevalence for genital HPV infection in women with cytologically normal Pap smears has ranged from about 1.5% in sexually inexperienced women to about 45% in sexually active women (147,180). Studies that have conducted repeated testing over time have demonstrated prevalences exceeding 50% in young women [179]. HPV 16 has been the most common HPV type detected among cytologically normal women and it is also the most common HPV type detected in cervical cancers worldwide [151]. An extensive review detailing existing PCR-based data has been published by Xi and Koutsky [186].

HPV infection is similarly common among men however studies in male populations are far more limited. In male sex partners of women attending an STD clinic, 63% of penile samples were HPV positive by PCR [7]. In healthy men aged 18 to 23 years, HPV DNA was detected by PCR in urethral specimens from 12% of 66 men with normal penile epithelium and in 26% of 39 men with aceto-white epithelium [84]. Lazcano-Ponce and coworkers [100] demonstrated that in urethral and coronal sulcus swab samples, HPV was not detected in men who reported not having engaged in sexual intercourse but was present in 43% of men who reported sexual activity. Case-control studies have been conducted to consider the potential role of the male factor in cervical cancer [18,123]. Twenty-six percent of husbands of 210 women with cervical cancer and 19% of husbands of 262 control women were positive for HPV DNA by PCR in Colombia whereas 18% of husbands of 183 women with cervical cancer and 4% of husbands of 171 control women were positive for HPV DNA in Spain.

HPV incidence provides a measure of newly acquired HPV infections in a population of persons during a specific time interval. Estimates of HPV incidence are generally limited to very defined study populations such as family planning clinics, sexually transmitted disease (STD) clinics, or university student populations. Ho and coworkers [76] reported a 14% annual incidence rate of subclinical HPV infection detected by PCR assays in college students. Woodman and coworkers [184] reported that in 1075 women who were cytologically normal and HPV negative at recruitment, the cumulative risk at 3 years of any HPV infection was 44% (95% CI 40–48); HPV 16 was the most common type. Population-based data based on HPV DNA results are essentially non-existent for incident HPV infection although clinical observations have been used to propose population-based incidence. The use of clinical measures to estimate HPV incidence are likely to represent underestimates since direct measurement of HPV DNA would be expected to result in greater detection sensitivity. Based on Pap smear cytology, an estimated crude annual HPV incidence of about 7% was reported for a cohort of women 22 years of age in Finland [167]. For genital warts, an incidence of 106 per 100,000 persons was reported in a

population-based study of genital warts conducted in the United States [32]. Although data are extremely limited, HPV incidence is likely to be similar among women and men.

Data examining time trends for HPV infections are relatively limited and are confounded by the fact that awareness of HPV infections and their clinical manifestations increased significantly over the past 30 years. In addition, significant changes in diagnostic classifications occurred over this same time period. A U.S. survey of physicians reported that genital wart infections increased 4.5 fold during the years 1966 to 1984 [14]. Consistent with this observation, the incidence of genital warts reported in England and Wales doubled from 1971 to 1979 [5]. Increases in incident HPV infection might be expected to correlate with increasing incidence rates for other STDs. Increases in STDs have been observed when the proportion of individuals in the population who were young and sexually active increased. HPV seroprevalence studies have suggested an increase in HPV seropositivity across similar time periods. A population-based sample of pregnant women in Stockholm, Sweden between 1969 and 1989 found a 50% increase in HPV seroprevalence from 1969 to 1983 but stable seroprevalences during the 1980s [2]. The seroprevalence of herpes simplex type 2 (HSV-2) in these same samples showed similar trends [2], reflecting the increased rate of sexual activity in the population.

Genomes of HPV types and their variants are stable, since identical variants have been found in unrelated individuals residing in different countries who have no known contact with each other [30]. For HPV 16, five distinct phylogenetic branches have been reported. These branches have been designated E (European), As (Asian), AA (Asian American), Af-1 (African-1), and Af-2 (African-2) [30,190]. Studies conducted in numerous populations support the notion that representative variants from all of these five major HPV 16 lineages can be detected worldwide, although specific prevalences differ by geography [189]. Differences in HPV type and variant prevalences may be explained by founder effects and/or may reflect selection by the host population.

Determinants of genital HPV detection and persistence

Epidemiological studies in diverse populations that consider sexual, behavioral and demographic factors have generally concluded that detection of HPV decreases with age [10,27,38,119] and increases with number of sex partners both lifetime and recent. An extensive review of risk factors associated with detection of genital HPV infections was published by Xi and Koutsky [186]. Other sexual behaviors such as age at first sexual intercourse, years since first intercourse, frequency of sexual intercourse, sexual intercourse during menses, and anal intercourse have not been consistently associated with HPV DNA detection. Additional risk factors for detecting HPV may be population dependent and some are probably markers of sexual behavior. Studies examining the association of smoking and HPV detection have generally been negative. The relationship between oral contraceptive (OC) and sexual activity has made it difficult to determine the relationship between HPV

detection and OC use. Several studies have reported positive associations between HPV detection and OC use although the majority of studies have not confirmed these findings. The association between HPV infection and reproductive history such as age at menarche, stage of menstrual cycle, age at first pregnancy, number of pregnancies and current pregnancy has also been inconsistent.

