

Beyond Human Papillomavirus: The Cervix, Exogenous Secondary Factors, and the Development of Cervical Precancer and Cancer

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■ **Abstract:** Human papillomavirus (HPV) is the necessary but probably not sufficient cause of cervical precancer and cancer. Secondary exogenous and endogenous factors, HPV cofactors, may contribute to the probability of a cancer-associated (oncogenic) HPV infection progressing to cervical precancer and cancer. For these cofactors to influence the natural history of HPV infection, they must act on cervical tissue to promote viral persistence, progression to precancer or cancer given viral persistence, or both. The aim of this review was to examine briefly the impact these factors may have on carcinogenesis of the cervix. Specifically, the roles of the cervical transformation zone, cervical immunity, inflammation and coinfection, and exposure to the main HPV cofactors (smoking, oral contraceptive use, and multiparity) are discussed. ■

Key Words: human papillomavirus, cervical cancer, cofactors, transformation zone

Cervical infection by one of approximately 15 cancer-associated (oncogenic) human papillomavirus (HPV) types is generally accepted as the necessary cause of cervical cancer [1–3]. However, oncogenic HPV infections are common sexually transmitted infections

(STI) that most often are self-limiting. Occasionally, oncogenic HPV infections persist, and it is these women with persisting infections who are at the greatest risk of precancer (histopathologic diagnosis of cervical intraepithelial neoplasia grade 3) and cancer of the cervix [4, 5].

It is largely unknown why a few oncogenic HPV infections persist and progress to cervical precancer or cancer. Several secondary non-HPV risk factors (HPV cofactors) that contribute to the risk of developing cervical cancer have been implicated based primarily on epidemiologic evidence. Candidate exogenous HPV cofactors include smoking, prolonged oral contraceptive use, and non-HPV STIs [6, 7]. Endogenous factors may include host immune responses and multiparity [6, 7]. These factors may contribute to the risk of viral persistence, progression to precancer or cancer, or both. Mechanistically, it seems likely that expression of HPV oncoproteins interferes with programmed cell death, thereby rendering infected epithelial cells vulnerable to secondary assaults that in turn can cause genomic damage and tumorigenesis [8]. Greater viral persistence affords a greater opportunity for cumulative and potentially genotoxic exposures in an infected cell [8]. In addition, persistence may increase the likelihood of viral integration and concomitant dysregulation of viral protein expression, leading to greater expression of oncoproteins. However, it seems unlikely that there is a strong selective advantage for the virus to cause cancer, a concept supported by the observation that the greatest viral production sometimes occurs in the mildly abnormal tissue surrounding a high-grade lesion rather than

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from the high-grade lesion itself [9]. Women without a cervix after undergoing a total hysterectomy have high prevalence of HPV, including oncogenic types (Castle P, unpublished observations, 2004), but they are at very low risk of HPV-induced cancers of the vagina, again suggesting little selective advantage for cancer causation.

Given the localized nature of an HPV infection, that is, there is no apparent systemic viremia, and the predominance of cancers occurring in the transformation zone of the cervix, it seems intuitive that these HPV cofactors must act on the cervical tissue to alter the natural history of an HPV infection by increasing the likelihood of viral persistence and progression to cervical precancer and cancer. However, the link between epidemiologic evidence and the physiologic and immunologic state of the cervix (cervical microenvironment) has not been established fully and will likely be necessary to understand the natural history of infection leading to cervical precancer and cancer.

THE TRANSFORMATION ZONE

Ironically, it is the role of the cervix itself that may be underappreciated in the development of cervical cancer. Specifically, the transformation zone (TZ) of the cervix, a zone of active squamous metaplasia proximal to the original squamocolumnar junction established at birth and distal to the current squamocolumnar junction, is uniquely susceptible to HPV-induced carcinogenesis. Approximately 99% of HPV-related genital cancers occur in this annulus of tissue, where columnar epithelium is replaced by squamous epithelium in a reparative process called “squamous metaplasia,” probably in response to the pubertal acidification of the lower genital tract as the result of lactobacilli colonization [10]. Similar transformation-like tissue in the anus also are prone to HPV carcinogenesis. The TZ, like HPV, perhaps should be considered a near prerequisite for HPV-induced cancer.

