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Cervical Intraepithelial Neoplasia

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Abstract Cervical intraepithelial neoplasia (CIN) has been traditionally defined as a continuum of intraepithelial squamous abnormalities which exhibit nuclear atypia in all epithelial layers and possess some potential for progression to invasive carcinoma if not removed. Efforts to subdivide this spectrum into categories of low and high cancer risk have been based previously on the strong association between CIN III (carcinoma *in situ)* and subsequent invasive carcinoma. However, in practice, this distinction has been discouraged because CIN I and II may be associated with CIN III and a small proportion may progress to invasive carcinoma. As human papillomaviruses (HPV) have emerged as potential markers for subdividing precursor lesions, so-called "high-risk" HPV types have been associated with all grades of CIN, whereas "low-risk" HPV types have segregated primarily in lesions closely resembling condylomata. The place of condyloma in the spectrum of CIN, as well as the precise definition of CIN I, has been controversial. Some authors distinguish condyloma from CIN I and others use similar criteria for the diagnosis of both. Currently, the trend among pathologists and cytopathologists is to classify CIN I as a process either identical to or closely resembling condyloma (low-grade), and CIN II and III as lesions falling within the spectrum of CIN as classically described (high-grade). As new etiologic perspectives (HPV), classifications (Bethesda) and outpatient managements (LEEP) evolve, morphologic definitions of CIN will remain important to patient care, particularly if management decisions are based on nuances of histologic or cytologic grade. When using cervical lesion morphology as an endpoint in chemoprevention studies, investigators must understand that "morphologic progression" of CIN may not be synonymous with biologic progression, that discrepancies between HPV type and morphology exist, and that cytology and histology provide variable, and at times conflicting, information.

Key words: Cervical intraepithelial neoplasia, cytology, human papillomavirus, morphology

Strategies aimed at chemoprevention of cervical cancer include preventing its precursor lesion, cervical intraepithelial neoplasia (CIN). Because cervical carcinomas are presumably preceded by CIN in most cases, carcinomas could be prevented by preventing either the development of CIN or its progression to invasive carcinoma. This discussion will focus on three aspects of CIN which may be relevant to prevention: the relationship of papillomaviruses to CIN; grading systems for CIN and their biologic validity; and other aspects of cervical biology which may influence the natural history of cervical neoplasia and should be considered when devising strategies for prevention.

PATHOGENESIS OF HPV-RELATED CERVICAL NEOPLASIA

Important relationships between papillomaviruses and cervical neoplasia include specific HPV types associated with advanced CIN and invasive carcinoma. These viruses share charac-

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teristic influences on cultured keratinocytes. Specifically, HPV types 16, 18, and others can immortalize cells in culture. Immortalization capacity is linked to the E6 and E7 proteins, which bind the p53 and Rb gene products. This binding is thought to be important in the pathogenesis of HPV-related neoplasms because unlike HPV-negative tumors, HPV-positive tumors rarely manifest with mutations in Rb or p53 genes. This is still controversial, however. Nevertheless, E6/E7 gene products exert influences over squamous cell proliferation in culture, verifying their role in the genesis of neoplasia [1].

Also important is that integration of papillomavirus DNA into the cell genome occurs preferentially in invasive squamous cell carcinomas. Disruption of the virus, which occurs between the E1 and E2 open reading frames, preserves the E6/E7 region while sparing this region from control by the E2 open reading frame. It is not clear whether integration is a critical event, since one-fourth of HPV 16-associated carcinomas exhibit integration. However, virtually all HPV 18-related carcinomas exhibit this phenomenon, an interesting issue in light of the data discussed below [2].

