squamous cells, viral replication occurs in maturing squamous cells. Normally, these more mature cells are arrested in the G_1 phase of the cell cycle, but they continue to actively progress through the cell cycle when infected with HPV, which uses the host cell DNA synthesis machinery to replicate its own genome. As you will recall from Chapter 7, viral E7 protein binds the hypophosphorylated (active) form of RB and promotes its degradation via the proteasome pathway, and also binds and inhibits p21 and p27, two important cyclin-dependent kinase inhibitors. Removal of these controls not only enhances cell cycle progression, but also impairs the ability of cells to repair DNA damage. This defect in DNA repair is exacerbated by the viral E6 proteins of high-risk HPV subtypes, which bind to the tumor suppressor protein p53 and promote its degradation by the proteasome. In addition, E6 up-regulates the expression of telomerase, which leads to cellular immortalization. The net effect is increased proliferation of cells that are prone to acquire additional mutations that may lead to cancer development. By contrast to high-risk HPVs, the E7 proteins of low risk HPVs bind RB with lower affinity, while the E6 proteins of low-risk HPVs fail to bind p53 altogether and instead appear to dysregulate growth and survival by interfering with the Notch signaling pathway.

Another factor that contributes to malignant transformation by HPV is the physical state of the virus. The viral DNA is integrated into the host cell genome in most cancers. This configuration increases the expression of E6 and E7 genes, and may also dysregulate oncogenes near the sites of viral insertion, such as *MYC*. By contrast, viral DNA is extrachromosomal (episomal) in precursor lesions associated with high risk HPVs and in condylomata associated with low risk HPVs.

Even though HPV has been firmly established as a common cause of cervical cancer, it is not sufficient to cause cancer. This conclusion is supported by the fact that a high percentage of young women are infected with one or more HPV types during their reproductive years, but only a few develop cancer. Thus, other factors, such as exposure to co-carcinogens and host immune status, influence whether an HPV infection regresses or persists and eventually progresses to cancer.

Cervical Intraepithelial Neoplasia (Squamous Intraepithelial Lesions)

The classification of cervical precursor lesions has evolved over time and the terms from the different classification systems are currently used interchangeably. Hence a brief review of the terminology is warranted. The oldest classification system grouped lesions as having mild dysplasia on one end and severe dysplasia/carcinoma in situ on the other. This was followed by the *cervical intraepithelial neoplasia* (CIN) classification, with mild dysplasia termed *CIN I*, moderate dysplasia *CIN II*, and severe dysplasia termed *CIN III*. Because the decision with regard to patient management is two-tiered (observation versus surgical treatment), the three-tier classification system has been recently simplified to a two-tiered system, with CIN I renamed low-grade squamous intraepithelial lesion (LSIL) and CIN II and CIN III combined into one category referred to as high-grade squamous intraepithelial lesion (HSIL) (Table 22-1).

Table 22-1 Classification Systems for Squamous Cervical Precursor Lesions

Dysplasia/Carcinoma in Situ	Cervical Intraepithelial Neoplasia (CIN)	Squamous Intraepithelial Lesion (SIL), Current Classification
Mild dysplasia	CIN I	Low-grade SIL (LSIL)
Moderate dysplasia	CIN II	High-grade SIL (HSIL)
Severe dysplasia	CIN III	High-grade SIL (HSIL)
Carcinoma in situ	CIN III	High-grade SIL (HSIL)

CIN, Cervical intraepithelial neoplasia; SIL, squamous intraepithelial lesion.

LSIL is associated with a productive HPV infection. In LSIL, there is a high level of viral replication and only mild alterations in the growth of host cells. *LSIL does not progress directly to invasive carcinoma and in fact most cases regress spontaneously; only a small percentage progress to HSIL*. For these reasons, LSIL is not treated like a premalignant lesion. In HSIL, on the other hand, there is a progressive deregulation of the cell cycle by HPV, which results in increased cellular proliferation, decreased or arrested epithelial maturation, and a lower rate of viral replication, as compared with LSIL. Derangement of the cell cycle in HSIL may become irreversible and lead to a fully transformed malignant phenotype, and thus *all HSILS are considered to be at high risk for progression to carcinoma*. LSILs are ten times more common than HSILs.

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The diagnosis of SIL is based on identification of nuclear atypia characterized by nuclear enlargement, hyperchromasia (dark staining), coarse chromatin granules, and variation in nuclear size and shape (Fig. 22-14). The nuclear changes are often accompanied by cytoplasmic "halos." At an ultrastructural level, these "halos" consist of perinuclear vacuoles, a cytopathic change created in part by an HPV-encoded protein called E5 that localizes to the membranes of the endoplasmic reticulum. Nuclear alterations with an associated perinuclear halo are termed **koilocytic atypia**. The grading of SIL into low or high grade is based on expansion of the immature cell layer from its normal, basal location. If the immature squamous cells are confined to the lower one third of the epithelium, the lesion is graded as LSIL; if they expand to the upper two thirds of the epithelial thickness, it is graded as HSIL.

The histologic features of LSIL correlate with HPV replication and changes in host cell growth and gene expression (Fig. 22-15).

- The highest viral loads (assessed by HPV DNA in situ hybridization, Fig. 22-15*B*) are found in maturing keratinocytes in the upper half of the epithelium.
- HPV E6 and E7 proteins prevent cell cycle arrest. As a result, cells in the upper portion of the epithelium express markers of actively dividing cells, such as Ki-67 (Fig. 22-15*C*), that are normally are confined to the basal layer of the epithelium. Disturbed growth regulation also leads to overexpression of p16, a cyclin-dependent kinase inhibitor (Fig. 22-15*D*).
- Both Ki-67 and p16 staining are highly correlated with HPV infection and are useful for confirmation of the diagnosis in equivocal cases of SIL.