Although the TZ is necessary for cervical cancer, it is not required for an HPV infection. As mentioned, women without a cervix after undergoing total hysterectomy have a vaginal oncogenic HPV prevalence similar to the cervical prevalence in nonhysterectomized women. There is also evidence that vaginal infection precedes cervical infection, suggesting that the vagina could act as a reservoir for infection of the cervix. It can be inferred from these data that the squamous metaplastic epithelium of the TZ is not necessary for HPV infection but is uniquely affected by the oncogenes of HPV.

The molecular characteristics of the TZ, as compared with the proximal squamous epithelial in the ectocervix and vagina and distal columnar epithelial in the endocervical canal, have not been elucidated. New technologies, such as the combination of gene expression microarrays,¹ protein microarrays, and tissue microarrays with laser capture microdissection, may be used to examine the molecular physiology of the TZ. For example, paired tissue biopsies of squamous, squamous metaplasia, and columnar epithelium from cytologically normal, HPV DNA-negative and HPV DNA-positive women could be used to compare gene expression in each cell type, using laser microdissection to isolate pure cell types and gene expression microarrays on pools of isolated cells. Differences in molecular profiles may suggest mechanisms of susceptibility to oncogenic transformation for this tissue and also may be relevant to squamocolumnar junctions in other mucosal epithelium, which are similarly susceptible to oncogenic transformation by exogenous exposures. Similarly, a comparison of messenger RNA or protein levels of HPV-infected squamous and squamous metaplastic cells may inform about differences in cell-viral molecular interactions that are important to cervical carcinogenesis.

CERVICAL IMMUNITY

Persistence versus clearance of infection implicates differences in host immune responses as an important cofactor in cancer development. Genital HPV may occupy a unique evolutionary niche by infecting the cervix where the induction of immune responses is typically poor. The immune response in the female lower genital tract may be particularly refractory to infection, given the reproductive need to avoid responses to “foreign” sperm. Vaccination of the genital tract does not typically result in robust immunity, and by comparison, other routes of mucosal vaccination (e.g., nasal) typically result in greater antibody titers in mucosal secretions of the genital tract via a common mucosal immune system [11, 12]. In the case of a natural cervical HPV infection, seroconversion is rather slow and weak, occurring 6 to 12 months after viral acquisition as measured by DNA detection and not occurring for all infected women [13,

¹A microarray is a miniaturized two-dimensional array, often on a small glass, filter, or silicon wafer, on which molecular probes (e.g., genes, gene fragments, and antibodies) are deposited or synthesized (“spotted”) in a predetermined spatial order for high-throughput, parallel assays. Each spot typically has a unique molecular specificity (e.g., the gene probe hybridizes to a unique genetic element), and these arrays can have hundreds or thousands of spots.

14], further suggesting poor induction of immune responses to HPV infection at the cervix.

Cell-mediated immune responses to HPV are believed to be critical to viral clearance, but relevant biomarkers or cell types have not been defined. Robust natural immune responses to HPV may be hindered by the absence of organized lymphatic tissue, like the Peyer's patch in the gastrointestinal tract, and a replicative cycle that avoids immune surveillance [15]. Some studies have observed greater HPV-specific proliferative lymphocyte responses and lesion regression [16, 17], stronger cytotoxic T-cell responses against HPV-infected cells and reduced viral persistence [18, 19], and greater release of interleukin 10, a marker of cellular immune responses that is associated with the absence of lesions among women with HPV infection [19].

Immune responses to HPV also may be type specific. Preliminary data have demonstrated that HPV 16 is more apt to persist over more than 5 years than other HPV types, whereas oncogenic types not including HPV 16 persist, on average, no longer than nononcogenic types (Schiffman M, personal communication, 2004). Similarly, the prevalence and incidence of HPV 16 infection seems to be the least influenced of all HPV types by CD4+ cell counts in HIV-infected women [20]. Together, it can be inferred from these data that HPV 16 uniquely avoids immune surveillance; however, the molecular basis of HPV 16 evasion is unknown.

