RESEARCH COMMUNICATION

Utility of Ki-67 and p53 in Distinguishing Cervical Intraepithelial Neoplasia 3 from Squamous Cell Carcinoma of the Cervix

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Abstract

The differentiation between cervical intraepithelial neoplasia 3 (CIN 3) and early squamous cell carcinoma (SCC) of the cervix may be difficult in certain situations. Identification of invasion beyond the basement membrane is the gold standard for the diagnosis of the latter. The objective of this study was to determine whether the use of Ki-67 and p53 could help in solving the above dilemma. This was a retrospective study on 61 cases of cervical neoplasms comprising of 25 cases of CIN 3 and 36 SCC. All cases were evaluated by immunohistochemistry using Ki-67 and p53 monoclonal antibodies. Results showed that the differences of Ki-67 and p53 expression between CIN 3 and SCC were statistically significant. In conclusion, Ki-67 and p53 may serve as helpful adjuncts to routinely-stained histological sections in differentiating between CIN 3 and SCC.

Key Words: Cervical neoplasms - cervical intraepithelial neoplasia - Ki-67 - p53

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Introduction

Cervical cancer is one of the leading causes of mortality in Malaysia and ranked as the second commonest cancer for women after breast cancer (Parkin et al., 1999). A recent study showed that the age of developing cervical neoplasia tends to be younger and younger (Parkin et al., 2001). Cancer is a disease feared by the public and Malaysian patients usually present themselves to the hospital at an advanced stage due to ignorance or unawareness of the potential of distant metastasis.

The International Agency for Research concluded that there is a strong association between Human papillomavirus (HPV) and cervical cancer development, and the risk of developing cancer depends on the HPV type (Muñoz et al., 2003). However, not all women who have HPV infection develop cervical cancer, suggesting that HPV alone is not sufficient for cervical carcinogenesis. Researchers are currently focused on other important cell cycle factors such as loss of growth suppression, increased cell growth rates, and angiogenesis (Stanley et al., 2001; Tjalma et al., 2001; Araujo Souza et al., 2003). Two of the cell cycle genes which may be involved include Ki-67 and p53.

The Ki-67 protein is a cellular marker for proliferation which can be detected within the cell nucleus. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Ki-67 is an excellent marker to determine the growth fraction of a given cell population (Gerdes et al., 1983). Ki-67 expression is normally confined to the parabasal and basal layers in the normal cervical epithelium (al-Saleh et al., 1995; Bulten et al., 1996). Increased Ki-67 reactive cells in the intermediate and superficial layers of the squamous epithelium correlate with the presence of cervical dysplasia, while the percentage and superficial location of positive cells in the cervical epithelium also correlates with the grade of cervical intraepithelial neoplasia (CIN) (Konishi et al., 1991; al-Saleh et al., 1995).

The p53 gene functions include cell-cycle arrest and apoptosis in response to DNA damage. Normal p53 protein has a very short half-life, and thus the protein level is too low to be identified immunohistochemically. In contrast, mutant p53 proteins have a longer half-life (Finlay et al., 1988) and can be easily detected by immunohistochemical methods. The p53 gene is one of the most important targets of the HPV E6 gene. It was found that E6 protein has the ability to stimulate p53 degradation, and inhibits several functions of the wild-type p53 including the suppression of malignant growth (Storey et al., 1998; Kisseljov et al., 2000).

One of the commonly faced challenges in the pathology of cervical lesions is the differentiation between

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CIN 3 lesion and early invasive squamous cell carcinoma (SCC), whereby identification of invasive foci is the mainstay in the diagnosis of the latter. Although the histologic features of preinvasive cervical neoplasms are well recognized, the nature of the biopsy specimen can result in significant intra-observer and inter-observer diagnostic variability (Robertson et al., 1989; de Vet et al., 1990). The aim of this study was to investigate the Ki-67 and p53 proteins as possible biomarkers in distinguishing CIN 3 from cervical SCC.

Materials and Methods

Study Design

This is a retrospective study on cases diagnosed as CIN 3 and SCC, obtained from the histopathology records of the Department of Pathology in a tertiary hospital in Malaysia for a period of seven years. The total number of cases was 61, comprising of 25 cases of CIN 3 and 36 cases of squamous cell carcinoma.

