CHAPTER 3

Pathology of HPV infection at the cytologic and histologic levels: Basis for a 2-tiered morphologic classification system

Thomas C. Wright, Jr.

KEYWORDS
Squamous intraepithelial lesion (SIL); Cervical intraepithelial neoplasia (CIN); Cervix; Cancer precursors; Human papillomavirus (HPV)

Abstract Over the last 2 decades the pathogenesis and natural history of cervical cancer has become clearer. As a result, the cytologic and histologic terminology used to refer to cervical cancer precursors has needed to change. Today we recognize that almost all cervical cancers are due to infection with specific high-risk types of human papillomavirus (HPV). Most women become infected with these viruses within several years of initiating sexual intercourse and a productive HPV infection frequently results in characteristic morphologic changes within the infected cervical squamous cells. Cells demonstrating the morphologic changes associated with a productive HPV infection are referred to as low-grade squamous intraepithelial lesions (LSIL) when observed in cytologic specimens and low-grade cervical intraepithelial neoplasia (CIN 1) when observed in histologic specimens. In some women, HPV gene expression becomes unlinked to the state of differentiation of the infected epithelial cells and deregulated expression of the early region of the viral genome results in a dramatic increase in expression of two HPV oncoproteins (E6 and E7). This results in loss of normal cell cycle control of the epithelium and genetic instability. When this occurs the epithelium develops characteristic morphologic features, with immature “basaloid-type” squamous cells and mitotic figures in the upper half of the cervical epithelium. Such lesions are felt to represent “true” neoplasia and are referred to as high-grade squamous intraepithelial lesions (HSIL) when observed in cytologic specimens and high-grade cervical intraepithelial neoplasia (CIN 2,3) when observed in histologic specimens.

© 2006 International Federation of Gynecology and Obstetrics. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The histopathologic and cytopathologic classification of a disease should reflect both its clinical behavior and the current concepts regarding its pathogenesis. Over the last 2 decades our understand-
tive. Inevitably, many will question the need for the change and it takes years for training resources such as textbooks and management guidelines to be updated. The recent change in the histopathologic and cytopathologic classification of cervical cancer precursors seems to have been particularly disruptive, perhaps because so many clinicians are involved in different aspects of cervical cancer prevention. In this article we review the pathogenesis of cervical cancer as well as the histopathologic and cytopathologic classification and features of cervical cancer precursors.

2. Older terminologies

From the 1930s though the 1960s, noninvasive intraepithelial lesions of the cervix were classified using a 4-tiered terminology [1]. The 4 categories of precursor were mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma in situ (Figure 1). It was not widely accepted that dysplastic lesions were cervical cancer precursors, and carcinoma in situ was believed to be the only "true" precursor. Therefore, women with dysplasias of all grades tended to be followed up cytologically, whereas treatment for those with carcinoma in situ was frequently a cone biopsy or a hysterectomy. By the early 1970s, laboratory studies as well as prospective clinical follow-up studies showed that the dysplasia/carcinoma in situ terminology reflected neither the clinical behavior nor the pathogenesis of cervical cancer precursors [2]. The laboratory studies available at that time indicated that the cellular changes observed in dysplasia and carcinoma in situ were qualitatively similar and remained rather constant throughout the histologic spectrum of putative precursor lesions. Both dysplasia and carcinoma in situ could be monoclonal proliferations of abnormal squamous epithelial cells, and both were frequently aneuploid [3]. By electron microscopy and time-lapse cinematography, all grades of dysplasia and carcinoma in situ shared features [4]. Moreover, studies of intraobserver and interobserver variability in the histologic diagnosis of severe dysplasia and carcinoma in situ demonstrated that pathologists could not reproducibly differentiate the 2 entities [5]. Perhaps most importantly, prospective follow-up studies demonstrated that dysplastic lesions of all grades could progress to carcinoma in situ, and that all, therefore, could progress to cervical cancer. Based on the information available in the early 1970s, Richart [6] introduced the concept that all types of precursor lesions to squamous carcinomas of the cervix represented a single disease process. He termed this process cervical intraepithelial neoplasia (CIN).