A third important point relates the integrity of the normal viral life cycle (vegetative viral functions), directly or indirectly, to the biologic effect of papillomaviruses. It is significant that a high proportion of HPV 6 or 11 infections manifest with vegetative replication, production of capsid proteins, and koilocytotic atypia. In contrast, HPV 16 frequently manifests with minimal viral maturation and generally much less evidence of koilocytosis [3]. Whether the lack of viral cytopathic effect in HPV 16-related lesions is a function of biologic effects of the virus (*i.e.*, with disruption of normal cell maturation), or signifies a defect in the capacity of this virus to maintain viral maturation, is unclear. However, the implication is that HPV 16, while able to infect squamous epithelium, is not efficient in maintaining a normal life cycle. This could result in autoregulation defects and over-expression of viral oncogenes. Indirectly supporting this hypothesis is the presence of two distinct morphological phenotypes associated with HPV 18 infection. The most publicized is the spectrum including adenocarcinoma *in situ*, invasive adenocarcinoma, and small cell undifferentiated carcinoma of the cervix [4–6].

Interestingly, less than 5% of pure squamous cell carcinomas generally contain HPV 18, and as mentioned above, the viral DNA is invariably integrated [2]. However, a more recently recognized and far more common manifestation of HPV 18 is a low- to moderate-grade (CIN I–II) precursor lesion. These have been shown to contain abundant viral DNA; they produce capsid proteins characteristic of condylomata in general [7,8]. Studies of DNA sequences indicate that the same virus is responsible for both processes, in contrast to HPV 16, which segregates in higher grade precursors and cancers [8]. This suggests that under certain circumstances HPV 18 pursues normal viral replication despite its potent transforming potential *in vitro*. This preservation of normal viral functions may effectively inhibit the expression of gene products (E6/E7) responsible for the tissue effects observed in advanced neoplasia. The invariable presence of viral genomic integration in HPV 18-associated cancers supports this scenario [2]. Because genomic integration eliminates normal vegetative replication, this would explain advanced disease associated with a virus which normally maintains a normal life cycle and regulation of its oncogenes.

The location of the infection in the cervix is also related to CIN. Another poorly understood factor which influences risk is the cervical transformation zone itself. Several studies have addressed this in depth, emphasizing several important issues. Precursor lesions do not develop on the portio *per se*, virtually always occurring in the region into which the native squamous epithelium of the portio has extended. CIN virtually never occurs in squamous metaplasia deep within the transformation zone (T-zone). CIN lesions, including those designated as condyloma, generally become progressively less differentiated as they extend cephalad into the endocervix. These phenomena point to important factors in the T-zone which influence not only lesion development, but the morphologic progression of lesions once they develop [9].

Lastly, the relative "oncogenesis" of different HPV types is related to CIN. Despite different HPV types being associated with different general morphologies, certain types emerge as potent risk factors for invasive carcinomas.

For instance, low-risk HPV types are not identified in invasive carcinomas of the cervix except under very unusual circumstances. These types

comprise approximately 5% of precursor lesions, where they predominate in the low- grade category, and 0% of cancers. They include types 6, 11, and others [10].

Prototypical high-risk HPV types, specifically types 16 and 33, correlate strongly with CIN II and CIN III or invasive carcinoma. We have found HPV 16 in approximately 60% of both high-grade CIN and invasive carcinomas [11].

Type 31 is the second most common HPV type detected, comprising approximately 15% of high-grade CIN lesions, predominating in lesions classified as CIN II [12]. The association of HPV 31 with CIN is considerably stronger than invasive carcinoma in our experience, with less than 2% of carcinomas containing this HPV type [C.P. Crum, unpublished observations].

Type 18 is a "biphasic" HPV type with respect to phenotype. It comprises 4–5% of cervical squamous precursor lesions, segregating primarily in lesions in the CIN I to CIN II category [7,8,11]. It is rarely identified in CIN III unless it is accompanied by adenocarcinoma *in situ*. In our experience, HPV 18 is very uncommon in pure squamous cell carcinomas (about 2%), paralleling HPV 31 [C.P. Crum, unpublished observation]. In contrast, it is the dominant HPV type in adenocarcinomas *in situ* and invasive adenocarcinomas, adenosquamous carcinomas, and small cell undifferentiated carcinomas, detected in at least 50% of these lesions [4–6]. Thus, HPV 18 does the most "damage" via non-pure squamous neoplasms. The other route, including pure squamous carcinomas, appears to be a minor one for this HPV type.

At least 20% of CIN lesions are associated with as yet untyped HPVs [11].

