Human Papillomaviruses: Their Clinical Significance in the Management of Cervical Carcinoma

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By Jeffrey F. Hines, MD [2], A. Bennett Jenson, MD [3], and Willard A. Barnes, MD [4]

Studies have shown a strong association between certain human papillomaviruses and the development of cervical carcinoma and its precursor lesions. The oncogenic potential of papillomaviruses has been clearly

Introduction

Papillomaviruses are ubiquitous in a wide variety of vertebrate species, including humans. They infect and cause proliferative lesions of cutaneous and mucosal squamous epithelium [1]. More than 60 types of human papillomaviruses (HPVs) have been described (Table 1) [2,3], each of which shows a particular predilection for tissue sites and has defined oncogenicities. Particular interest has focused on HPVs associated with anogenital tract disease. There is a well-established association between HPV infection, cervical dysplasia, and cervical carcinoma [4-6].

In 1994, 15,000 new cases of invasive cervical cancer and 4,600 deaths attributable to cervical cancer are projected in the United States. Statistics on cervical cancer worldwide are much more staggering, with 500,000 deaths per year caused by this cancer [8]. These figures highlight the public health significance of controlling cervical cancer and its precursor lesions.

Recent advances in immunology and molecular biology have broadened our understanding of the biology of HPV. In particular, insights into the molecular mechanisms of HPV-mediated tumorigenesis may be the basis for new preventive and therapeutic strategies for HPV-associated disease (Figure 1).

Characteristics of Human Papillomavirus

The viral etiology of warts was first described by Licht in the late 19th century [8]. In 1933, Shope and Hurst described the first DNA tumor virus isolated from the papillomavirus of cottontail rabbits [9]. Condyloma acuminata, long recognized to be sexually transmitted, were linked to a viral etiology when virus particles were detected in genital warts by electron microscopy in the 1970s [10,11]. Papillomaviruses have been classified into the papovavirus group because of similarities among papillomavirus, polyoma virus, and the vacuolating virus of monkeys [12]. The structure and genetic organization of all the papilloma-viruses are strikingly similar. Papillomaviruses consist of a 55-nm, nonenveloped, icosahedral-shaped virion whose genome is organized as closed, circular, double-stranded DNA of approximately 8,000 base pairs in length [13] (Figure 2).

The papillomavirus genome can be divided into three functional regions (Table 2) [14]: The "early" region contains eight open reading frames, or genes, whose products are responsible for viral DNA replication, transcriptional control, and cellular transformation. The "late" region encodes the two structural capsid proteins, L1 and L2, of the virion. The "long control region" contains the origin of DNA replication, promoter elements, and transcriptional enhancer sequences.

The true prevalence of HPV infection in the general population is unknown. This is due to a number of variables, including coital activity, host response to infection, and the diagnostic modality used to detect HPV infection. Cutaneotropic HPVs cause verruca plana, which is common among young children, and verruca vulgaris, which is common among adolescent children [15]. Mucosotropic HPVs produce a variety of lesions of the conjunctiva, oropharynx, and larynx, in addition to anogenital tract lesions.

Pathogenesis of HPV Infection

Sexual transmission of anogenital warts is supported by data confirming the presence of similar HPV types on cervical and penile lesions of sexual partners [16]. Several factors affect the rate of transmission of mucosotropic HPVs. These include coitus with multiple sexual partners,
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immunodeficient states, and pregnancy. These highly infectious viruses have a relatively long incubation period following inoculation. Lesions caused by mucosotropic HPV types usually appear within 4 to 6 weeks in humans. The same incubation time is observed with mucosotropic viruses that infect keratinocytes in the athymic nude mouse model [11,17].
The host response to infection is similar for all HPV types. All three types of squamous epithelia (cutaneous-keratinized, mucosal-nonkeratinized, and metaplastic) are susceptible to HPV infection. While infection may manifest itself differently for different HPV types, infection begins in the basal layer of squamous epithelia. Presumably, virus from infected cells is released into epithelial breaks of the susceptible host [18].
Human papillomaviruses demonstrate specific tissue tropism to anatomic sites. Equally important is the fact that HPVs can only synthesize structural proteins that encapsidate the genome and form virions in the most differentiated keratinocytes.