The activation of antigen-presenting cells is critical to triggering innate immune responses to pathogens. Innate immune responses are the primary immune response that, unlike adaptive immune responses (e.g., cytotoxic T cells and antibodies), are nonadaptive (do not improve with each exposure to the pathogen) and are nonspecific, relying on molecular motifs common to pathogens rather than pathogen-specific molecular sequences (epitopes). Innate immune responses, characterized by nonspecific phagocytosis and inflammation, lead to seroconversion and cell-mediated immunity, the later of which includes the development of CD8+ T cells that may be necessary for viral clearance [21]. Human papillomavirus is an efficient activator of dendritic cells [22], antigen-presenting cells found throughout the body. As such, HPV is an effective adjuvant for overcoming tolerance to self-antigens [23]. However, HPV may be a poor activator of Langerhans's cells (LCs), a specialized antigen-presenting cell that is found in epithelia, including the epithelial layer of the cervix. Immune responses to HPV may require the addition of costimulatory molecules to overcome this block to acti-

vation [24, 25]. Poor immunologic responses to HPV may be the result of poor immune surveillance by LCs specific to HPV. It is noteworthy that the experiments using LCs were performed with HPV 16 virus-like particles, and the unresponsiveness of LCs may be HPV 16 specific, consistent with the aforementioned observations in HIV cohorts. Complementary data for the other HPV types are needed.

Genetic epidemiologic evidence also points to the importance of human immune responses, including innate immunity, in the viral natural history. Specific human leukocyte antigens (HLA) alleles and haplotypes, which encode for cellular membrane proteins for presentation of foreign (pathogen) molecules to the host immune system, have been shown to be related to HPV natural history and cervical cancer. Specifically, there is now consistent evidence that HLA class II *DRB1*13/DBQ1*0603* alleles are protective against HPV infection, cervical neoplasia, and cancer, but there has been no single allele has been found consistently to increase risk in epidemiologic studies conducted to date [26]. These data suggest that certain alleles alone or in combination may be advantageous for presentation of HPV antigens for viral clearance. However, given that no single HLA risk allele has been identified, it may take the presence of multiple suboptimal alleles to increase the risk of disease. Human leukocyte antigen class I allele *HLA-CW*0202* was negatively associated with having low-grade and high-grade cervical neoplasia, regardless of HPV type, in three studies of cervical neoplasia, suggesting that this allele may be associated with HPV persistence [27]. In addition to its role in acquired immune responses, HLA-C molecules stimulate a specialized cell of the innate immune system, natural killer cells, via killer cell immunoglobulin-like receptors. The interactions between natural killer cells and dendritic cells promote and regulate adaptive immune responses to infection [28]. The functional differences in HLA class I haplotypes and the development of an immune response to HPV have not been elucidated.

INFLAMMATION AND COINFECTION

Although acute inflammation, a characteristic of innate immune responses, may play a role in host immunity-mediated clearance of an HPV infection, there is an expanding body of literature suggesting that chronic inflammation may contribute to the development of cervical precancer and cancer as it may for other carcinomas. High rates of cervical cancer often coincide with endemic and epidemic cervicitis.

Chronic inflammation, unlike acute inflammation, may result in downregulation of cell-mediated immune responses, and therefore may increase the likelihood of HPV persistence. Chronic inflammation also may increase the exposure of cells to reactive oxygen species [7], which may result in increased genomic damage and possible progression to cervical precancer and cancer. A recent study demonstrated an association of cervicitis with high-grade cervical lesions among oncogenic HPV-infected women [29]. Another study reported increased cyclooxygenase-2 expression, a prostaglandin G/H synthetase that is specifically upregulated in inflammatory processes, in human cervical cancer [30]. Use of nonsteroidal anti-inflammatory drugs, which target the cyclooxygenase-2 pathways, may decrease the risk of cervical cancer [31]. Some studies, but not all, have found diets high in vitamin E, an antioxidant that could neutralize the potentially genotoxic by-products of inflammation-induced oxidative stress, were protective against high-grade cervical neoplasia and cancer [7]. Together, this evidence suggests a role for chronic inflammation in cervical carcinogenesis.

