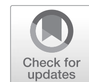

6 Cervical Squamous Intraepithelial Lesions: A Pathologist's Perspective

Charanjeet Singh and Grace N. Kim

6.1 Introduction

The histologically well-defined precursor squamous lesions and their temporal relationship with invasive squamous carcinoma were recognized more than a century ago; however, their association with human papillomavirus (HPV) is relatively recent (1970s). The evolution from precursor to cervical cancer is not only temporal; the lesion also has a spatial preference. Anatomically, the squamocolumnar junction of the cervix is a sharp border between the stratified squamous epithelium of ectocervix and the mucin-producing columnar epithelium of endocervix. However, puberty and menarche-associated physiological changes result in a more gradual and functional border characterized by metaplastic squamous epithelium, the so-called "transformation zone" mucosa. HPV infection and, therefore, virtually all cervical neoplasms and their precursors have a predilection for this transformation zone.

The clinical ease of recognition of this colposcopically visible transformation zone perhaps underlies the success of cervical cancer screening and that of the management of the precursor squamous lesions of cervix.

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6.2 Evolution of the Terminology of Cervical Squamous Precursor Lesions

The terminology for histopathological classification of cervical squamous precursor lesions has evolved over the last century, driven primarily by the understanding of the natural history of HPV infection and secondarily by evolution of the management options. Historically, the terms surface carcinoma, intraepithelial carcinoma, and carcinoma in situ (CIS) were used to describe the precursor lesions in which the cells looked malignant but did not invade into the stroma. The two-tiered CIS and non-CIS terminology meant hysterectomy for women with CIS and no treatment for the latter group. Studies by Reagan, Hicks [1], Seidemann [2], and other investigators showed that some of these surface lesions of the cervix, despite having abnormal looking cells, were not as aggressive as CIS and had lower risk for progressing to invasive cancer. These lesions were termed variously as anaplasia, basal cell

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hyperplasia, and atypical hyperplasia. Reagan et al. proposed the term "dysplasia" in 1953 [2] and graded it as mild, moderate, or severe based on the degree of squamous epithelial differentiation with respect to CIS, giving rise to a four-tiered system of precursor lesions. Based on this system, the women with CIS underwent hysterectomy, while the patients with "severe dysplasia" were subjected to cold knife conization.

The seminal investigation from Richart in 1969 [3] established that morphologic changes in the form of mild dysplasia to cervical cancer represented a disease continuum and that there was an absence of objective evidence to separate severe dysplasia from CIS. This led to the proposal of cervical intraepithelial neoplasia (CIN) terminology as follows: CIN1 for mild dysplasia, CIN2 for moderate dysplasia, and CIN3 for severe dysplasia. Due to proposed disease continuum of all lesions, CIN1 and CIN2 were treated with ablation (such as laser, CO_2, etc.), and CIN3 was treated with hysterectomy.

The work by zur Hausen [4] and colleagues in 1976 hypothesized the role of HPV in cervical cancer with identification of types HPV16 and HPV18 in cervical cancers in 1983–1984. Further understanding of the HPV biology led to increasing recognition that CIN1 was a more indolent lesion, while CIN2 was at the action threshold with CIN3. Based on this, the lesions were biologically regarded as "low-grade squamous intraepithelial lesion" (LSIL, which included CIN1/mild dysplasia) and "high-grade squamous intraepithelial lesion" (HSIL, which included CIN2/moderate and CIN3/severe dysplasia). The discovery of two-tiered biological significance of the cervical lesions coincided with the US Congress passing the Clinical Laboratory Improvement Amendments (CLIA) in 1988. The Bethesda system (TBS) [5] for reporting of cervical cytology was a by-product of CLIA 1988 amendment. TBS adopted the terminology of "LSIL" and "HSIL" for reporting cervical precursor lesions, along with the use of terms "negative for squamous intraepithelial lesion and malignancy (NILM)" and its most controversial term "atypical squamous cells of undetermined significance (ASCUS)," for lesions that were indeterminate morphologically (Fig. 6.1).

In the 1990s and early 2000s, despite the usage of LSIL and HSIL terminology for reporting cervical cytology, the three-tiered system of CIN1, CIN2, and CIN3 remained in use for cervical biopsy, cone, and LEEP reporting.

