

# Immunocytochemical detection of p16<sup>INK4a</sup> protein for the identification of patients at risk of cervical cancer

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## SUMMARY

**Background:** p16<sup>INK4a</sup>, cyclin-dependent kinase inhibitor that inhibits cyclin dependent kinase 4 and 6, is a product of the INK4a gene, involved in the phosphorylation of the retinoblastoma protein (pRB). HPV E7 protein binds specifically to pRB. The inactivation of pRB leads to enhanced p16<sup>INK4a</sup> protein levels in the affected cells. Overexpression of p16<sup>INK4a</sup> has been proposed as a biomarker for the identification of dysplastic lesions in histologic and cytologic samples. The aim of this study was to evaluate whether p16<sup>INK4a</sup> could be used as an additional marker in the detection of patients with cervical intraepithelial lesions at risk of progression to invasive cancer.

**Methods:** A total of 49 Thin Prep samples with cytologic diagnoses of Low Grade and High Grade SIL, and 20 with normal cervical cells, were included in the study. The mean age of the patients was 46 years. p16<sup>INK4a</sup> immunostaining was performed using the CINtec<sup>TM</sup> p16<sup>INK4a</sup> Cytology Kit (Dako Cytomation) according to the instructions of the manufacturer. Samples were considered positive if a brown reaction product was present in both the nucleus and the cytoplasm (predominantly in the nucleus.). Histologic diagnoses of punch biopsies from the SIL cases were available and could be compared to the cytologic findings.

**Results:** 17 cases from the control group were negative (85%), whereas 3 presented only a few positive metaplastic cells. In 34/37 (94.2%) cases of low grade lesions immunoreactive cells were not present but 3/37 (8.1%) showed focal staining. All but one cytologic sample with HGSIL showed positive p16<sup>INK4a</sup> immunoreactivity. The intensity of p16<sup>INK4a</sup> protein detection correlated well with the degree of abnormality.

**Conclusion:** Our preliminary study reveals that p16 immunostaining can be applied successfully as an adjunct to cytologic diagnostic methods in order to improve diagnostic results and detect patients at risk of cervical cancer.

**Key words:** Cyclin-Dependent Kinase Inhibitor p16; Biological Markers; Immunocytochemistry; Uterine Cervical Neoplasms; Cytology

## INTRODUCTION

p16<sup>INK4a</sup> is a cyclin-dependent kinase (CDK) inhibitor that inhibits cyclin dependent kinase 4 and 6, product of the INK4a gene, involved in the phosphorylation of the retinoblastoma protein (pRB). HPV E7 oncoprotein binds specifically to pRB (inactivation of pRB).

pRB inhibits transcription of the cyclin dependent kinase inhibitor gene p16<sup>INK4a</sup>. The inactivation of pRB leads to enhanced p16<sup>INK4a</sup> protein levels in the affected cells. Increased expression of the p16<sup>INK4a</sup> is a result of active expression of the hrHPV oncogenes E6 and E7, which both interact with various cell cycle-regulating proteins, in undifferentiated basal or parabasal cells, induce neoplastic transformation of the cervical epithelium.

p16<sup>INK4a</sup> has been proposed as a specific biomarker for the identification of dysplastic lesions in histologic and cervical smears or liquid-based cytologic slides.

The aim of this study was to evaluate whether p16<sup>INK4a</sup> could be used as an additional marker in the detection of patients with cervical intraepithelial lesions at risk of progression to invasive cancer.

## MATERIAL AND METHODS

A total of 69 Thin Prep samples, 49 with cytologic diagnoses of Low Grade and High Grade SIL, according to the 2001 Bethesda system, and 20 with

normal cervical cells, were included in the study. All cytologic specimens were prepared using the Thin Prep 2000 system (Cytyc Corp, Boxborough, Massachusetts, USA).

The mean age of the patients was 46 years (22-59 years). p16<sup>INK4a</sup> immunostaining was performed using the CINtec<sup>TM</sup> p16<sup>INK4a</sup>, clone E6H4, Cytology Kit (Dako Cytomation) according to the instructions of the manufacturer.

Samples were considered positive if a brown reaction product was present in both the nucleus and the cytoplasm (predominantly in the nucleus). Histologic diagnoses of punch biopsies from the SIL cases were available and could be compared to the cytologic findings

## RESULTS

The immunocytochemical detection demonstrated that 17 cases from the control group were completely negative (85%), whereas 3 presented only a few positive metaplastic cells (Table 1). They showed regular and euchromatic nuclei with nuclear and cytoplasmic reaction for p16<sup>INK4a</sup>. In 34/37 (94.2%) cases of low grade lesions immunoreactive cells were not present but 3/37 (8.1%) showed focal staining. These three women showed a follow-up cytological diagnosis of moderate dysplasia (HGSIL). All but one cytologic sample with HGSIL showed positive p16<sup>INK4a</sup> immunoreactivity presenting various numbers of positive dysplastic cells (Figure 1 and 2). The reevaluation of the case with p16<sup>INK4a</sup> negative cells revealed that the slide prepared for immunocytochemistry had a low cell number. There were only a few degenerated dysplastic cells.