The origins of chronic cervical inflammation are uncertain, because HPV itself is not inflammatory, but may be the result of STIs other than HPV. Herpes simplex virus 2 seropositivity [32] and genital tract *Chlamydia trachomatis* DNA positivity [33, 34] have been linked with invasive cervical cancer, although other studies have failed to confirm these findings. Herpes simplex virus 2 is a chronic infection with acute viral activation and expression, and *C. trachomatis* may be chronic if untreated and may cause pelvic inflammatory disease. Thus, there is some biologic plausibility to the STI coinfection increasing the risk of cervical precancer and cancer, although the high-risk behavior leading to these infections also leads to HPV acquisition. Therefore, these associations between other STIs and cervical cancer may simply reflect an increased likelihood of exposure to the necessary causal agent, HPV, and non-causal agents (i.e., confounded by HPV).

SMOKING, ORAL CONTRACEPTIVE USE, AND MULTIPARITY

Smoking, prolonged oral contraceptive use, and multiparity have been implicated as main HPV cofactors based on strong epidemiologic evidence [6]. From an etiologic perspective, the roles of these cofactors in the development of cervical cancer require additional scru-

tiny because of the incomplete biologic evidence complementing the epidemiologic evidence. More exactly, there must be a measurable effect of these cofactors at the cervix to rule out the aforementioned residual confounding by HPV, that is, these cofactors may reflect differences in lifestyle that increase the likelihood of HPV exposure.

The evidence that smoking is an HPV cofactor is compelling, given recent prospective data showing exposure precedes the development of cervical precancer and cancer [35, 36] and the detection of smoking metabolites in cervical mucus [37] and smoking-related DNA adducts in cervical tissue [38]. Several questions remain. First, is the effect of smoking on the risk of cervical cancer the result of genotoxicity, immune suppression, or both? If smoking causes genotoxicity, is the formation of smoking-related adducts in cervical tissue associated with cervical precancer or cancer, and does greater adduct formation strengthen this association? That is, do oncogenic HPV-infected smokers in whom cervical intraepithelial neoplasia 3 or worse develops have greater adduct formation than those in whom the disease does not develop? If smoking is an immune suppressant, what is being modulated? For example, there is some evidence that heavy smoking decreases the number of cervical LCs, CD8+ T cells, and total lymphocytes [39].

Long-term oral contraceptive (OC) or hormonal contraceptive use has been linked to an increased risk of in situ and invasive cervical cancer [40], but is not associated with having an HPV infection [41]. The proposed mechanism by which OC use increases the risk of cervical cancer is via hormonal responsive elements that increase the transcription, and presumably translation, of E6 and E7 oncoproteins [42]. Thus, OC use may lead to an increased viral productivity and may result in greater persistence of the viral infection, which is consistent with the stronger association of longer-duration use and cervical cancer.

Cervical cancer also has been linked to high parity [43]. However, the risk was most strongly related to full-term vaginal births. The effects of parity seemed stronger in younger women, possibly indicating that the frequency of full-term births, presumably while carrying a concurrent HPV infection, leads to a greater risk. The most plausible mechanisms for the risk associated with multiparity are cervical trauma, maintenance of the transformation zone, thereby increasing HPV exposure to the susceptible tissue, or the production of cellular

oxidative and nitrate stresses that may lead to DNA damage [44].

FINAL COMMENTS

A number of factors secondary to oncogenic HPV infection of the transformation zone of the cervix have been implicated as cofactors. The strongest of these seem to be (innate and adaptive) cell-mediated immunity, smoking, prolonged OC use, STIs or inflammation, and the number of lifetime births. The impacts of these discussed factors are summarized in Figure 1.