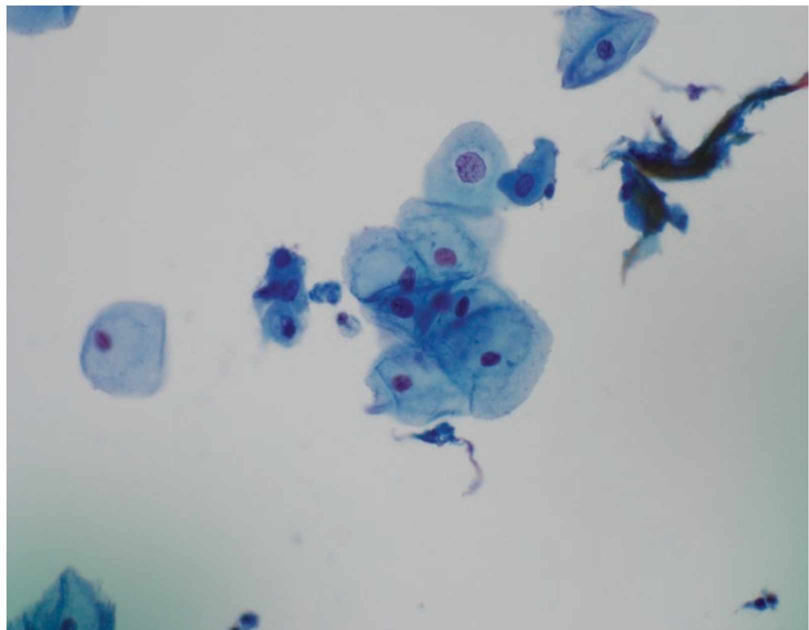

Fig. 6.1 Note a single cell in the group shows slight nuclear enlargement and irregularity, along with cytoplasmic clearing, consistent with atypical squamous cell of undetermined significance. This cell, by itself, is not diagnostic of an HPV infection-related lesion (Pap stain, ThinPrep smear, 600× magnification)

This discrepant use of terminology by pathologists was a result of utilization of three-tiered terminology for clinical management by the American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Guidelines: expectant management was advocated for CIN1, and in-office excision using cold knife cone or LEEP was advised for CIN2 and CIN3.

As is now well-recognized that HPV is associated with intraepithelial lesions and invasive cancers in the entire anogenital region and in both genders, a task force called the Lower Anogenital Squamous Terminology (LAST) Project was co-sponsored by the College of American Pathologists (CAP) and the ASCCP in order to unify the terminology between cytology and histology. The LAST terminology recommendations of 2012 [6] unified the terminology across all lower anogenital sites and created a nomenclature system that reflected the current knowledge of HPV biology and current use of HPV biomarkers, in order to facilitate clear communication for management of these lesions, across different medical specialties. As per the LAST recommendations:

- A two-tiered nomenclature is recommended for noninvasive HPV-associated squamous proliferations of the lower anogenital tract.
- The recommended terminology for HPV-associated squamous lesions is LSIL and HSIL, which may be optionally classified by the –IN subcategorization.
- The –IN refers to intraepithelial neoplasia. For a specific location, the appropriate complete term such as CIN (cervix), VaIN (vagina), and VIN (vulva) should be used.

6.3 Morphology of Squamous Intraepithelial Lesions

Cytological examination of Pap smears is the primary method of recognition of SIL lesions, followed subsequently by histopathological examination of tissue based on the ASCCP guidelines. Herein, we are using the two-tiered system and the most current LSIL and HSIL terminology to describe the morphological changes associated with these lesions. The cytological appearances of these lesions are discussed first, followed by their histological counterparts.

6.3.1 Cytological Diagnosis of LSIL and HSIL

The Bethesda system of cervical cytology provides criteria for diagnosing various categories, beginning at NILM, ASCUS, LSIL, HSIL, and cancer, for liquid-based cytology (such as ThinPrep and SurePath) and conventional cytology.

The cytological criteria used for the diagnosis of LSIL (Fig. 6.2) include:

- Enlarged superficial cells with distinct borders; cells present singly or in groups.
- Enlarged nuclei of the squamous cells with at least 3× nuclear enlargement compared to the background intermediate cell nuclei; nuclear-to-cytoplasmic ratio is only slightly increased.
- Perinuclear cytoplasmic vacuolation (the so-called koilocytic change), which has sharp delineation from dense, peripheral orangeophilic cytoplasm, in the presence of appropriate nuclear changes.
- The nuclei tend to show hyperchromasia and slight irregularity (raisinoid appearance) and often show binucleation.
- The chromatin is coarsely granular to dense opaque, and nucleoli are either absent or small and inconspicuous.

As opposed to LSIL, the cytological changes of HSIL are seen more so in the intermediate and basal-like cells, which have cytoplasmic appearance like that of "metaplastic" squamous cells. The criteria diagnostic of HSIL (Fig. 6.3) are:

- Affected cells are present singly more often than in LSIL, and when present in clusters, the cells tend to have syncytial appearance with ill-defined borders.
- Nuclear hyperchromasia with variation in nuclear size and shape, marked nuclear