Immunohistochemistry

Ki-67: Monoclonal Mouse Anti-Human Ki-67 antigen (DAKO code No. M7240) at a dilution of 1:100 was applied on three-micron thick, formalin-fixed, paraffin-embedded tissue sections. This was performed by using 15-minute heat-induced epitope retrieval in 10 mmol/L citrate buffer at pH 6.0, followed by 30 minutes incubation at room temperature with the primary antibody. Finally, the slides were counterstained with haemotoxylin and eosin. The same technical personnel performed the immunohistochemical staining for all the cases. Sections of normal tonsil tissue were used as positive control.

p53: Monoclonal mouse anti-human p53 protein (DAKO code No. M7001), at a dilution of 1:50 was applied on three-micron thick, formalin-fixed, paraffin-embedded tissue section. This was performed by using 20-minute heat-induced epitope retrieval in Dakocytomation target retrieval solution, followed by 30 minutes incubation at room temperature with the primary antibody. This was followed by staining using the DAKO LSAB+/HRP and EnVision+/HRP kits. Streptavidin and diaminobenzidine (DAB) were then added. Finally, the slides were counterstained with haemotoxylin and eosin. The same technical personnel performed the immunohistochemical staining for all cases. Sections of breast cancer tissue were used as positive control.

Interpretation of results

All slides were examined under light microscopy. Strong nuclear staining was regarded to denote p53 and Ki-67 positivity. 100 cells were evaluated in representative high-power fields to obtain the percentage of cell positivity. Two independent pathologists who determined the immunohistochemical staining results were blinded to the clinical diagnoses and origin of the samples.

Statistical analysis

The percentage of p53 and Ki-67 protein staining for CIN 3 (pre-malignant) and SCC (malignant) were evaluated using the chi-square test. p value was calculated by SPSS program version 12.0 (SPSS Inc., Chicago, IL, USA). Any p value < 0.05 was considered to be statistically significant.

Results

Clinical Presentation

The age of clinical presentation for all the patients with cervical neoplasm (CIN 3 and SCC) ranged from 28 to 72 (mean 43.8) years. Patients with cervical squamous cell carcinoma (32 to 72, mean 51.1 years) were older than those with CIN 3 (20 to 58, mean 38.2 years); one of the patients who had CIN 3 was below 30 years of age. The distribution of CIN 3 amongst the major ethnic groups in Malaysia was 69.6% Chinese, 21.7% Malays and 8.7% Indian, while the distribution of SCC was 50% Chinese, 38.9% Malay and 11.1% Indian.

Ki-67 Expression

Positive staining for Ki-67 was localized in the nuclei of dysplastic and carcinoma cells (Figure 1). Thirteen of the 25 (52%) premalignant (CIN 3) cases were positive for Ki-67 protein. In contrast, 34 of the 36 (94.4%) malignant (SCC) cases were positive. The average percentages of Ki-67 expression were 12% and 64.9% for CIN 3 and SCC respectively. The relationship between the percentages of cells with Ki-67 expression and their corresponding histological diagnosis is summarized in Table 1. The difference of Ki-67 protein expression between CIN 3 and SCC was statistically significant (p value <0.0001).

p53 Expression

Positive staining for p53 expression was localized in the nuclei of dysplastic and carcinoma cells. Eighteen of the 25 (72%) pre-malignant (CIN 3) cases were positive for p53. In contrast, 34 of the 36 (94.4%) malignant (SCC) cases were positive. The average percentages of p53 expression were 33% and 71.1% for CIN 3 and SCC respectively. The relationship between the percentage of cells with p53 expression and their corresponding histological diagnosis is summarized in Table 2.