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The intensity of p16<sup>INK4a</sup> protein detection correlated well with the degree of abnormality. The staining was not easily detectable in degenerated dysplastic cells.

**Table 1. p16<sup>INK4a</sup> immunoreactivity**

PAP TEST	Negative results	Positive results	Total
Normal	17 (85%)	3 (15%)	20
LGSIL	34 (91.9%)	3 (8.1%)	37
HGSIL	1 (8.3%)	11 (91.6%)	12
TOTAL	52 (75.3%)	17 (24.7%)	69

## CONCLUSION

Various immunocytochemical markers have been investigated in order to resolve the problem concerning the discrimination of patients with cervical dysplasia at risk of recurrence or progression to invasive cancer. It has been shown that persistent hrHPV infection is a prerequisite of the development of SIL (3). The hrHPV E7 oncogene product blocks the activity of the pRB gene product. The cyclin-dependent kinase inhibitor p16<sup>INK4a</sup> is regulated via a negative feedback control by pRB so that transcription of the p16<sup>INK4a</sup> gene is released when pRB function is reduced or lost in the affected cells.

In our study normal cervical epithelium was not stained. A few metaplastic cells (easily distinguished from dysplastic ones), displayed a focal faint staining pattern, whereas HGSILs showed a strong and diffuse expression of p16<sup>INK4a</sup>.

These observations confirm previous studies suggested that p16<sup>INK4a</sup> is overexpressed in cells transformed by hrHPV types. Using this antibody exfoliated dysplastic epithelial cells could easily be detected in liquid-based cytology specimens.

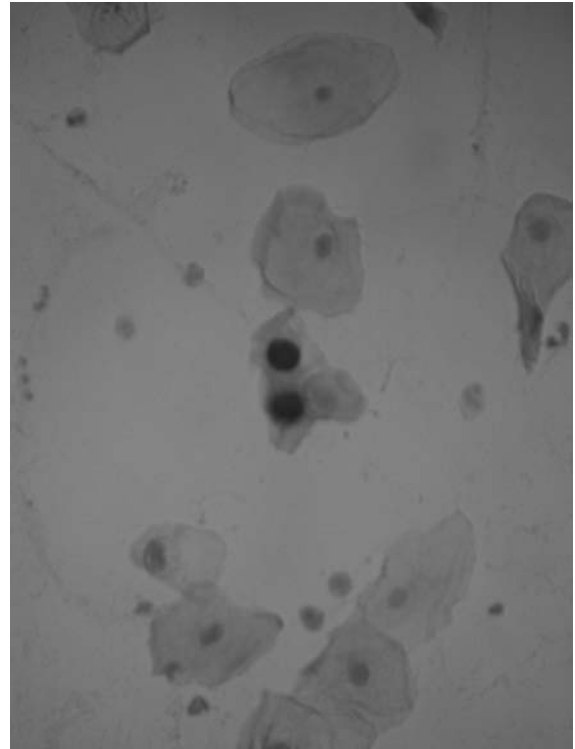
Therefore, p16<sup>INK4a</sup> immunostaining can be applied successfully as an adjunct to cytologic diagnostic methods in order to improve diagnostic results and detect patients at risk of cervical cancer.

## Conflict of interest

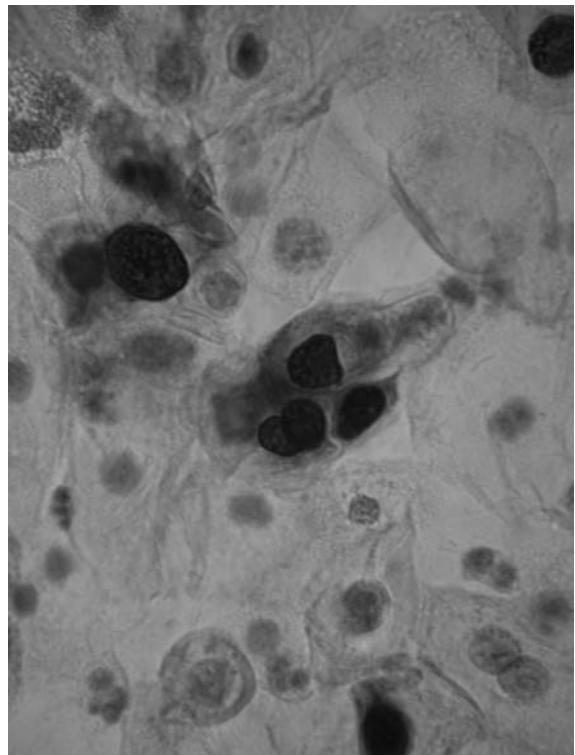
We declare no conflicts of interest.

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**Figure 1. Metaplastic cells positive for p16<sup>INK4a</sup>, x400**



**Figure 2. Positive staining in a dyscaryotic cells, x400**