Three Sequelae of Infection
Following infection with HPV, three sequelae are possible:
First, the HPV genome can stabilize as a nonintegrated episome and remain latent in the host without producing clinical or morphologic changes in the squamous epithelium.
Second, active infection can be established with vegetative replication of HPVs, which induces the proliferation of squamous epithelia into benign tumors (warts, papillomas).
Third, the HPV genome can become integrated into the host genome, which interrupts its control of oncoproteins of highly oncogenic viruses.
Expression of early and late viral gene products accounts for the morphologic changes seen in affected epithelia. Early gene expression causes cellular proliferation, which results in acanthosis. Late gene expression results in production of viral capsid proteins, which are evident (by electron microscopy and immunocytochemistry) only within nuclei of terminally differentiated, superficial epithelial cells (keratinocytes). In latently infected cells and benign tumors, the HPV genome is present in nonintegrated, episomal form. Viral capsid assembly in productively infected, terminally differentiated keratinocytes causes degenerative changes in the nuclei and cytoplasm, which are recognized histologically as koilocytosis [19].
Latent infections with no pathologically identifiable lesions comprise a large reservoir of virus that may be reactivated for transmission and autoinfection. It is believed that 10% of sexually active individuals harbor latent HPV infections [20]. Active infections occur in up to 5% of sexually active women, and appear as flat or exophytic condylomas of the cervix, vagina, or vulva. Condyloma acuminata are usually associated with HPV types 6 and 11.

Association with Dysplasia and Malignancy
Infection with certain HPV types has a high probability of being associated with dysplasias (types 6 and 11) or with malignancies (types 16 and 18). Squamous intraepithelial lesions show characteristic disorderly, undifferentiated, proliferating basaloid and parabasaloid cells that occupy different portions of the epithelium, from the lower third in mild cervical intraepithelial neoplasia to full epithelial involvement in carcinoma in situ. Conversely, invasive cervical carcinomas extend beyond the basement membrane.
The HPV genome in malignancies is usually not episomal, but rather, is integrated into the host genome in at least 80% of cervical cancer cases. The viral E2 gene serves as the most significant site of integration into the host genome. With integration, normal regulatory function of E2 is interrupted. This event appears to be critical for tumorigenesis. Loss of regulation results in overexpression of viral E6 and E7 oncoproteins, which are known to inactivate the cellular tumor-suppressor gene products, p53 and pRb, respectively [21].
It is not known which stage of HPV genome integration correlates with the change from dysplasia to malignancy. Integration of the viral genome is not always required for tumorigenesis. In some HPV-16 and other HPV-associated carcinomas, viral DNA exists in an extrachromosomal state [22]. In vitro biologic assays have enabled investigators to study the transforming activity of cloned human papillomavirus DNA on primary and immortalized cells, and thereby evaluate the role of viral genes in tumorigenesis. Assays of infected and transfected murine and human cells have been important in determining whether continually expressed E6 and E7 are necessary and sufficient for in vitro transformation [23]. Some cell lines produced by these assays are tumorigenic in nude mice alone; others require cooperation with the expressed ras oncogene to become tumorigenic. Tumorigenicity in these instances is dependent upon HPV type [24].

Host Immune Responses
Immunologic response to HPV infection is an important aspect of host response. Persistence or spontaneous regression of lesions is related to cell-mediated immunity. Patients with altered cellular immunity (immunosuppression, immunodeficient states, pregnancy) have a higher incidence of warts and condylomas [25]. Patients with the congenitally acquired disease of impaired cellular immunity, epidermodysplasia verruciformis, have skin warts and increased rates of detection of HPV types 5 and 8. These warts have a high likelihood of transforming into squamous or basal cell carcinomas [26].

Finally, HPV-associated primary, metastatic, and recurrent cervical cancers frequently exhibit a reduction in or total loss of allelic expression of critical major histocompatibility complex class I molecules, which are involved in antigen presentation at the cell surface and in antigen recognition. Downregulation of these molecules may enable cervical cancers to escape cell-mediated immune surveillance [27].

Humoral immune responses to HPV infection have been incompletely quantified. Sera from animals and humans with a history of infection generally react positively to enzyme-linked immunosorbent assays with denatured papillomavirus capsid proteins [28-30]. Antibodies are detected in rabbits and mice inoculated with intact HPV virions [28,31]. Humoral immunity appears to protect the host against HPV infection and its transmission.

Recent advances in molecular biology have enabled investigators to synthesize recombinant virus-like particles and capsid proteins of papillomaviruses that react with conformational-dependent, neutralizing antibodies [32-35]. This development will allow for the investigation of the role of humoral immunity in the natural history of papillomavirus infection.