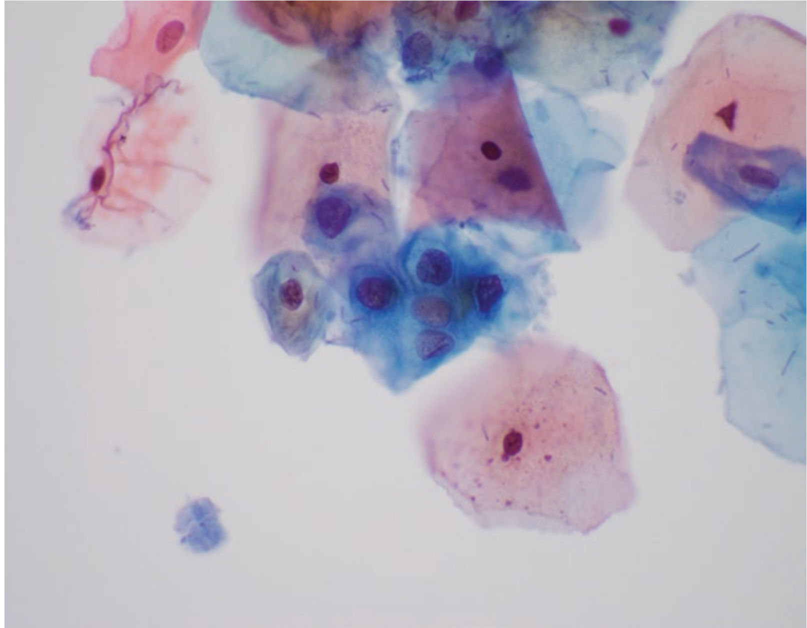

Fig. 6.2 Classic example of "koilocytic change" diagnostic of low-grade squamous intraepithelial lesion. The superficial cells show nuclear hyperchromasia, irregularity, and enlargement. The cytoplasm shows a clearly demarcated perinuclear halo (Pap stain, ThinPrep smear, 600× magnification)

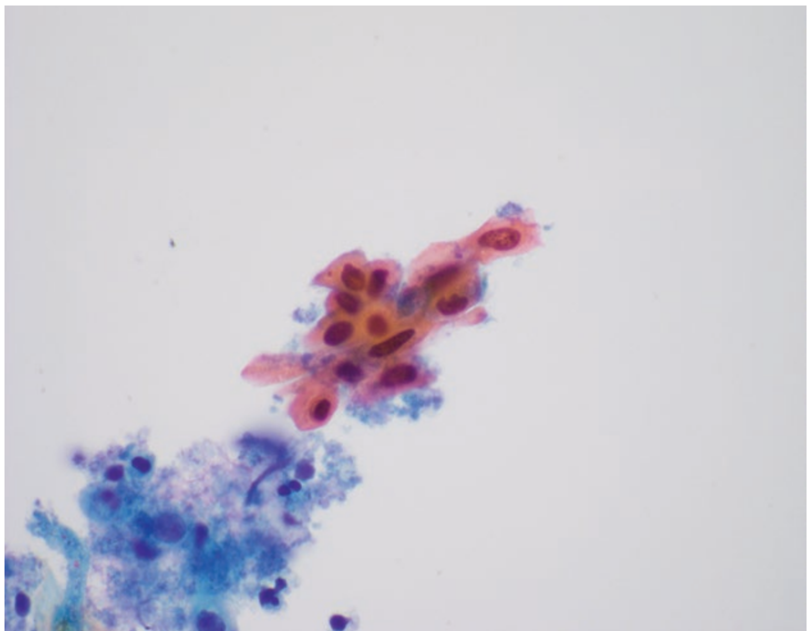

Fig. 6.3 Compared to the cells of LSIL (see Fig. 6.2), note the HSIL cells have marked variation in sizes of individual nuclei and thickened nuclear membranes (Pap stain, ThinPrep smear, 600× magnification)

enlargement, and high nuclear-to-cytoplasmic ratios.

- Nuclear irregularities are marked and grooves are common. The nuclear membranes are thicker and pronounced.
- The chromatin is finer and evenly distributed. Similar to LSIL, the nucleoli are still uncommon.
- Also uncommon is keratinized cytoplasm in HSIL lesions; and when present, differential diagnostic consideration is with squamous carcinoma especially together with necrosis.

6.3.2 Histological Findings of Squamous Intraepithelial Lesions

As discussed in detail elsewhere in this book, patients with cytological diagnosis of LSIL, depending on the age, may undergo co-testing for HPV and/or colposcopy-guided biopsy of the cervix and/or endocervical curettage. Usually, on the other hand, patients with HSIL on cytology almost always have colposcopy followed by a biopsy or LEEP or cold knife cone. The purpose of the biopsy is either to confirm the cytological diagnosis or find a more worrisome component so that definitive management can be performed timely. As previously alluded to, the changes are usually first and often best seen at the functional border of endocervical and squamous epithelium, the so-called "transformation zone" mucosa. The normal transformation zone cells show proliferation of immature/basal layer and early squamous differentiation, but not keratinization/epidermidalization.

The LSIL (CIN1) lesions are generally flat; however, less commonly they may be exophytic (condyloma) or papillary. The major histologic criteria for diagnosis are prominent nuclear enlargement in superficial cells, at least three times the normal nuclear size. The transition from normal epithelium to LSIL is generally discrete. As previously noted, the cells may show binucleation and/or multinucleation, and at least two such cells [7] are needed for a convincing diagnosis. Parakeratosis may be present, but is not required for the diagnosis. The basal layers are normal and do not show dysplastic features in LSIL. When the surface epithelial features of LSIL coexist with loss of polarity, the presence of abnormal mitoses or a high mitotic rate, and atypia beyond the parabasal layers, it should invoke the diagnosis of HSIL and more particularly CIN2.