At the time of its introduction, CIN was believed to describe a spectrum of histologic and cytologic changes that shared a common etiology, biology, and natural history. Although the CIN terminology divided the precursor lesions into 3 groups – CIN 1 for mild dysplasia; CIN 2 for moderate dysplasia; and CIN 3 for both severe dysplasia and carcinoma in situ (Figure 1) – these groups were considered to represent different stages of a single biological continuum. Therefore, inherent in the CIN terminology was the belief that CIN lesions of all grades were "true" cancer precursors and that all, even CIN 1 lesions, had the potential to progress to invasive cancer if untreated. The CIN terminology was introduced concordantly with the development of simple outpatient ablative techniques for treating CIN lesions, such as cryotherapy and electrofulguration. Because of this convergence it became widely recommended that CIN lesions of all grades, including CIN 1, be treated. The CIN terminology became widely adopted for use in both histopathology and cytopathology. Yet, as our understanding of the pathogenesis of cervical cancer and its precursor lesions improved, it became clear that the basic premise underlying the CIN terminology was incorrect.

3. Pathogenesis of cervical cancer

Today it is recognized that human papillomavirus (HPV) infection plays an essential role in the pathogenesis of cervical cancer [7]. Most women become
exposed to and infected with HPV shortly after initiating sexual activity. The target cells for an initial HPV infection are the immature basal cells of the epithelium, and HPV is thought to reach these cells through microabrasions or cracks within the epithelium. Viral replication is tightly linked to the differentiation state of the virally infected epithelial cells [8, 9]. Replication of the HPV genome is tightly controlled by cellular mechanisms within the basal cells, and appears to be linked to cellular replication so that the viral DNA replicates with the host’s genome. The number of copies of the HPV genome, as a circular or episomal form, is thus low in the basal cell’s nucleus, and virally encoded proteins are expressed at very low levels. As a result, HPV-infected basal cells show no specific cytologic or histologic changes and cannot be distinguished from uninfected cells. This stage of an HPV infection is referred to as a “latent” or “clinically unapparent” infection since the woman is HPV DNA positive, but no lesions can be detected, even by microscopy.

As HPV-infected epithelial cells differentiate and move upwards in the epithelium, viral transcription of the early region of the HPV genome dramatically increases [8, 9]. The early region encodes for a number of proteins, including E1, E2, E6, and E7, which are important for viral replication. The HPV genome is relatively small (approximately 8000 base pairs) and does not encode the enzymes required for viral DNA replication. The only proteins provided by the viral genome that are directly involved in viral replication are 2 regulatory proteins, E1 and E2, and HPV must therefore rely on the host’s DNA replication machinery for viral DNA synthesis [10]. However, as the epithelial cells differentiate, the cellular DNA replication machinery is normally inactivated. In order to undergo vegetative DNA amplification in the differentiating epithelial cells, the virus needs to reactivate the cellular DNA replication machinery. Studies of cultured human keratinocytes have shown that the viral E7 protein is capable of reactivating the cellular DNA replication machinery in differentiated cells [11]. The viral E6 protein also appears to play an important role by blocking the apoptosis that would normally occur in the differentiated cells [12]. Together, these changes provide in the cell the synthetic phase environment necessary for vegetative viral DNA replication and complete virion formation. Infectious virus is eventually released as the differentiated cells are shed from the epithelium. In most women immunity develops against HPV after a period of months or years, and productive viral infection ceases [13]. These women eventually become HPV DNA negative.

In some HPV-infected women viral gene expression becomes unlinked to the state of differentiation of the infected epithelial cells, resulting in a change in the topography of viral gene expression within the epithelium (see Chapter 1) [8, 9]. One of the results of this other mode of viral infection is the dramatic increase in the expression of E6 and E7 HPV in the lower layers of the epithelium due to the proteins’ deregulated expression [8, 9]. The deregulated expression of HPV proteins E6 and E7 results in the disruption of normal cell cycle regulation; abrogation of apoptosis mechanisms; and genetic instability. Genetic instability, which is a characteristic feature of most malignant neoplasms, occurs early in the development of precancers, thereby allowing for the stepwise acquisition of multiple mutations. Produced by alterations in the mitotic spindle apparatus, genetic instability permits aberrant mitotic events that can produce a disequilibrium in the distribution of chromosomes, leading to changes in the number and structure of chromosomes. It can eventually produce a change in the overall DNA content, referred to as aneuploidy. Genetic instability is thought to play a critical role in the development of cervical cancer [14].