**Oncogenic Potential of HPV Types**

In an epidemiologic study of 2,627 women, Lorincz et al examined the prevalence of anogenital HPV infection among normal women and women with premalignant and invasive lesions using southern hybridization. The oncogenic potential of 15 anogenital HPV types was defined. Low-risk HPV types (6, 11, 42, 43, and 44) were found in 20% of low-grade squamous intraepithelial lesions, and were absent in all cancers. Intermediate-risk HPV types (31, 33, 35, 51, 52, and 58) were detected in 23% of high-grade squamous intraepithelial lesions and in 10% of cancers. High-risk HPV types (16, 18, 45, and 56) were detected in 53% of high-grade squamous intraepithelial lesions and in 74% of cancers. Adenocarcinomas of the cervix were usually caused by HPV type 16 or 18.

**Continuum of Disease Progression**

Studies have documented a continuum of disease progression from squamous intraepithelial lesions to invasive carcinomas. If left untreated, 16% of cases of mild dysplasia will progress to carcinoma within 84 to 96 months, two-thirds will regress, and 22% will persist as mild dysplasia [36]. The majority of high-grade lesions will persist or progress. Two-thirds of cervical intraepithelial stage III lesions will progress to invasive cancer within a mean of 10 years [37].

Finally, human papillomavirus DNA has been detected, via sensitive polymerase chain reaction assays, in more than 95% of squamous and adenocarcinomas of the cervix [38]. This finding, combined with data showing odds ratios for highly oncogenic HPV types ranging from 31 to 296 for the occurrence of cervical cancer, supports the association of certain HPV infections with cervical neoplasia [3]. Clearly, other factors are probably operative in the development of cervical cancer (i.e., tobacco, oncogenes), but HPV is necessary although perhaps not sufficient. Eradication or protection against HPV infection would decrease the number of cervical cancers, perhaps by as much as 95%.

**Traditional Approach to Detection and Treatment**

**Cytology and Colposcopy**

Cytologic examination of exfoliated cells from the cervix (the Pap smear) is an effective screening method for premalignant and malignant lesions of the cervix. The traditional approach to the classification of Pap smears was based on a numerical system, which proved to be inadequate. In 1988, the National Cancer Institute convened a workshop charged with revising and standardizing cytologic reports. A new classification system, the Bethesda system, was adopted. This classification has undergone several revisions, and a simplified version was released in 1994 [39].

Under the Bethesda system, premalignant lesions fall into three categories: atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, and high-grade squamous intraepithelial lesion. Moderate and severe cervical intraepithelial neoplasias and carcinoma in situ are classified as high-grade lesions. Low-grade squamous intraepithelial lesions include mild cervical intraepithelial neoplasia and other HPV-associated lesions previously described as "condylomatous
Atypical squamous cells of undetermined significance is the designation given to abnormal cells that do not fit into the other two categories but display minor abnormalities unrelated to infection or regeneration and previously described as atypical. "Undetermined significance" relates both to the lack of defined criteria for their description and the uncertain relationship of these cells to HPV infection, cancer precursors, or other conditions.

Colposcopy with directed biopsy for histologic confirmation of cervical lesions is the traditional means of evaluating abnormal Pap smears. Results of the colposcopic findings in conjunction with tissue histology direct additional therapy.

**Role of Molecular Hybridization and Polymerase Chain Reaction Tests**

Molecular hybridization and polymerase chain reaction tests may be useful as adjuncts to cytology and colposcopy in evaluating cervical disease. Molecular hybridization tests detect human papillomavirus DNA in cells and tissue. DNA is extracted from or identified in archival, formalin-fixed, and fresh cells and tissue. The availability of DNA hybridization tests, such as southern blotting, dot blotting, and in situ DNA hybridization allows for HPV detection and typing. Some kits have been approved by the Food and Drug Administration and are commercially available (eg, ViraType).

The polymerase chain reaction has become an extremely useful and powerful tool for detecting and typing human papillomavirus DNA. With this technology, a single copy of viral DNA can be amplified exponentially and used for qualitative and quantitative investigations. The polymerase chain reaction has the advantage of being extremely sensitive for human papillomavirus DNA, but the disadvantage of decreased specificity.

**Treatment Approaches**

Historically, cutaneous and mucosal warts have been treated with a variety of destructive and surgical techniques, including topical salicylic acid, glutaraldehyde, liquid nitrogen, podophyllum, laser ablation, and surgical excision. Some warts will recur regardless of the form of treatment employed.