The cells in CIN2 show surface epithelial koilocytosis or abnormal keratinization and/or bizarre nuclei; on the contrary, a complete lack of maturity characterizes CIN3 (HSIL). In the CIN3 lesions, nuclear hyperchromasia involves full thickness of epithelium, with minimal to no surface maturation and with irregularly spaced nuclei. The mitoses, both typical and atypical, can be seen in any layer of the squamous epithelium.

6.4 Morphology of Glandular Intraepithelial Lesions

Endocervical adenocarcinoma in situ (AIS) is a premalignant, high-risk HPV-related glandular counterpart of HSIL. Most cases of AIS are associated with HPV18 followed by HPV16. Despite the continuity of glandular epithelium of endocervix with squamous epithelium of ectocervix, at the transformation zone, AIS is less frequent than HSIL. However, most cases of AIS tend to have coexistent SIL.

Cytological criteria for diagnosis of AIS (Fig. 6.4), as detailed in the Bethesda system for reporting of cervical cytology, include:

- Sheets, clusters, or strips of glandular cells with nuclear crowding and overlap
- Nuclear elongation, stratification, and variation in size
- Hyperchromatic nuclei with coarsely granular to evenly distributed chromatin
- Presence of mitoses and/or apoptosis
- Inconspicuous to absent nucleoli
- Absence of tumor diathesis (tumor necrosis)

It is noteworthy that in glandular lesions, on cytological examination, the presence of prominent nucleoli (Fig. 6.5) and/or diathesis should invoke the consideration for an invasive adenocarcinoma. A well-differentiated invasive adenocarcinoma can lack both the nucleoli and diathesis and is a challenging differential of AIS on cytology and histology.

Histologically, AIS can involve the epithelium of a group of glands or a single gland, either in entirety or in patches. Paramount to its diagnosis are preserved glandular architecture and enlarged, hyperchromatic, stratified nuclei with high mitotic and apoptotic rate. The cytoplasm can be muco-depleted to abundant and basophilic or

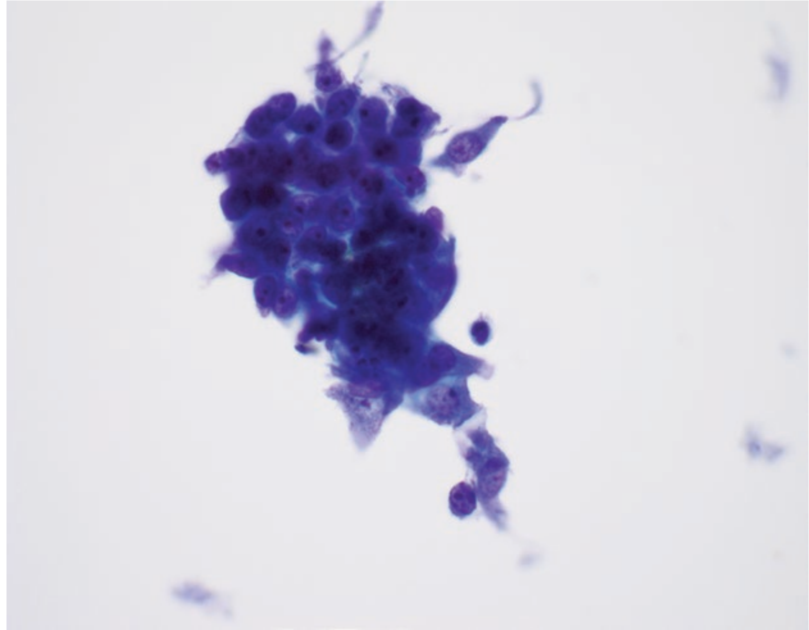

Fig. 6.4 Endocervical adenocarcinoma in situ with a hyperchromatic cell group that shows nuclear crowding. Noted at the periphery of the clusters are individual cells with "feathering" and high nuclear-to-cytoplasmic ratios (Pap stain, ThinPrep smear, 600× magnification)