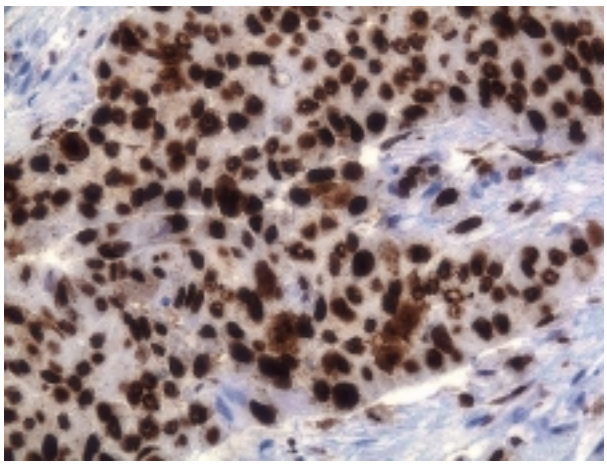

Figure 2. Ki-67 Expression in the Nuclei of Carcinoma Cells (Ki-67, x200)

Table 1. The Average Percentage of Ki-67 Expression in CIN 3 and SCC Cases

	Positive	Negative
Malignant (SCC)	64.9	35.1
Premalignant (CIN 3)	12.0	88.0

P value < 0.0001, SCC- Squamous cell carcinoma, CIN-cervical intraepithelial neoplasia

Table 2. The Average Percentage of p-53 Expression in CIN 3 and SCC Cases

	Positive	Negative
Malignant (SCC)	71.1	28.9
Premalignant (CIN 3)	33.0	67.0

P value < 0.0001, SCC- Squamous cell carcinoma, CIN-cervical intraepithelial neoplasia

Discussion

Our study showed a higher incidence of cervical neoplasia in the Chinese community compared with Malays and Indians, implying a higher prevalence of HPV infections in the former. This finding suggests that HPV vaccine could be recommended in Chinese women. When comparing the ratio of CIN 3 and SCC in various ethnic groups, we found that Chinese have a higher incidence of CIN 3 compared to SCC. In contrast, the ratio was reversed in Malays and Indians. A possible reason for this could be that the Chinese were more health conscious and sought early medical attention, or that some of the Malays and Indians may have initially opted for traditional therapy, and therefore presented with an advanced stage of the disease.

Because of the significant inter- and intra-observer variability in interpreting invasive and non-invasive cervical biopsy specimens, there is a need to search for biomarkers to assist in diagnosis. Oncogenes and cell-cycle regulators that may play a role in the genesis of cervical cancer include c-erbB 2, p27, p53, p16INK4a and Ki-67. Ki-67 is a cell proliferation marker associated antigen and hence an excellent measure of proliferation of a neoplasm. It has been applied in the histopathological diagnosis of malignant tumours such as skin, brain, lung and conjunctiva. p53 is linked with the control of cell growth, cell cycle, and apoptosis (Levine et al., 1992; Greenblatt et al., 1994). It has been found to be the most commonly targeted gene for alteration in human tumours such as carcinomas of the lung, colon and breast.

This study revealed that Ki-67 expression correlates well with the invasive nature of SCC, whereby the average percentage of malignant cell reactivity was 64.9%, compared to CIN 3 which was 12%. However, two of the malignant cervical lesions were completely negative for Ki-67. Hence, absence of Ki-67 staining does not exclude SCC. The average percentage of p53 expression was 71.1% in invasive carcinoma and 33% in CIN 3.

Song et al. found that Ki-67 expression was a strong predictor of the presence of HPV, and rising HPV loads were also found to be significantly correlated with elevated Ki-67 expression levels (Song et al., 2007). HPV infection activates host cell cycle progression increasing cell cycle kinetics, which is reflected as increased Ki-67 staining. Agoff et al. noted that although Ki-67 expression is a sensitive marker for cervical neoplasia, increased expression may also be seen in inflammatory conditions. This is expected because inflammation is also associated with increased cell turnover (Agoff et al., 2003).

CIN may remain unchanged, regress to normal or to a lesser grade, or progress to a higher grade or invasive carcinoma (Hu et al., 1997). Kruse et al studied Ki-67 expression in 87 early CIN lesions (25 CIN 1 and 65 CIN 2) to predict high grade CIN in the follow-up biopsies. Their results confirmed that quantitative Ki-67 features have strong prognostic value for progression in early CIN lesions (Kruse et al., 2004).

In conclusion, immunohistochemical markers such as Ki-67 and p53 may serve as helpful adjuncts in differentiating CIN 3 from SCC in difficult situations. However, morphological identification of invasive foci remains the gold standard for the diagnosis of invasive squamous cell carcinoma.

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