Condylomas respond to topical podophyllum, bichloroacetic and trichloroacetic acid, carbon dioxide laser ablation, intralesional interferon injection, topical fluorouracil, and surgical excision [2]. High-grade squamous intraepithelial lesions, after confirmatory biopsy, can be ablated or excised with cryosurgery, a carbon dioxide laser, loop electrosurgery excisional procedure, or knife conization.

There is controversy over the best scheme for triaging patients who have atypical squamous cells of undetermined significance or a low-grade squamous intraepithelial lesion on their Pap smear. Each year, such abnormalities are found on the Pap smears of approximately 2.5 million US women. The associated cost of colposcopic evaluation and intervention to remedy these lesions approaches $6 billion annually [40]. Prospective trials are warranted to evaluate the efficacy of other triage strategies for these lesions.

The treatment of invasive cervical cancer is not necessarily affected by HPV type. Until more prospective information is available on the impact of HPV type on tumor aggressiveness and recurrence, the approach to treating primary invasive cancers limited to the cervix consists of radiation therapy, chemotherapy, or radical surgery.

**Future Directions in Prevention, Detection, and Treatment**

**Prospects for a Prophylactic Vaccine**

As we begin to better understand the humoral immune response to HPV infection, the prospects for developing a prophylactic vaccine will improve greatly. Vaccines directed against HPV infection will result in diminished rates of condylomas, squamous intraepithelial lesions, and cancer. It is believed that vaccines against HPV-16 alone could save the lives of 225,000 women yearly worldwide [40].

A successful prophylactic vaccination program for hepatitis B virus transmission has been accomplished using a yeast-recombinant hepatitis B vaccine. Studies in animals and humans have confirmed production of antibodies against hepatitis B surface antigen following immunization with this vaccine, as well as its efficacy in preventing viral hepatitis B transmission [41].

Successful papillomavirus vaccine programs exist in animal models. Much of this work has been accomplished in cattle using bovine papillomaviruses. Calves vaccinated with bovine papillomavirus wart extract were protected against infection when challenged by bovine papillomavirus virions. Sera from these animals showed an increase in protective neutralizing antibodies, which target antibody-binding sites on the surface of virions [30,42].

Until recently, little data had been reported on the immune response to papillomavirus infections
due to an inability to successfully propagate these viruses in vitro. Reports that native virions and papillomavirus subunits induce protective antibodies stimulated investigations into the development of prophylactic vaccines.

**Conformational Epitopes**—A brief discussion of papillomavirus conformational epitopes is critical to understanding papillomavirus immunology. Conformational, or discontinuous, epitopes (antibody-binding sites) are defined by the spatial arrangement of amino acids that stems from the tertiary and quaternary structure of proteins. The most important class of conformational epitopes are neutralizing epitopes found on the surface of intact virions [43,44]. Antibodies generated against these epitopes are sufficient to prevent infection in vitro and in animal models [30,42,43]. Bovine papillomavirus-1 L1 protein expressed in insect cells assembles into empty capsids and induces high-titered neutralizing antibodies [34]. Recombinantly synthesized papillomavirus capsid proteins and virus-like particles that mimic the conformation of empty native virions can react with conformation-dependent, neutralizing antibodies.

Several authors have recently reported on a variety of methods that can be used to express the major capsid protein of papillomaviruses that mimics intact virions [32-35,45]. Hines and colleagues [45] used mammalian and insect cell systems to express the major capsid proteins for HPV types 1, 6, 11, 16, and 18. Both systems expressed L1 protein mimicking the conformation displayed by intact virions. Using a series of well-defined antibodies, these researchers were able to detect type-specific, neutralizing epitopes in certain HPV types. Other authors have successfully purified virus-like particles that assemble themselves into empty capsids and react with neutralizing antibodies [32,34].

These investigations confirm the fidelity of L1 protein expression in displaying conformational, neutralizing, immunodominant epitopes characteristic of intact virions. More importantly, these immunologically active, empty viral capsids are likely to be successful as prophylactic subunit vaccines against naturally transmitted infection because they are antigenic, protective in animal models, and lack viral DNA that might be carcinogenic to the host.