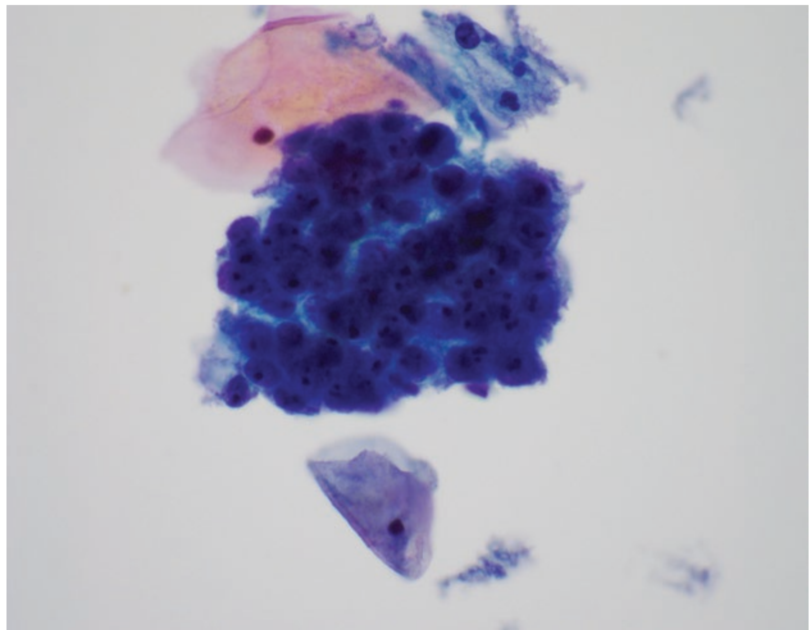

Fig. 6.5 In this group, compared to that in Fig. 6.4, the nuclei have prominent nucleoli, and there is a variation in nucleolar size. Some cells have more than one nucleoli. These features are more suggestive of an invasive adenocarcinoma, compared to AIS (Pap stain, ThinPrep smear, 600× magnification)

eosinophilic. Presence of glandular complexity and/or desmoplasia, AIS-like features in deeper glands and/or marked nuclear atypia even in superficial glands, should invoke the consideration for invasive adenocarcinoma. This is an important distinction to make, and in difficult cases, review by multiple pathologists and/or consultation with an expert gynecologic pathologist should be considered. Compared to the risk of squamous carcinoma in HSIL, AIS has a higher risk to transform to invasive adenocarcinoma, which when stage-matched with squamous carcinoma has higher risk of nodal involvement.

6.5 Stratified Mucin-Producing Intraepithelial Lesion (SMILE)

These are uncommon lesions which are thought to arise from the reserve cells at the transformation zone. The current Bethesda terminology for cytologic reporting does not recognize SMILE as a diagnostic category, given that it would be a challenging lesion to diagnose on cytology and that its histologic features are like that of HSIL. SMILE have stratified immature cells that display intracytoplasmic mucin or cytoplasmic vacuoles. These mucinous cells are typically seen in the mid to lower layers of the epithelium. AIS-like gland formation is not identified in SMILE. Most of the cases with SMILE-like lesions have coexistent HSIL or AIS or both.

6.6 Morphologic Evaluation of Cone and LEEP Excision Biopsies

Both cone and LEEP biopsies are procured once the diagnosis of cervical squamous or glandular intraepithelial lesions has been established. The role of the pathologist in LEEP and cone biopsies includes:

- To estimate the burden of intraepithelial lesion or adenocarcinoma in situ (i.e., the number of quadrants involved)
- To identify if there is a potential more worrisome component (e.g., invasive carcinoma in the setting of a previous CIN-3)
- To establish if the dysplasia extends higher into the endocervical canal

As such, during the evaluation of these specimens, the endocervical margins of the cone and endocervical curetting specimen (which may be separately submitted depending on the local practice) must be evaluated carefully. Due to the location at the transformation zone, it is not uncommon for CIN3 to involve and extend into the endocervical glands; this phenomenon should be carefully distinguished from stromal invasion. In such cases, evaluation of the deep margin of cone or LEEP is equally important.

6.7 Differential Diagnoses

Apart from extension of the CIN into endocervical glands, and mimicking invasion, the other significant differential diagnoses of cervical squamous intraepithelial lesions include radiation atypia, immature repair, atrophy, immature metaplasia, polyp-associated atypia, and pregnancy implantation-associated atypia. The differential diagnoses for AIS include tubal or endometrioid metaplasia, reactive endocervical gland atypia, Arias-Stella-like reactions, etc.

While LSIL, HSIL, and AIS can be distinguished from each other and from reactive atypia in most cases, ancillary studies are both useful and required in others. Studies [8] have also shown that routine use of ancillary studies lowers the rate of major cytohistologic discrepancies and is associated with a higher rate of HSIL (CIN2+) diagnoses and lower CIN1/CIN2+ ratios. To understand HPV ancillary testing, an understanding of the HPV types and life cycle is critical.

6.8 The Human Papillomavirus

HPV, for which more than 200 different types have been studied thus far, is a circular, double-stranded DNA virus. It has strains that range from innocuous, nearly commensal to pathologic and infectious. While HPV infections are commonplace, and most infections are cleared by the host's robust immune system, a persistent HPV infection can lead to stepwise and temporal progression from preneoplastic lesions to neoplasia [6]. Within the lower anogenital tract, HPV viruses are recognized as part of the alpha genus and are generally divided into two broad, mutually exclusive families: "low-risk" HPV and "high-risk" HPV. The most common types of "low-risk" HPV include HPV 6 and 11, while "high-risk" types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, to name a few. Viral oncogenic potential is the main distinguishing characteristic between the "low-risk" and "high-risk" groups, in that "low-risk" HPV types contribute to the pathogenesis of benign mucosal lesions without potential for significant clinical

progression, while the "high-risk" HPV types carry notable malignant potential, not only as mucosal carcinomas but also as precursor mucosal lesions [4–6, 9].