LATENT PAPILLOMAVIRUSES AND THEIR RELEVANCE TO MORPHOLOGY

HPV may exist in the genital tract in the absence of morphologic abnormalities. Latent or occult papillomavirus infection is defined as the presence of HPV DNA in the absence of a visible, histologic, or cytologic abnormality. It is not synonymous with subclinical infection unless the latter is defined as the absence of a colposcopic abnormality. Latent infection was first implied by Oriel [12], who observed that the lag period from exposure to clinical warts was approximately four months [12]. Kreider *et al.* [13] also noted that a period of several weeks separated experimental infection and a morphologic abnormality. Steinberg *et al.* [14] first reported HPV nucleic acids in normal epithelium in the larynx; a wealth of data has been collected in the past 10 years which signify that HPV nucleic acids may exist in normal-appearing squamous epithelium. Significantly, no studies have verified the precise location of such occult viral sequences since they were demonstrated by blot hybridization or polymerase chain reaction rather than *in situ* hybridization. Hence, the concept of latent HPV nucleic acids and their relationship to clinical disease has received intense scrutiny. The risk of clinical disease in women with occult HPV DNA infection is correlated with recurrent disease.

Ferenczy *et al.* [15] determined that recurrences in women following laser therapy were related to the presence of HPV DNA in the normal squamous epithelium adjacent to the laser site. This suggests that recurrences are most likely due to endogenous activation of the virus rather than re-infection from the sexual partner. It is important to stress that this process of reactivation cannot be averted by extensive physical destruction of epithelium, as one study painfully demonstrated. Rather, it appears that with increasing time the host immune response reduces the risk of recurrence. In the above study, virtually all recurrences developed within six weeks of therapy, presumably because those infections were in their early stages. Long-term follow-up after ablation failed to demonstrate persistent virus in most individuals. These observations indicate that re-infection is endogenous rather than attributable to the sexual partner. Krebs [16] found that recurrence rates of cervical lesions were not influenced by treating the male partners.

Recurrent disease which develops after ablation of cervical lesions may contain HPV types other than the original lesion [17]. In the initial years of cryotherapy, it was generally assumed that persistence of abnormalities in the cervix after therapy signified inadequate therapy. However, with the advent of HPV DNA testing, it became possible to determine if recurrent abnormalities were the result of the initial infectious virus. Nuovo and Pedemonte [17] observed that in many cases, lesions developing after cryotherapy were frequently associated with HPV types

other than those associated with the original lesion. These findings implied that a "recurrence" may signify a new infection not hindered by the immune response to the previous infection. Indirect evidence for immunity was provided in the study by Rosenfeld *et al.* [18] who found that only 1% of adolescents tested twice actually harbored the same HPV type on both occasions. Follow-up studies of treated women likewise argue for immune phenomena which reduce recurrence rates [19]. This is in contrast to the data by Ferenczy *et al.* [15]; however, the latter study involved treating lesions which had been visible for a short time on the external genitalia and conceivably would be at greater risk for recurrence. In contrast, cervical lesions detected by Papanicolaou (Pap) smear would have been present longer; the elapsed time would permit an immune response which would effectively prevent re-infection by the original virus. Predictably, infections in immunosuppressed patients are more likely to persist, with the same HPV type present in both original and recurrent lesions [20].

The index of HPV DNA in the population is dependent upon detection technique, age, sexual activity, and timing of sexual partners. Studies detailing the profile of HPV DNA in the population have provided conflicting data on the index of HPV DNA in women. These conflicts arise from using detection techniques with variable sensitivity and from the nature of the target population. Studies using polymerase chain reaction technology have provided an increased index of HPV-positives in contrast to Southern blots, the index of the two techniques being approximately 20 and 10%, respectively [21]. However, the nature of the target population significantly influences the index, irrespective of the technique used. Using the technique with the highest specificity (Southern blot hybridization), Rosenfeld [18] observed a positive rate as high as 39% by a single test in young, sexually active teenage women. Using polymerase chain reaction for HPV detection, Melkert *et al.* [22] observed a 14.1% detection rate in sexually active women between 15 and 30 years of age. In contrast, the same authors observed an HPV-positive rate of 4.1% in women 35–55 years of age in cervical cancer screening programs. One of us [unpublished observation] found the index of HPV-positives is less than 2% in women with the lowest risk (women from 35–55 undergoing hysterectomy for benign disease).