Few population-based studies have investigated the prevalence of type-specific infection for a broad spectrum of HPV types. Most studies have combined all genital HPV types detected into a single group for analytical purposes. Several studies have examined the determinants of high-risk and low-risk HPV types grouped separately [57,73,75,80,90,143,146]. Low-risk HPV types have been reported to be less associated with sex history and age than high-risk HPV types, although differences in associations have been reported. More recent investigations using highly sensitive and type-specific HPV DNA detection have demonstrated two peaks of increasing HPV prevalence. Of 9175 women in Guanacaste, Costa Rica, 3024 women were tested for more than 40 types of HPV with a PCR-based system [73]. Among women with normal cytology, HPV infections peaked first in women younger than 25 years, and then peaked again at age 55 years or older with predominantly low-risk and uncharacterized HPV types. Another population-based study was conducted in Mexico between 1996 and 1999 [99]. The sampling was based on an age-stratified random sample of 1,340 women with normal cytological diagnoses and 27 HPV types were distinguished. A first peak of 16.7% was observed in women under 25 years. HPV DNA prevalence declined to 3.7% in women 35–44 years and then increased progressively to 23% among women 65 years and older. This second peak of HPV infections in postmenopausal women demonstrated a clear predominance of cancer-associated or high-risk HPV types. The second peak of HPV prevalence in older women differed between these two studies in that one study reported a predominance of low-risk HPV types and the other reported a predominance of high-risk HPV types. The reason for these differences is not clear but further investigation are warranted to determine if the second peak of HPV prevalence in older women might reflect reactivation of latent HPV infections or newly acquired HPV infections following changes in immune and hormonal status. Certainly these data are intriguing given the possibilities as they relate to the natural history of cervical cancer outcomes. This second increase in HPV population prevalence in part overlaps the peak of cervical cancers in the population.

Studies on the persistence of cervical HPV DNA have been primarily limited to prevalent infections and time intervals between HPV DNA measurements have varied between studies. In addition, determinants of persistence have not been extensively examined. Despite these limitations, most studies have found that genital HPV infection is transient. Various investigations have demonstrated associations between HPV persistence and older age, types of HPV associated with cervical cancer, infection with multiple types of HPV and use of oral contraceptives [25,48, 75,76,103]. In one study the median duration of new HPV infections was 8 months (95% CI, 7 to 10 months) [76]. HPV type 16 has been shown in several studies to be the most persistent HPV type [25,45,103] followed by other high-risk HPV types.

Persistent detection of HPV 16 has demonstrated that the same dominant variant persists for months and sometimes years suggesting that reinfection by the same HPV type or of multiple variants is uncommon [185]. These data must be considered in the context of earlier discussions of expected risk for infection with particular HPV variants given population prevalences. Persistence has also been reported to increase with higher quantities of HPV DNA [25] although additional investigations in this area are needed. In a study conducted by Liaw and coworkers [101], persistence of concomitantly detected HPV was examined prospectively among 1124 cytologically normal women. Preexisting HPV 16 was generally associated with an increased risk for subsequent acquisition of other HPV types. HPV 16 did not affect the persistence of concomitant infections, regardless of type. This study suggests that prevention or removal of HPV 16 may be unlikely to promote the risk of infection with other HPV types. This has been a theoretical concern given prophylactic vaccination efforts that will be discussed later.

HPV and cancer

Epidemiological evidence has convincingly demonstrated that infection with high-risk HPVs is the greatest risk factor for cervical cancer [19,78]. Furthermore, the role of HPV in the development of CIN has been well established [15,93,152]. The relative risks for the association of HPV with CIN are commonly in the range of 20–70. This magnitude of risk is far greater than the association between smoking and lung cancer. Thus, HPV infection is considered a necessary but insufficient factor for malignant transformation.

Invasive cervical cancer occurs in approximately 400,000 women per year worldwide with an estimated 200,000 deaths per year [55,135,136]. The greatest burden of cervical cancer is in developing countries where it is often the most common female malignancy. Pap smear screening has reduced the incidence of cervical cancer in developing countries but in the U.S. alone, it has been estimated that over 5 billion dollars per year are expended to achieve a 75% reduction in cervical cancer [97].

An international study of invasive cervical cancers collected from 22 countries demonstrated that essentially all cervical cancers (99.7%) contained HPV [19,177]. Other studies reporting a proportion of HPV negative cervical cancer cases [56,102, 113,156] may have had specimens that were inadequate for testing, had extremely low copy numbers of HPV genomes or could have harbored integrated HPV forms that interfered with detection of targeted genomic segments. Numerous studies reporting HPV negative tumors have not included histological review to confirm the presence of tumor cells within the biopsy material and furthermore paraffin-embedded tissues have been used. The efficiency of amplification in paraffin-embedded tissues can be compromised [6,68] especially when the PCR targets are greater than a few hundred base pairs in length. In addition, the age of the specimen and variability in fixation methods can affect the amplification efficiency. The absence of HPV in a small proportion of cervical cancers has been reported as a poor prognostic factor for survival.