

PROPATH

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Immunohistochemistry

Utility of p16 (INK4a) Immunostaining in Cervical Biopsies

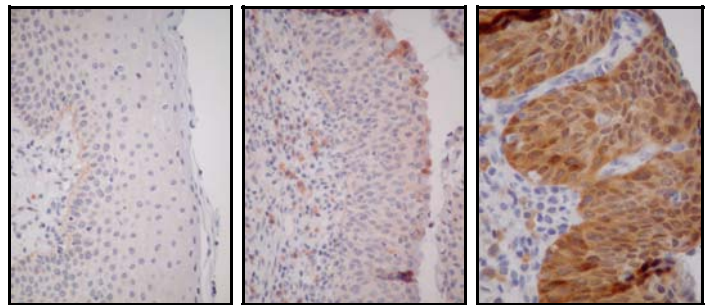
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Recognition of cervical dysplasia or human papilloma virus (HPV) effect can be a very difficult task, particularly in those cases with "borderline" morphologic features. Interpretation in such cases is highly subjective, and it is not surprising that interobserver reproducibility of HPV effect and mild squamous dysplasia is poor. Because the clinical (and social) implications of these diagnoses can be significant, ancillary methods that can assist in accurate diagnosis of these conditions have been sought by a number of investigators. This month, we review the utility of immunostaining for p16 (INK4a) for this purpose, a regulatory protein that decelerates the cell cycle.

The role of HPV in the development of cervical cancer is well established, and it is known that HPV infection causes a number of alterations in gene or protein expression within the infected host cells. One of the proteins produced by infection with high risk HPV is the E7 oncoprotein. This protein binds to the retinoblastoma gene product (RB), resulting in its functional inactivation. Since expression of the cyclin-dependent kinase inhibitor gene p16 (INK4a) is under negative feedback control of functional RB, overexpression of p16 (INK4a) ultimately occurs in cells infected by high-risk HPV. Because the p16 (INK4a) protein is detectable immunohistochemically, it offers a logical surrogate marker for high risk HPV, particularly since this protein is not expressed in normal cervical squamous epithelium. On immunohistochemical staining, expression of p16 (INK4a) protein is observed both within the nucleus and the cytoplasm of the affected cells.

In 1998, Sano and colleagues studied a series of 139 formalin-fixed paraffin embedded cervical and genital biopsies for p16 (INK4a) using immunohistochemistry, and correlated their findings with the results of HPV typing performed on the same samples. Marked overexpression of p16 (INK4a) protein (reflected by diffuse and strong immunostaining) was found in all preneoplastic lesions



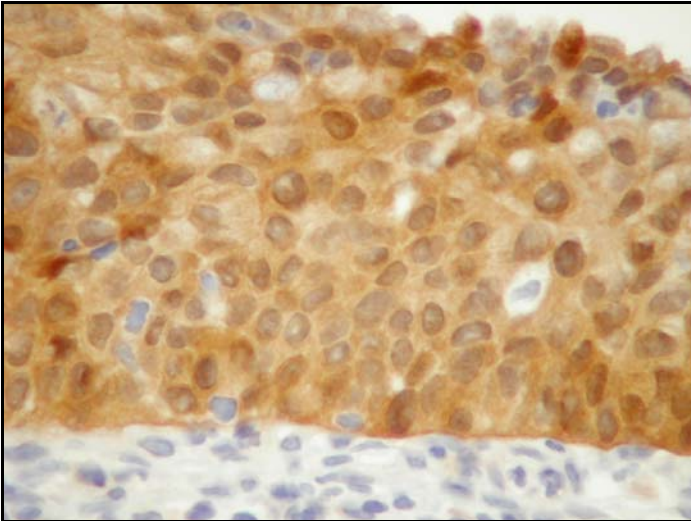
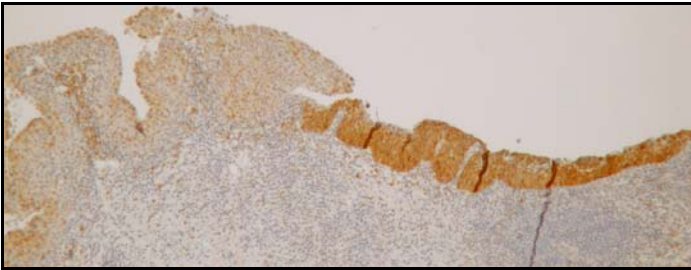
A p16 immunostain on normal cervical epithelium (left) is negative except for focal weak staining of the basement membrane. Squamous metaplasia (center) shows focal weak or patchy staining. In contrast, dysplastic cervical epithelium (right) shows strong and diffuse staining.