Specific indices of sexual activity also influence HPV positives. Bauer *et al.* [23] observed a relationship between number of lifetime sexual partners and HPV positivity. Other studies have produced conflicting data concerning the role of sexual activity. Kjaer *et al.* [24] noted a lower index of HPV-positives in women with a history of many sexual partners, and postulated that multiple partners may produce immune responses which eventually clear the virus. Studies supporting this concept have found that the index of HPV positivity is related to timing rather than absolute number of sexual partners, with the index highest in women with new sexual contacts in the months preceding DNA testing [25]. Other risk factors, including a history of sexually transmitted diseases, also influenced the index of HPV-positives.

The risk of detecting a lesion in an HPV-positive individual is variable and depends upon the target population, clinical history, and thoroughness of follow-up. In several scenarios, HPV-positives can be correlated with lesion detection. The first is the patient with an abnormal Pap smear who undergoes colposcopic evaluation. In this instance, it is assumed that Pap smear abnormalities associated with precursor lesions will be more likely to register as HPV-positive. Predictably, one study found that approximately 70% of HPV-positives in this group had a confirmed precursor lesion; approximately 20% of HPV-negatives were histologically confirmed [26]. These findings illustrate that the association between the presence of HPV DNA and a cervical lesion is strengthened when women are selected through an abnormal Pap smear. A portion of women with lesions, however, will not register HPV-positive.

The predictive value of HPV DNA-positives in women without an abnormal Papa smear varies with the population under study. Approximately 20% of consecutively evaluated HPV-positive women will have an abnormal Pap smear. Of those with a negative smear, the chance of histologic confirmation depends heavily on clinical history. Cox *et al.* [26] found 30% of HPV-positives histologically confirmed (versus 3% of HPV-negatives) in women referred for an abnormal smear whose second smear was negative.

Other studies found a powerful association be-

tween HPV-positives (in women with currently normal Pap smears) and histological confirmation of disease [27]. Relatively few studies have followed women without an abnormal smear. Lorincz *et al.* [28] found that HPV-positive women with a current normal smear were more likely to have a histologically confirmed lesion on follow-up if they had a history of prior disease. A study following HPV-positive women from a sexually transmitted disease clinic found that virtually all lesions developing in the first two years after conversion to HPV-positive occurred more commonly in women under age 20; two-thirds presented cytologically as CIN II/III without an intervening CIN I on cytology [29]. Importantly, this study population consisted of patients from an STD clinic, who had a high rate of sexual activity and co-existent sexually transmitted diseases. The latter has been correlated with risk of a co-existing or subsequent cervical lesion in HPV-positive individuals. The presence of HPV 16/18 also correlated the strongest with subsequent lesions, but other HPVs correlate as well, indicating that those HPVs were indirect markers for risk. In low-risk populations (office practice), the risk of developing a lesion in two years was much lower. One study found no significant difference between HPV-positive and -negative women with no history of a prior abnormal Pap smear [28].

Preliminary studies of closely followed HPV DNA-positive patients indicate that a significant proportion will manifest an abnormality in the cervix. In many cases, however, this appears to be a transient phenomenon, indicating that infection is cleared in many cases [L. Koutsky, personal communication]. This is supported indirectly by the much lower incidence of HPV-positives in women over age 35, in contrast to much younger women.

HPV testing by *in situ* hybridization correlates with the presence of morphologic atypia. This concept is particularly relevant to lesion identification and classification. The great majority of HPV-positive epithelia, as determined by direct demonstration of HPV nucleic acids, are abnormal in appearance.

Precisely where HPV DNA resides in women with "latent" infection remains to be determined, although it is probably harbored in small copy number in the basal epithelium for a period of time prior to lesion development. This has been implied by Kreider [J. Kreider, personal communication], who found little morphologic evidence of infection in the first few weeks after infecting grafts of foreskin and placing them under the renal capsule of nude mice. However, detecting HPV nucleic acids in tissues in the absence of morphologic change is uncommon, indicating that when viral DNA increases to the point of detection, it will usually be accompanied by some degree of morphologic change.

