The Role of Cytology in the 21st Century: The Integration of Cells and Molecules

Christine Bergeron^a Magnus von Knebel Doeberitz^b

^aDepartment of Pathology, Laboratoire Cerba, Cergy Pontoise, France; ^bDepartment of Applied Tumor Biology, Institute of Pathology, University of Heidelberg and German Cancer Research Center, Heidelberg, Germany

Key Words

Cervical cancer · Cytology · p16^{INK4a}/Ki-67 · Human papillomavirus · Screening

Abstract

Objectives: Cervical cancer screening test performance has been hampered by either a lack of sensitivity in Pap cytology or a lack of specificity of human papillomavirus (HPV) testing. This is disturbing for patients and a cause of high costs for health care providers. **Study Design:** The identification of p16^{INK4a} as a specific marker for the neoplastic transformation of cervical squamous epithelial cells by HPVs allows the identification of HPV-transformed cells in cytopathology specimens. **Results:** When compared to molecular HPV tests for triaging minor cytologic atypia, such as atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesions, the immunochemical detection of dual p16^{INK4a}/Ki-67-stained cells demonstrates a significantly improved specificity with good relative sensitivity. **Conclusions:** HPV testing has shown earlier detection of persistent high-grade squamous intraepithelial lesions (HSIL) compared to cytology and is more effective in preventing invasive cervical cancer. The next challenge for the HPV primary screening program is to find the best method(s) for selecting, among HPV-positive women, those patients in need of immediate colposcopy because they are at a higher risk of developing a precancerous lesion. An HSIL cytology result

and/or dual p16/Ki-67 staining could be the best candidates, but further randomized studies are required before these approaches can be used in routine practice.

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Pathogenesis of Human Papillomavirus-Triggered Neoplastic Lesions: The Detection of p16^{INK4a} as a Biomarker for Transforming Human Papillomavirus Infections

Precancerous cervical lesions and invasive cancers are associated with persistent high-risk human papillomavirus (HPV) infections. High-risk HPVs are highly infectious agents. Most infections regress spontaneously without intervention, while only few may persist and eventually cause squamous intraepithelial lesions (SIL). Three different phases of HPV infections are characterized by distinct viral gene expression patterns and reflect the nature of clinical lesions provoked by the respective HPV infections: (i) the latent phase, (ii) the productive phase, and (iii) the transforming phase. The productive phase is characterized by some expression of the viral E6 and E7 genes in basal cells, just enough to permit the lateral expansion of the HPV-infected cell clone. However, the expression of the E6 and E7 genes in this phase is well controlled, apparently by the viral E2 gene that initially activates early gene expression, although the expression of E6

and E7 is inhibited if the E2 gene product accumulates with increasing early HPV gene expression. If these basal cells start differentiating and progress during the normal differentiation pathway upwards to the intermediate cell layer, the squamous cells lose their capacity to proliferate and exit the cell cycle irreversibly. In these differentiated cells the papillomavirus genes become expressed at high rates and trigger the replication of the episomal viral genomes within the nuclei of the infected cells. The late gene products permit packaging of the replicated viral genomes and the newly produced viral particles are released from the disintegrating keratinocytes at the very surface of the infected squamous epithelium.

The transforming phase of HPV infection is characterized by marked overexpression of the E6 and E7 genes in the basal squamous cells that apparently manage to evade control by the E2 protein.

Due to the interaction of the E7 protein with the retinoblastoma gene product pRB1, overexpression of the E6 and E7 genes results in the inactivation of pRB and release of the cell cycle from control by pRB. Since pRB is directly involved in the control of the cyclin-dependent kinase inhibitor p16^{INK4a}, increased expression of E7 in proliferation-competent cells also results in the substantial overexpression of p16^{INK4a} in all cells that overexpress the viral oncogenes and retain the capacity to proliferate [1].