The association between infection with high-risk types of HPV and high-grade cervical cancer precursors as well as cervical cancer is so strong that it has prompted the use of HPV DNA testing in the clinical management of women with the “borderline” cytologic abnormalities referred to as atypical squamous cells of undetermined significance (ASC-US), and as an adjunct to the cytologic evaluation of women aged 30 years and older who undergo routine cervical screening [15, 16]. When HPV DNA testing is used in the management of women with ASC-US, molecular testing for high-risk types of HPV is performed, typically with residual fluid after a liquid-based cytologic specimen is processed. If a woman tests positive for high-risk HPV DNA she is referred for colposcopy; and if she tests negative, routine screening is resumed [16, 17]. In women who test positive for high-risk HPV DNA but are found to be cytologically negative, both tests are repeated 6 to 12 months after the initial screening. Because the negative predictive value of being both cytologically negative and testing negative for high-risk HPV DNA is greater than 98.8% in all studies, and 99.9% or 100% in most, women for whom the results of both tests are negative do not require rescreening for 3 years [15].
4. Two-tiered morphologic classification system of noninvasive HPV-associated cervical lesions

It used to be believed that the multistep nature of the pathogenesis of cervical cancer described above was mirrored by a defined sequence of morphologic changes, identified as CIN 1, CIN 2, and CIN 3, and that these changes reflected the natural history of CIN lesions. Numerous studies have demonstrated over the past several decades that a higher CIN grade correlated with a greater risk that lesions progress to a higher grade or to invasive cervical cancer. A comprehensive review of natural history studies of CIN lesions was conducted by Ostor (Table 1) [18]. The investigator concluded that spontaneous regression during the different follow-up studies occurred in 57%, 43%, and 32% of cases for CIN 1, CIN 2, and CIN 3 lesions, respectively, and that persistence occurred in 32%, 35%, and 56% of these lesions. Only 1% of CIN 1 lesions and 5% of CIN 2 lesions, but more than 12% of CIN 3 lesions, progressed to invasive cervical cancer during follow-up.

Moreover, it is now recognized that lesions that would in previous days have been considered high-grade CINs (CIN 2,3) often develop quickly after initial infection with high-risk types of HPV, and that such lesions are not necessarily preceded by lesions with the morphological appearance of a CIN 1 [19]. For example, in a long-term follow-up study of college students, it was found that the median time from first detection of HPV DNA to identification of a histologically confirmed CIN 2,3 lesion was only 14.1 months [19]. Similarly, in a long-term follow-up study of a prophylactic vaccine against HPV-16, most CIN 2,3 lesions associated with HPV-16 in the placebo arm appeared to develop rapidly after the initial infection with HPV-16 [20]. Therefore, the older concept that it took years to progress from a CIN 1 to a CIN 2 or CIN 3 lesion appears to be incorrect.

We thus recognize that high-grade CINs (CIN 2,3) often develop quickly after an incident infection with high-risk types of HPV, and that most CIN 1 lesions are not “neoplasia” but simply virus-producing lesions. Moreover, a lesion with the morphologic appearance of CIN 2 does not necessarily have fewer chromosomal alterations than a lesion with the morphologic appearance of CIN 3. Therefore, the terminology used for noninvasive HPV-associated cervical lesions needs to be changed to better reflect the biology of HPV infections. Instead of classifying noninvasive HPV-associated cervical lesions as a continuum of lesions (CIN 1, CIN 2, and CIN 3) reflecting a single underlying biological process at different stages of development, we need to represent noninvasive HPV-associated cervical lesions as 2 distinct biological entities (Figure 2) [21]. One represents a productive viral infection caused by oncogenically low- or high-risk types of HPV. These lesions are usually self-limited and spontaneously resolve when the patient stops shedding HPV. The other represents a true neoplastic process confined to the epithelium, but with the capacity to progress to invasive cervical cancer if not treated. These neoplastic lesions are usually histologically high grade; they almost always are associated with high-risk types of HPV; and they show deregulation of E6 and E7 expression. They also are monoclonal and have chromosomal alterations.