EVOLUTION OF THE CLASSIFICATION OF CIN

The CIN Classification

In the late 1960s, Richart [30] proposed unifying the dysplasia-CIS classification into the CIN classification, because of the biological similarity between different grades of dysplasia. Ploidy and cell culture studies linked lesions at either end of the spectrum, notwithstanding a higher rate of polyploidy in the lower grade lesions [31]. Also, subdividing lesions into two categories (dysplasia/CIS) could prompt excessive treatment of the latter, whereas CIN defined the same disease process, and its management was predicated upon clinical as well as histologic parameters. Criteria for subdividing each grade paralleled that of the dysplasia-CIS category, with emphasis on the spectrum as indivisible, notwithstanding the presence of three grades (CIN I–III). Lesions at the lower end of the CIN spectrum as defined were more likely to be diploid/polyploid; it was assumed that despite the common name, CIN comprised a spectrum of abnormalities with different risks of progressing to carcinoma. Notably, requirements for a diagnosis of CIN included nuclear atypia in the full thickness of the epithelium, with degrees of maturation separating individual grades [9].

Discovery and Definition of Condyloma

When first described in the 1950s, koilocytotic atypia was provisionally classified as a form of dysplasia [32]. However, the epithelial characteristics of this process differed significantly from those of classic dysplasia/CIS. Significantly, atypia in the most bland form of koilocytosis was confined to the superficial cell nuclei; comparisons with vulvar intraepithelial lesions dem-

onstrated distinct differences between condyloma and vulvar intraepithelial neoplasia [33]. The latter, including CIN (as classically described), was characterized by nuclear atypia throughout the epithelium. A number of authors noted a strong relationship between koilocytosis and dysplasia, with lesions containing both koilocytotic atypia and nuclear atypia in the lower cell layers. Such lesions were variously categorized as koilocytotic dysplasia, atypical condyloma, and CIN with koilocytosis. In the early 1980s, one of us devised a classification which subdivided cervical and intraepithelial lesions into condylomata, intraepithelial neoplasia with (CIN K) and without (CIN) koilocytosis [34]. This approach made no attempt to define CIN I, although CIN with koilocytosis denoted lesions in the CIN I–II category and CIN without koilocytosis designated lesions in the CIN II–III category. Based on a number of subsequent molecular epidemiologic studies, a strong correlation between CIN lesions and high-risk HPVs was established [7,11,35].

Revisions in the CIN Classification

One of the problems produced by the discovery of condyloma and the prominent role of HPV in the natural history of precursors was inclusion of condyloma within the CIN classification. In a consensus meeting sponsored by the ISGYP in 1988, a panel of gynecologic pathologists defined CIN I using the criteria previously used to differentiate condyloma, with CIN II exhibiting the cellular abnormalities characteristic of dysplasia [36]. What was traditionally termed CIN I was now classified as CIN II [36]. This is not universally accepted. For example, others distinguish condyloma from CIN I (the traditional approach), marking CIN I as the earliest point in the onset of nuclear atypia in all layers of the epithelium [37]. Either approach is valid as described, but the revised approach, by reducing a four-grade system to three, effectively combines CIN I and CIN II into a single category of CIN II, with CIN III corresponding to CIS as originally described.

The Bethesda System

The Bethesda System proposed contracting CIN into two categories, including low- and high-grade lesions. Low-grade lesions, placed at the low end of the spectrum, are synonymous with CIN I (condyloma) [38]. High-grade lesions correspond to CIN II–III, or well and poorly differentiated CIN lesions as classically described. The distinction between CIN I and CIN II is based on relatively reproducible criteria (the presence or absence of nuclear atypia throughout the epithelium); the distinction between CIN II and CIN III is based upon less reproducible parameters (nuances in maturation).