High-risk HPV types distinguish themselves from low-risk types by their ability to integrate the viral genome into the host DNA. However, depending on the specific high-risk HPV type, a viral genome may or may not be integrated within the affected tumor cells of cervical carcinoma. In particular, the most common high-risk HPV strain that occurs in cervical carcinoma, HPV 16, exhibits integration of viral genome into the host's DNA in approximately 70% of cervical cancer cases. Similarly, nearly 100% of HPV 18-infected carcinomas have integration of viral sequences [10]. The remainder of the cases demonstrates viral episomes within the affected cell without integration.

6.8.1 Life Cycle of the Human Papillomavirus

Despite the significant differences between low-risk and high-risk HPV types, the broad principles of the HPV life cycle are comparable. The episomal genome of the HPV virus has three distinct sets of encoding regions: (a) the early genes (E1, E2, E4, E5, E6, E7, E8), (b) the late region (proteins L1 and L2 coding the viral capsid), and (c) the long control region (LCR) or the upstream regulatory region (URR) [9]. The first inciting event in the HPV life cycle is infection, which introduces virions into the nucleus of the squamous epithelial basal cells secondary to surface epithelial trauma. At this point in the life cycle, the viral episome remains extrachromosomal and undergoes viral genome replication, with copy numbers ranging from 50 to 200 copies per infected cell. Once a constant copy number is reached, the life cycle enters a maintenance phase [10]. The vital players in the steps of replication and maintenance are the E1 and E2 genes which regulate transcription and replication.

After replication, a daughter cell of the infected undifferentiated basal cell travels away from the basal layers and into the superficial layers where it enters a period of squamous differentiation. Differentiation normally halts replication within suprabasal cells; however, in infected cells, replication is maintained. This process is called "cell cycle reentry," signifying aberrant reentry of superficial cells into S-phase of the cell cycle to allow for viral amplification. Of note, in some scenarios of high-risk HPV infection, the infection may remain dormant in the basal cells without further propagation into the upper squamous layers and without clinical evidence of an HPV-driven lesion. As such, the detection of high-risk HPV by ancillary testing does not necessarily equate to the presence of a dysplastic lesion [11]. In such cases of dormancy, as well as in low-risk HPV infections, the significance of the two proteins vital for neoplastic cellular proliferation, E6 and E7, is not well known. On the contrary, the role of E6 and E7 protein in high-risk HPV types is critical for neoplastic growth [10].

E6 and E7 are proteins transcribed from early viral genes, which are critical in high-risk HPV types as oncogenic drivers. E6 binds and targets tumor suppressor protein p53 for inactivation, which renders p53 incapable of its normal function of pausing cell cycle progression and signaling cell death when cell cycling is overstimulated. Only in high-risk HPV types is p53 marked for ubiquitination and degradation [10]. E7, on the other hand, binds with retinoblastoma (Rb) protein, also signaling this protein for degradation. The absence of Rb protein function prevents the proper functioning of proteins that usually regulate S-phase entry [4]. Interestingly, a critical difference in low-risk and high-risk HPV types is the affinity of E7 protein to Rb. The binding affinity of E7 to Rb in low-risk HPV is ten times weaker when compared to that of high-risk HPV types. Low-risk HPV also lacks affinity to the entire family of Rb proteins, while high-risk HPV can target all members. In addition, integration of the viral genome into the host's DNA is not a calculated event but rather one of chance, often occurring at weak points in the host's genome [10]. During integration, the E2 locus, which is responsible for keeping E6 and E7 gene products at lower levels, is often damaged allowing for uncontrolled E6 and E7 production [6].

One of the late events during the HPV life cycle is the assembly of virions that solely occurs in the superficial squamous cells. L1 and L2 are late gene products for major and minor capsid proteins. A single viral genome is packaged in the capsid, and this virion is then released during natural cell shedding of terminally differentiated keratinocytes [4, 6, 12].

6.8.2 Laboratory Testing for HPV

The confirmation of HPV in tissue samples has become an adjunctive test in the diagnosis and management of dysplastic lesions and, more recently, the prognosis of malignant tumors. For instance, the finding of high-risk HPV in a head and neck squamous cell carcinoma has been shown to have improved therapy response and disease-free survival compared to HPV-unrelated carcinomas [13]. HPV detection methods are of two types, indirect methods and direct methods. The indirect methods rely on the life cycle of HPV, and since HPV virus cannot be cultured in-vitro, the direct methods of detection are predominantly molecular-based [14].

Various tests for HPV detection and their clinical implications are discussed in Chap. 9, and the reader can refer to that chapter for details. Only a brief mention of the tests will be done here.

6.8.2.1 Indirect Methods

The indirect methods use surrogate markers of HPV infection, such as p16 immunostaining and Pro-Ex C.