The 2-tiered classification system can account for all noninvasive HPV-associated cervical lesions. Lesions that are most likely the manifestation of a productive viral infection are referred to as “low-grade” and those that potentially represent a cervi-
Figure 2 Low-grade/high-grade terminology for noninvasive HPV-associated epithelial lesions. This terminology is based on the realization that morphologically low-grade lesions generally represent productive HPV infections and do not act as precursors to invasive cervical cancer. In contrast, morphologically high-grade lesions tend to be neoplastic and have the potential to act as precursors to invasive cancer. Low-grade and high-grade lesions are separated on the diagram to emphasize the fact that high-grade lesions can develop de novo.

Almost half of these lesions are self-limited, and have been observed to regress spontaneously within short periods without any treatment [24]. However, most of these lesions are associated with high-risk types of HPV and many are aneuploid and demonstrate loss of heterozygosity (LOH) at specific chromosomal loci. These are characteristic features of CIN 3 lesions and invasive cervical cancer [26]. A histopathologic diagnosis of CIN 2 is also poorly reproducible. Because a considerable proportion of lesions with the histologic features of CIN 2 have the biologic features of neoplasia, they are classified as high-grade in the 2-tiered terminology [21,22,26].

5. Histologic and cytologic features of noninvasive HPV-associated cervical lesions

5.1. Low-grade productive HPV infections

During the productive viral infection stage, large numbers of complete viral genomes are produced, which are subsequently packaged with the capsid proteins into infectious virions. Characteristic cytologic changes occur in the squamous cells in which productive HPV infection is taking place. These are multinucleation, nuclear enlargement, nuclear hyperchromasia, irregular nuclear outlines, and perinuclear halos. Squamous epithelial cells demonstrating these cytologic features are referred to as koilocytes, a word derived from the Greek koilos, which means hole (Figure 3). These changes are recognizable in both cytologic and histologic specimens.
Classic cytologic features low-grade CIN:
- Cellular enlargement
- Multinucleation
- Nuclear hyperchromasia
- Nuclear irregularity
- Presence of perinuclear halos

Classic cytologic and histologic features high-grade CIN:
- Immature basaloid-type cells with high nucleus to cytoplasm ratio
- Immature basaloid-type cells in the upper half of the epithelium
- Mitoses occurring in the upper half of the epithelium
- Irregular, hyperchromatic nuclei
- Abnormal mitotic figures

In addition to the cytologic changes occurring in individual squamous cells, architectural changes also occur in a squamous epithelium in which productive HPV infection is taking place. The squamous epithelium then frequently becomes acanthotic (or thickened), and sometimes forms papillary projections with central fibrovascular cores (Figure 4A and B). Lesions with this histologic appearance are referred to as low-grade CINs (CIN 1), whereas specimens showing the cytologic features of HPV effects are referred to as LSILs.

In-situ hybridization techniques can demonstrate the presence of HPV DNA in koilocytes within tis-

Figure 3 Low-grade squamous intraepithelial lesion (LSIL). Epithelial cells removed using a spatula or broom from the more differentiated layers of the squamous epithelium of the cervix demonstrate the characteristic features of a productive human papillomavirus (HPV) infection. They show multinucleation, perinuclear halos, nuclear enlargement, and hyperchromasia. Cells with these features are frequently referred to as koilocytes.

Figure 4 Low-grade cervical intraepithelial neoplasia (CIN 1). (A) During productive HPV infection the cervical squamous epithelium becomes somewhat thickened, or acanthotic. (B) At higher magnification the cytologic effects of a productive HPV infection are obvious, with multiple koilocytes.
In-situ hybridization has been performed with a probe against the DNA of high-risk types of HPV. Cells that have blue nuclei have large numbers of HPV genomes indicating that a productive viral infection is taking place. Most of the productively infected cells are in the more differentiated layers of the epithelium. Although this lesion has the morphologic features of a low-grade lesion, it is caused by infection with a high-risk type of HPV.

S28 T. C. Wright, Jr.

Figure 5 In-situ hybridization of HPV in a low-grade CIN (CIN 1). In-situ hybridization has been performed with a probe against the DNA of high-risk types of HPV. Cells that have blue nuclei have large numbers of HPV genomes indicating that a productive viral infection is taking place. Most of the productively infected cells are in the more differentiated layers of the epithelium. Although this lesion has the morphologic features of a low-grade lesion, it is caused by infection with a high-risk type of HPV.