Despite the convincing epidemiologic evidence and some biologic plausibility, more analyses are needed linking the epidemiology with the biology. In particular, there must be a measurable effect at the cervix to obviate the possible residual confounding by HPV. If these cofactors are biologic, it also can be anticipated that exposure to cofactors will be correlated with new more specific biomarkers of risk such as p16^{INK4a}, which is overexpressed in productive oncogenic HPV infections causing cervical neoplasia [45].

From an etiologic perspective, it will be interesting to determine whether the impact of an HPV cofactor is on viral persistence, the likelihood of progression to cervical intraepithelial neoplasia 3 or worse given persistent infection, or both stages. Thus, prospective studies of HPV-infected women are required. Such insights may inform the molecular mechanisms of HPV carcinogenesis.

To understand the biologic mechanisms of clearance versus persistence and progression, molecular measurements of the cervix may be required to describe and detail the carcinogenic process. Biomarkers in blood often correlate poorly with those in the cervix [7], suggesting that blood measurements of biomarkers will be less accurate than measurements at cervix. Messenger RNA and protein expression can be measured from bi-

opsied tissues and cervical scrapes such as those routinely collected in Pap smear screening, and protein expression can be measured from cervical secretions collected using a variety of well-tolerated collection devices, including ophthalmic sponges placed at the os of the cervix [46]. New technologies, such as gene expression microarrays for messenger RNA measurements and protein microarrays [47], recycling immunoaffinity chromatography [48], and flow cytometric methods [49] for protein measurements may be used to examine broad molecular states related to clearance, persistence, or progression. However, it is uncertain how representative measurements in these specimens are of the true immunologic and physiologic state of the cervix. The reproducibility of these measurements also must be addressed. Thus, intensive methodologic work is needed before the cervical microenvironment can be assessed and related both to exposures and to the risk of developing cervical cancer.

From a public health perspective, it is of less concern whether these exogenous cofactors are etiologic or are simply surrogates of HPV acquisition. These secondary risk factors have minor impacts on relative risk above infinite increase in relative risk associated with having an HPV infection versus not having one. In the case of OC use, the increase risk must be weighed against the impact of contraceptive use on the decrease of unwanted pregnancy and the morbidity and mortality attributable to these births in resource-poor regions. The cervical cancer risk associated with OC use also must be evaluated against the risk associated with not using OCs, namely greater parity, which is perhaps more prevalent in high-risk regions than OC use. A formal risk-to-benefit analysis for OC use is needed.

In conclusion, oncogenic HPV infection is the starting point for cervical carcinogenesis, but other factors, in the context of infection, may contribute to the development of cervical cancer. The biologic mechanism by which these cofactors confer risk needs greater exploration, as do their methods of action, that is, viral persistence or progression. The latter may be explored in large population-based cohort studies with HPV testing, such as those in Costa Rica [50], Brazil [51], and Denmark [52]. The relative contribution (i.e., attributable risk of these cofactors, given oncogenic HPV infection) of these cofactors will depend on the frequency of exposure in a given population. However, it remains unclear how much of cervical cancer can be attributed to these cofactors in the context of an oncogenic HPV infection

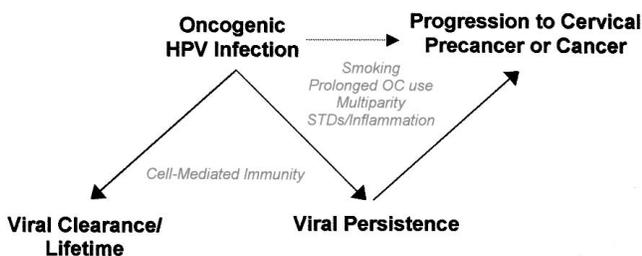


Figure 1. Summary of secondary factors that may affect the natural history of human papillomavirus (HPV) infection. OC, oral contraceptives; STDs, sexually transmitted diseases.

[53] and whether there is some fraction of cancer cases independently caused by HPV.

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