that showed infection by high and intermediate risk HPV subtypes (16, 18, 31, 33, 52, and 58), and also in all invasive cervical carcinomas. Lesions that were associated with low-risk HPV types 6 and 11, such as condyloma acuminata and low-grade SIL, showed focal and weak staining for p16 (INK4a).

In a similar study reported in 2001 by Klaes and associates, marked overexpression of p16 (INK4a) was detected in all CIN 1 lesions (n = 47) except 7 cases that were associated with low-risk HPV types. In addition, p16 (INK4a) protein was overexpressed in all cases of CIN 2 (n = 32) and CIN 3 (n = 60), as well as 58 of 60 (97%) invasive cervical carcinomas. As such, these results suggest that overexpression of p16 (INK4a) assists in the identification of high-risk HPV-related cervical squamous lesions.

Keating et al. reported the results of a study of 99 biopsies, including 24 cases of low-grade SIL, 36 cases of high-grade SIL, 29 cases of mature or immature squamous metaplasia, and 15 cases of atrophy or metaplastic epithelium with atypia. The immunohistochemical results with p16 (INK4a) were correlated with histologic diagnoses and with results of HPV status by PCR

analysis. A positive p16 (INK4a) immunostain was defined as moderate to strong diffuse or focal staining. 100% of the low-grade and high-grade SIL's were positive, and strong diffuse staining with p16 (INK4a) showed a significant association with high-risk HPV-associated lesions. In some normal and metaplastic epithelium, p16 (INK4a) staining was noted in a diffuse weak basal pattern with occasional focal stronger positivity.



In these p16 immunostains, the top photo demonstrates the contrast between the weak-staining area of papillary immature metaplasia (left side of epithelium) and the strongly-staining dysplastic area (right side of epithelium). Note that p16 stains both cytoplasm and nuclei (bottom photo).

At ProPath, we have been performing p16 (INK4a) immunostaining in cases referred to our laboratory for HPV typing by in situ hybridization, and these preliminary studies of small numbers of cases have shown findings similar to those discussed above. The cervical biopsies that have been positive for high-risk HPV types (16 and 18) have shown strong reactivity with p16 (INK4a), and cases that have been positive for low-risk HPV types 6 and 11 have been negative for p16 (INK4a) or shown only focal weak positivity. Cases that have been positive for intermediate-risk HPV (types 31, 33, and 51) have shown p16 (INK4a) staining in some cases but one such case was negative for p16 (INK4a). One case of papillary immature metaplasia that was studied at ProPath

showed scattered areas of weak positivity, which was clearly different from the strong reactivity noted in an area of high-grade dysplasia present in the same cone biopsy specimen (see photos on left side of this page).

In summary, p16 (INK4a) immunostaining shows great promise as a marker of lesions associated with high-risk HPV, and it may assist in improving the reproducibility of diagnoses in cervical dysplastic and reactive lesions. It appears to be particularly useful for distinguishing immature squamous metaplasia from high-grade SIL, which can be a very challenging morphologic differential diagnosis. P16 (INK4a) is now available in the ProPath immunohistochemistry laboratory.

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