Figure 5

5.2. High-grade neoplastic HPV-associated lesions

Lesions that have the histologic features of a neoplasia and may be precursor lesions are referred to as high-grade CINs (CIN 2,3), whereas cytological specimens with these features are referred to as HSILs. Although HPV-induced cytopathic effects are often present, they are usually less prominent in high-grade CIN (CIN 2,3) than in low-grade CIN (CIN 1). High-grade CIN (CIN 2,3) is characterized by immature basaloid cells and mitotic figures that extend into the upper half of the epithelium (Figure 6). The immature basaloid cells usually have nuclear crowding, pleomorphism, and loss of normal cell polarity. They have a minimal amount of cytoplasm and therefore a high nucleus to cytoplasm ratio. Another feature found in most high-grade lesions is the presence of abnormal mitotic figures (Figure 7). Most invasive cervical cancers have chromosomal abnormalities and LOH at specific chromosomal loci. In addition, they are aneuploid. This suggests that aneuploid intraepithelial lesions with LOH at these loci are at risk for progressing to invasive carcinomas if left untreated, and studies have documented that the percentage of aneuploid CIN lesions with LOH at the specific sites increases with CIN grade [28–30]. In one study that used an imaging system to measure the DNA content of Feulgen-stained cervical cells, it was found that 33% of mildly dysplastic lesions were aneuploid [28]. The percentage of aneuploid lesions increased to 75% for moderate dysplasias and carcino-
mas in situ. Similar results have been obtained using other techniques to assess DNA content. As 85% of CINs with aneuploid DNA patterns contain abnormal mitotic figures, the best histologic predictor of an aneuploid lesion is the presence of abnormal mitotic figures [31].

Another characteristic feature of high-grade lesions is overexpression of p16INK, a cyclin-dependent kinase inhibitor involved in the control of the cell cycle [32]. The intracellular expression of p16INK is increased upon the binding of high-risk, HPV-derived E7 oncoproteins to the retinoblastoma gene product. A number of studies have demonstrated that staining with p16INK is very uncommon in normal cervical squamous epithelium, but that a small proportion of CIN 1 lesions, a higher proportion of CIN 2 lesions, and the vast majority of CIN 3 lesions and cancers stain positively for p16INK (Figure 8) [33–36]. Moreover, prospective follow-up studies suggest that p16INK-positive lesions behave as precursor lesions. In one prospective study with women having low-grade CINs (CIN 1), the rate of regression of p16INK-negative lesions was found to be higher (71%) than that of p16INK-positive lesions (38%) [37]. Although some of the p16INK-negative low-grade CINs (CIN 1) progressed to high-grade CINs (CIN 2,3) in that study, the p16INK-positive lesions were much more likely to progress. In another prospective study, 44% of the women with lesions found to be p16INK positive on biopsy and classified as “not CIN 2,3” by consensus histopathologic diagnosis were subsequently diagnosed with CIN 2,3 [33]. Therefore, there is considerable data suggesting that p16INK immunostaining can help identify which high-grade CIN (CIN 2,3) lesions will behave as precursor lesions.
6. Conclusions

Over a decade ago, S.J. Gould thus described the significance of taxonomy to the sciences:

“The much maligned science of taxonomy, the ordering and classification of organisms, takes a culturally imposed backseat to the more interventionist and generalizing work style of experimentation and quantification. But taxonomy should be viewed as one of the most fundamental, and probably most noble, of scientific pursuits — for what can be more basic than the parsing of nature’s rich and confusing complexity? Our categories, moreover, record our modes of thought, and taxonomy therefore teaches us as much about our mental functioning as about nature’s variety.” [38]

This review of the evolution of the terminologies used for noninvasive HPV-associated lesions of the cervix provides greater insight into how pathologists, clinicians, and scientists view the pathogenesis of cervical cancer than into the actual morphologic expression of the lesions. While the latter have remained stable, each change in terminology, from the dysplasia/carcinoma in situ terminology of the 1950s, through the spectrum of CIN introduced by Richart in the late 1960s, to the Bethesda System’s low-grade/high-grade lesion terminology, has been based on new scientific information that has altered our fundamental views of the pathogenesis of cervical cancer.

References

Pathology of HPV infection at the cytologic and histologic levels