The Clinical Impact of Using p16^{INK4a} Immunohistochemistry in Histology

A recent consensus conference aimed to unify the histology terminology of HPV-associated squamous lesions of the lower anogenital tract considering the biological aspects of the various stages of HPV infections and their relation to biomarker expression (the Lower Anogenital Squamous Terminology Project) [2]. A two-tiered classification system was proposed, differentiating low-grade squamous intraepithelial lesions (LSIL) from high-grade squamous intraepithelial lesions (HSIL). LSIL mostly represent the productive phase of an oncogenic HPV infection, whereas HSIL represent the transforming phase. Diagnostic studies convincingly demonstrate that the use of p16^{INK4a} histopathology substantially improves the reproducibility and diagnostic accuracy of a histopathological diagnosis using this new terminology. Strong and diffuse staining of parabasal squamous cells with p16^{INK4a} is considered to be a positive staining. p16 immunohistochemistry is recommended for the differential diagnosis between HSIL and a mimic of precancer, such as imma-

ture metaplasia, atrophy, or reparative epithelial changes. p16 immunohistochemistry is also recommended if the pathologist is entertaining an HE morphological interpretation between an LSIL and HSIL. The 2014 WHO classification of tumors of the uterine cervix modified the terminology of the precancerous lesions of the cervix and replaced the term 'cervical intraepithelial lesion' with 'intraepithelial lesion' with only two grading systems, similar to both the LAST and the cytology terminology [3].

The Clinical Impact of Using p16^{INK4a}/Ki-67 in Triage of Minor Cytologic Atypia

When compared to molecular HPV tests, the immunohistochemical detection of p16^{INK4a}-stained cells demonstrates a significantly improved specificity with good relative sensitivity. However, p16^{INK4a} single-staining immunocytochemistry protocols required the morphological interpretation of immunoreactive cells to distinguish between p16^{INK4a}-positive cells showing intraepithelial lesions and those cervical cells occasionally overexpressing p16^{INK4a} to arrest their cell cycle as an emergency response to aberrant squamous epithelial differentiation, as is seen in squamous metaplastic cells or rare endocervical cells. Since the latter cells are consistently cell cycle arrested, the combination of antibodies detecting p16^{INK4a} and the cell cycle progression marker Ki-67 in one cell allows for the unequivocal identification of truly HPV-transformed cervical cells [4]. The clinical performance of this approach has been evaluated in the triage of atypical squamous cells of unknown significance (ASC-US) and LSIL cytology results. The use of the residual material from Pap cytology cases categorized as ASC-US or LSIL indicates that p16/Ki-67 dual-stained cytology provides a high sensitivity level for detecting underlying HSIL positivity, while the specificity rate of this morphology-independent dual biomarker approach is substantially improved over the specificity rates that are observed when morphology interpretation algorithms are applied on cervical cells showing single immunoreactivity for p16 [5–8].

Triage of HPV-Positive Women

Randomized controlled trials of HPV testing have repeatedly shown earlier detection of persistent HSIL compared to cytology and greater efficacy in preventing invasive cervical cancer [9]. However, directly referring all

HPV-positive women to colposcopy results in a marked increase in the number of colposcopies needed to detect a precancerous lesion. Therefore, methods are needed for selecting among HPV-positive women those who have a very low probability of carrying a colposcopy-detectable precancerous lesion and therefore not needing immediate colposcopy. Supplements to the European guidelines recommend HPV primary screening alone with triaging those with positive cytology, with HPV-positive women with abnormal cytology (ASC-US or more severe) being referred immediately to colposcopy, and retesting after 1 year in HPV-positive women with negative cytology [10]. Women positive at the time of either test are referred to colposcopy. Informed cytology of HPV positivity is more sensitive than blind cytology [11]. Screening programs with an informed cytology triage are expected to perform better than predicted in trials and could possibly allow longer intervals before the retesting of HPV-positive women with normal cytology.

Other strategies using p16^{INK4a} to triage women with HPV infection have also been evaluated [12]. The relative

sensitivity of an HPV primary screening approach with p16 triage versus conventional cytology was better and the referral rate for colposcopy was comparable to cytology. Dual p16/Ki-67 staining may assume an important role for the triage of HPV-positive women, as has been successfully shown using p16 staining alone [12].

The next challenge for the HPV primary screening program is to find the best method(s) for selecting, among HPV-positive women, those patients in need of immediate colposcopy because they are at a higher risk of developing a precancerous lesion. An HSIL cytology result and/or p16/Ki-67 dual staining could be the best candidates, but further randomized studies are required before they can be used in routine practice.

Disclosure Statement

The authors have no conflict of interest in relation to this work.

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