**Key Questions**—Animal models and clinical trials are clearly indicated to better define the ability of major capsid papillomavirus proteins to induce protective antibodies and prevent HPV-associated disease. The following key questions regarding a HPV prophylactic vaccination program will need to be addressed: Should both males and females be vaccinated? At what age should vaccination occur? Should only high-risk populations be vaccinated, or the general population? These issues will be addressed as strategies continue to progress and additional animal and human data become available.

**Serologic Screens**
Serologic assays have been used to measure antibody response to HPV infection in patients [28,29]. Correlation of antibody reactivities with the presence of active or latent HPV infection, squamous intraepithelial lesions, and cancer would be one benefit of serologic testing.

New methods of synthesizing recombinant papillomavirus capsid proteins and virus-like particles now permit putative seroepidemiologic studies of HPV infection and serologic typing. Limited seroepidemiologic studies of HPV infections are being accomplished using these recombinantly synthesized proteins [46,47]. These important studies will finally provide insights into the natural history of HPV infection, and possibly identify immunologic markers of lesion regression and progression. In addition, a serologic test may help identify women at risk for developing cervical dysplasias and cancers, who may benefit from prophylactic vaccination.

**Molecular and Immunologic Treatment Strategies**
In the continuum between HPV infection and the development of cervical cancer, there are three broad phases in which molecular and immunologic strategies may be employed. The first phase is the time between exposure to the virus and the development of infection. The second phase occurs after infection has occurred but prior to cellular transformation, and the final phase occurs after cellular transformation. As discussed above, prophylactic vaccination is an immunologic strategy that could prevent progression from the first to the second phase. The following sections outline additional strategies that could be directed at the final two phases.

**Immunotherapy**—Cellular immunity appears to play an important role in modulating the effects of HPV infection. Clearly, molecular signals are required to stimulate cytotoxic T cells. Viral peptides are presented to the cell surface after complexing in the cleft of major histocompatibility class I molecules, and serve as targets for cytotoxic T lymphocytes.

Several viral peptides or epitopes have been characterized. Feltkamp et al reported that an E7 epitope of HPV-16 injected into mice protects against tumor formation following challenge [48]. Immunization with these peptides stimulates cell-mediated cytotoxic destruction and prevents tumor
formation. Stimulation of cytotoxic T lymphocytes by early gene oncoprotein peptides is another attractive immuno-therapeutic strategy to prevent cellular transformation and tumorigenesis. Adoptive transfer of stimulated cytotoxic T lymphocytes by HPV oncoprotein epitopes to cancer patients may help accelerate tumor regression. Clearly, additional in vitro and animal studies are needed to further define the role of this strategy.

**Antisense Therapy**—Another innovative strategy for therapy involves messenger RNA (mRNA) antisense transcripts. These mRNA oligomers have been found naturally in prokaryotes, eukaryotes, and tumors, and can be synthesized for in vitro investigations. Messenger RNA transcripts have been reported in herpes simplex and Epstein-Barr viral infections, and may have a natural role in mediating viral gene function and expression and cell growth [49]. These transcripts bind to complementary coding and noncoding regions of nucleic acids. In vitro studies have shown that expression of antisense mRNA to HPV E6 and E7 open reading frames is associated with reduced cell growth [50]. It is possible that genetically engineered mRNA transcripts could be directed against E6 or E7 gene products and modulate tumorigenesis. Clinical translation of this technology deserves further investigation. However, as an efficient delivery system for antisense therapy has not yet been developed, this approach is less attractive at this time.

**Summary**

Human papillomaviruses are a diverse group of viruses of varied oncogenic potential that cause infections of cutaneous and mucosal epithelia. Much epidemiologic, clinical, and scientific data support the role of HPV infection in the development of anogenital warts, premalignant lesions, and carcinomas.

We have begun to unravel the basic molecular mechanisms that enable HPV-encoded oncoproteins to affect cellular proliferation and transformation. As we gain a better understanding of the molecular, immunologic, and biochemical mechanisms that permit HPVs and other factors to transform epithelial cells, new treatment strategies can be devised.

Strategies under current investigation are focusing on the induction of effective humoral and cell-mediated immunity, the expression of HPV gene products, and cofactors that interact with HPV gene products to affect cell transformation. As a result of these investigative efforts, prophylactic HPV capsid vaccines and other gene therapies may soon become clinically available.

The consequences of HPV infections, both benign and malignant, have a significant impact on society. Continued basic science and clinical research is needed to provide important information about mechanisms of carcinogenesis and the natural history of infection, to improve triage and treatment protocols, and to develop novel methods of detection, prevention, screening, and treatment.

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