P16 Immunostaining

Immunostaining for p16 exhibits block positivity (strong nuclear and cytoplasmic reactivity) in high-grade lesions (CIN-2 and CIN-3, and AIS). This relies on the principle that the p16 protein is upregulated with disruption of Rb protein function by E7 in high-risk HPV-associated lesions. Consequently, it is more so a surrogate marker of high-risk HPV type. The staining in reactive atypia and in low-risk HPV-associated lesions tends to be patchy and weak. The advantage in using p16 immunostain is the ease and objectivity in interpretation by pathologists on routine tissue biopsies, obtained for follow-up after Pap smear or a previous diagnosis of dysplasia. The published literature shows large inter-observer variability found by multiple studies in the diagnosis and grading of CIN by pathologists. This has been attributed variously to:

- Technical factors: Small biopsy, poor processing, incomplete representation, thermal crush, and other artifacts.
- Patient-related factors: Pregnancy, menopause, exogenous hormone, coexistent infections, and prior radiation.
- Pathologist-related factors: These are mainly seen in grading, especially when the epithelium is thin, metaplastic, or denuded.

Study by Singh et al. [8] has shown that the more frequent use of an objective marker such as p16, alone or together with Ki-67 (which is a marker of proliferation), in difficult cases, allows pathologists to modify their diagnostic thresholds and render a more objective diagnosis between low-grade (CIN-1) and high-grade (CIN-2 +) lesions. The LAST working group advocates the use of p16 in the following situations:

- To differentiate HSIL from benign mimics (such as immature metaplasia or atrophy)
- To classify indeterminate lesions (essentially CIN 2 in the old terminology) as either LSIL or HSIL
- To reach a consensus on possible cases of HSIL with differing pathologists' interpretation

Pro-Ex C Assay

Pro-Ex C is another immune-cytochemical assay, which is an S-phase proliferation marker with a specific pattern of staining. TOP2A and MCM2 are S-phase proteins, which are induced upon integration of HPV viral DNA into the host genome, leading to increased levels of E6 and E7 proteins. This leads to aberrant S-phase induction and at the morphological level correlates with high-grade dysplasia. In cervical biopsies, positive staining for Pro-Ex C is defined by staining of nuclei of more than half of the mucosal

thickness. While Pro-Ex C has been shown to be a more specific marker than Ki-67, its specificity and sensitivity are lower compared to p16. For this reason and due to difficulties in laboratory validation and standardization of Pro-Ex C stain, p16 immunostaining continues to be the preferred marker for indirect testing of high-risk HPV in dysplastic lesions.

6.8.2.2 Direct Methods

The predominance of innovation in HPV detection has been in the arena of direct methodology. The most frequently used assays currently are signal amplification assays and nucleic acid target amplification assays, five of which are FDA-approved in the United States. The majority of these tests detect HPV genomic DNA. The first to be FDA-approved was the Digene Hybrid Capture 2 in 2003, followed by Hologic's Cervista HPV HR and Cervista HPV 16/18 in 2009. The Roche Cobas HPV test was more recently approved for testing in 2011.

1. Signal Amplification Tests
   - The Digene Hybrid Capture 2 High-Risk HPV DNA Test: RNA probes are utilized directly against HPV DNA of 13 high-risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). If these viral genotypes are present, a DNA-probe hybrid is formed in a solution of isolated DNA from the patient's sample and recognized by a chemiluminescent compound. The intensity of the emitted light is proportional to high-risk HPV DNA content, and a semiquantitative value beyond a designated cutoff determines reporting of the specimen as positive or negative for HPV. This assay can be automated on the Qiagen Rapid Capture System resulting in high-throughput processing [14].
   - The Cervista HPV HR Test: This test by Hologic utilizes a specific proprietary method called Invader. A cocktail of oligonucleotide probes targeting 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and the Invader probes are added to the isolated specimen DNA, which ultimately results in a conformational change at the target. The Cervista assay is automated, and the presence of an internal control is beneficial in that it accounts for specimen cellularity. Cervista HPV 16/18 is a reflex test similar to the Cervista HPV HR and also uses the same Invader chemistry technology; however, the probes are specific to HPV strains 16 and 18 only [14].
   - Cobas 4800 HPV Test: It is the newest edition of a real-time PCR assay that is specific to 14 high-risk HPV types and also HPV16/18 as a distinct duo. Hence, the Cobas test is unique in that it can simultaneously report a pooled result on 12 high-risk HPV types and also individual HPV 16 and 18 genotype results without extra cost. In contrast, all prior PCR-based assays target a combination of both low- and high-risk HPV types. The Cobas 4800 HPV test is the only FDA-approved test that can be used singularly without an adjunctive Pap test for cervical cancer screening in women over the age of 25 years. Additionally, all the aforementioned FDA-approved HPV tests are approved only for ThinPrep cervical specimens and not SurePath, with the exception of the Cobas HPV test, which was approved for use on SurePath-collected cells on July 7, 2016 [14].
   - Aptima HPV Assay: A FDA-approved HPV assay which utilizes the fully automated GenProbe TIGRIS DTS System. It is the only assay which detects E6 and E7 mRNA via transcription-mediated amplification. Detection of viral oncogenes E6 and E7 serves as direct evidence of HPV transcriptional activity, and this is regarded as the gold standard in confirmation of clinically relevant HPV infection [13]. E6 and E7 mRNA transcripts are highly specific to HPV genotypes, and as such, the Aptima assay can detect mRNA of 14 high-risk HPV genotypes. Unlike the Cobas system, the Aptima assay cannot differentiate between the 14 genotypes nor specify the presence of HPV 16/18. In women in which

the Aptima HPV assay is positive, the specimen can be further tested by the Aptima HPV 16 18/45 genotype assay [14].