Weaknesses in the Concept of a Binary Classification System

There are several reasons to divide precursor lesions into two groups as defined above. First is the strong association between grade and certain HPV types, specifically types 6, 11 and 16; second is the simplicity with which the system can be applied; third is that the cytologic counterpart to abnormal parabasal cells would be classified as high grade, although they may or may not be available for cytologic evaluation; a fourth is the close association between CIN II and CIN III. This concept has several limitations, including a poor understanding of viral-host cell interactions and factors which predispose some women to cervical carcinoma. One factor is the relatively poor correlation between short-term natural history, lesion grade, and HPV type. Lesions within the entire spectrum of condyloma/CIN have been observed to both regress and persist [31]. Importantly, a relatively high proportion of flat condylomas of the cervix will not regress within six months [39]. Another factor is the different distribution of high-risk subtypes within the spectrum of high-grade lesions. For example, Types 31 and 18, two cancer-associated HPV types, are more likely to be associated with lesions classified as CIN I or CIN II than CIN III [7,11]. This is in contrast to HPV 16, and suggests important biologic differences between these HPV types and HPV 16. Moreover, there are important differences in the association of certain HPV types and precursors versus invasive carcinomas [10]. The latter observations suggest that host factors may play an important part in the genesis of certain HPV-related neoplasms. Moreover, they may challenge the popular concept that all cancers develop from precursor lesions which evolve over a long time. This is suggested by comparisons of mean age between

different precursor lesions. In our experience, the mean age of HPV 16-associated CIN III is approximately four years later, significantly higher than CIN II. This implies that CIN II occurs earlier and progresses over time to CIN III. However, differences in mean age between HPV 18-associated squamous and high-grade squamo/glandular neoplasia (CIN III/ACIS) is much greater (35 versus 22 years), implying that factors other than undetected progression of CIN I results in CIN III-ACIS [Crum and McLachlin, unpublished data]. One explanation is that alterations take place in the cervical T-zone during reproductive life, increasing cancer risk to certain HPV types. In this scenario, HPV 18 infections occurring in young women produce alterations which carry lower risk, whereas in older women the risk is much higher. In this scenario, increasing risk is accrued as a function of age, presumably due to biologic changes in the T-zone. This concept has its analogy in the vulva, where HPV 16-associated precursors carry a much greater risk of progressing to invasive carcinoma in older women [40–42]. Although such occurrences may reflect the progression of precursors which have been untreated for many years, it is possible that these lesions develop more rapidly in older women due to age-related changes in the vulvar mucosa. For example, we have observed HPV 16 infections superimposed on lichen sclerosis in older women, including associated invasive carcinomas [43]. The co-existence of two risk factors for vulvar carcinoma—lichen sclerosis and HPV—in the same patient leaves open the possibility that squamous mucosal changes associated with older age (lichen sclerosis) confer susceptibility to HPV infection. Similarly, the cervical T-zone may undergo changes during reproductive life which could influence risk.

IMPLICATIONS FOR CHEMOPREVENTION

The issues discussed above suggest that cervical cancer prevention must be addressed on several fronts. Least efficient is use of the Pap smear, which must be administered repeatedly to ensure the rate of cancer remains at a level considered acceptable in the United States. Equally inefficient are strategies aimed at managing precursor lesions, since a high level of surveillance must be maintained to identify women at risk and a large number must be treated. The most promising strategies would focus on preventing the development of precursor lesions, specifically those which carry the greatest risk of cancer development. Most compelling is the use of vaccines against papillomaviruses, a concept which is under considerable study with the use of unique vectors for producing capsid proteins *in vitro* [44]. The value of this approach will be determined by the ease with which vaccination can be achieved, its safety, and the efficacy with which mucosal immunity can be generated by systemic vaccination. A second approach of potential interest is altering the target epithelium (the cervical T-zone) to reduce its risk of neoplasia. Prophylactic obliteration of the T-zone by cautery or cryotherapy is supported historically; theoretically this would shorten the reparative phase from many years to a matter of days [9]. Alternatively, this protection may be achieved by other mechanisms designed to alter the biochemical makeup of this extremely sensitive mucosa. Whether such strategies are feasible remains to be determined, but they have implications not only for the uterine cervix, but for other mucosal sites in which a squamo-columnar junction is a critical part of the pathogenesis of cancer.

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