2. Nucleic Acid Hybridization Assays
   - Historically, the Southern blot and dot blot hybridizations have been available; however, due to their labor-intensive techniques, high DNA content requirements, and low overall sensitivity, these methods are not being used [15].
   - HPV DNA in situ hybridization (ISH) test: This test is no longer commercially available, though laboratory-validated editions do still exist. It targets 21 HPV genotypes inclusive of the most common high-risk HPV genotypes and few low-risk genotypes [16]. In situ hybridization is performed on a tissue section similar to immunohistochemistry, and thus, advantageously, the morphologic context of the lesion in question remains intact during the evaluation process, though the sensitivity of the DNA ISH test is low.
   - RNA in situ hybridization tests: These tests use a similar technique, and only one, the Ventana Inform HPV III, is currently commercially available for clinical use [17]. The Inform HPV III ISH test targets E6 and E7 mRNA of either a pooled high-risk HPV panel of 18 subtypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), a pooled low-risk HPV panel of 10 subtypes (6, 11, 40, 43, 44, 54, 69, 70, 71, and 74), or a focused detection of HPV 16/18. The Ventana Inform HPV III demonstrates different patterns of staining that can detect either episomal or integrated HPV genetic material; however, the test has been plagued by interlaboratory variability, with complications of background staining and low sensitivity [17].
   - RNA ISH test: The latest addition to the armamentarium of HPV tests is developed by Advanced Cell Diagnostics. It has been studied to have superior sensitivity and specificity compared to p16 immunohistochemistry, DNA ISH, and DNA PCR [11, 13, 18, 19], along with a high concordance rate with p16 immunostaining. Mills et al. [14] recently in mid-2017 demonstrated its usage on the clinically available Leica Bond III. Compared to DNA-based detection systems and particularly PCR assays, RNA ISH testing is promising in that it is not overly sensitive in detecting HPV DNA in samples without cytologic or histologic changes of viral infection but also direct detection of E6 and E7 mRNA viral oncogenes may be more clinically relevant since E6 and E7 mRNA detection correlates with active HPV transcription and proliferation of lesions [11, 18].

The choice between the abovementioned direct methods of HPV testing is largely based on extraneous factors such as the size of the laboratory, the test volume, the available infrastructure, and the preference of the clinicians between HPV testing and cytology for primary screening of cervical lesions. It is noteworthy though that in April of 2014, the FDA approved the use of the Roche Cobas HPV assay for primary cervical cancer screening in women over the age of 25 years, without the concomitant Pap test. This approval recommended either colposcopy or a Pap cytology for patients with specific high-risk HPV types detected by the HPV test.

6.9 Conclusion

In conclusion, this chapter highlights the different aspects of life cycle of HPV and upregulation of p16 due to integration of genome of hrHPV in the host DNA. In the biopsy specimens, p16 immunostain can serve as a surrogate marker for hrHPV detection and in differentiating low-grade squamous intraepithelial lesions from high-grade squamous intraepithelial lesions. Alternatively, in cellular material obtained for Pap smear, molecular methodologies detailed in this chapter may be used to detect and sub-type HPV into high-risk and low-risk groups. Detection of hrHPV and/or a HSIL lesion is the action threshold for a more frequent follow-up and/or a cone/LEEP procedure, based on the current ASCCP guidelines.

Key Points

- The cytological criteria used for the diagnosis of LSIL include enlarged superficial cells with nuclear enlargement to at least three times the reference intermediate cell and perinuclear cytoplasmic vacuolation.
- The important cytological features of HSIL include nuclear hyperchromasia with very high nuclear-to-cytoplasmic ratio, marked nuclear irregularities with grooves, and thick nuclear membranes.
- The cytological features of glandular intraepithelial lesions include sheets, clusters, or strips of glandular cells with nuclear crowding and overlap, nuclear elongation, stratification and variation in size, presence of mitoses and/or apoptosis, and inconspicuous to absent nucleoli.
- SMILE are uncommon lesions which are thought to arise from the reserve cells at the transformation zone; Bethesda terminology does not recognize SMILE as a diagnostic category, given that it would be a challenging lesion to diagnose on cytology and that its histologic features are like that of HSIL.
- Tests for HPV detection include indirect methods (p16 immunostaining and Pro-Ex C) and direct methods (signal amplification tests and nucleic acid hybridization assays).

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