The role of p53, Bcl-2 and Ki-67 in premalignant cervical lesions and cervical cancer

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Summary

Purpose: The aim of this study was to determine the role of p53, Bcl-2 and Ki-67 expression in the carcinogenesis of cervical carcinoma and aggressiveness of cervical intraepithelial neoplasia (CIN). *Methods:* The pathology specimens of 63 patients with a diagnosis of normal squamous epithelium (22 cases), CIN I (14), CIN II (5), CIN III (8) and squamous cell carcinoma (14) were evaluated immunohistochemically for the expression of p53, Bcl-2 and Ki-67 in paraffin sections. *Results:* The expression of p53 and Ki-67 increased proportionally to the grade of CIN and cervical cancer, but only the increase of p53 expression was statistically significant ($p = 0.002$). There was no significant correlation between Bcl-2 expression and premalignant and malignant cervical lesions. *Conclusion:* p53 expression may have a role in the carcinogenesis of squamous cell cervical carcinoma whereas Bcl-2 expression has no role. Ki-67 expression can not be used in determining the aggressiveness of CIN lesions.

Key words: Cervical intraepithelial neoplasia; Cervical cancer; p53; Bcl-2; Ki-67.

Introduction

A complex balance between proliferation and apoptosis maintains normal cellular turnover. Genetic events leading to an increase in proliferation and a reduction in apoptosis, therefore, can result in cancer development.

Tumor suppressor gene p53 and apoptosis control gene Bcl-2 are two important regulatory genes for cell survival. p53 is activated as a result of DNA damage and has a role in DNA repair and apoptosis. Its mutation impairs the repair or apoptosis of genetically damaged cells. The Bcl-2 gene is a member of the Bcl-2 gene family regulating apoptosis. Its expression prolongs cellular survival by preventing apoptosis.

Pathologists use the number of mitotic figures as well as cellular atypia in the cervical epithelium for grading of CIN [1]. The expression of Kİ-67 shows the proliferative activity of the cells [2]. It has been found that Kİ-67 expression can be used in grading of CIN and evaluating the progression potential of CIN [3].

p53 [4-6] and Bcl-2 [6-8] genes have been shown to be involved in cervical cancer carcinogenesis, which continues to be one of the major causes of female cancer-related death worldwide. The aim of this study was to assess the value of p53, Bcl-2, and Kİ-67 expression in the carcinogenesis of cervical cancer and the importance of these markers in determining the aggressiveness of premalignant cervical lesions.

Materials and Methods

The study adhered to the Declaration of Helsinki and Good Clinical Practice guidelines. After Institutional Review Board approval pathology specimens of cervical biopsy and hysterectomy materials of patients with a diagnosis of premalignant cervical lesions and squamous cell cervical cancer from February 2002 to March 2004 were included in the study. The pathological diagnosis included 22 cases of normal squamous cell cervical epithelium, 14 cases of CIN I, five cases of CIN II, eight cases of CIN III and 14 cases of squamous cell cervical cancer. All specimens were fixed in 0.4% formalin and processed routinely. Hematoxylin and eosin (H&E) stained slides of the cases were reviewed. One paraffin-embedded tissue block of each case demonstrating the representative areas were selected for immunohistochemical studies.

Immunohistochemical Analysis

Paraffin-embedded 5 μm serial tissue sections were mounted on poly-D lysine (Sigma) coated slides and deparafinized. Antigen retrieval was performed by either citrate buffer pH 6.0 or by EDTA pH: 8.0. Primary antibodies against Bcl-2 (monoclonal 100/D5, Neomarkers, Fremont CA, USA, 1:50), p53 (monoclonal DO-7+BP53-12, Neomarkers, Fremont, CA, USA, 1:100) and Ki67 (monoclonal MB1, DBS, CA, USA, 1:30) were used. A Ventana NexES automated immunostainer with Ventana DAB detection kit was used for secondary visuilization. Nuclear red-brown staining was accepted as positive for p53 and Kİ-67, whereas perinuclear cytoplasmic red-brown staining was accepted as positive for Bcl-2.

Histological Analysis

Immunohistochemical staining was semiquantitatively graded as grade 0: no staining; grade 1: weak staining; grade 2: intermediate staining; and grade 3: strong staining. The percentage of the stained cells was rated as: grade 1: staining in 0-5% of cells; grade 2: staining in 6-25% of cells; grade 3: staining in 26-50% of cells; grade 4: staining in 51-75% of cells; and grade 5: staining in 76-100% of cells. The staining index was calculated for each case as the product of staining intensity and staining ratio (Staining Index = Staining Intensity* Staining Ratio).

Statistical Analysis

Statistical analyses are carried out by employing the Statistical Package for Social Sciences software 11.0 for Windows package software (SPSS, Inc., Chicago, IL). Univariate analy-

Revised manuscript accepted for publication January 11, 2006

sis was performed using the Kruskal-Wallis test with multiple comparisons to investigate significant differences among the five groups. The difference between two groups was evaluated with the Mann-Whitney U-test. A p value less than 0.05 was considered as statistically significant.

Results

The mean values and standard deviations of the staining index of p53, Bcl-2 and Kİ-67 for CIN and squamous cell cervix cancer are given in Table 1. We observed staining for p53 (Figures 1 and 2), Kİ-67 (Figures 3 and 4) and Bcl-2 (Figures 5 and 6) in the basal layer in CIN I cases and in the intermediate and superficial layers as the grade of CIN increased. The average staining index (ASI) for p53 rose with increasing CIN grade till the cervical cancer stage. There was a statistically significant difference for ASI for p53 among the five groups. (Kruskal Wallis test; $p = 0.002$). Intergroup differences between the control group and CIN III ($p = 0.002$), control group and cervix cancer ($p < 0.001$), CIN I and cervix cancer ($p = 0.009$), CIN II and CIN III ($p = 0.045$) and CIN II and cervix cancer ($p = 0.002$) were found to be significantly different by the Mann-Whitney U-test.

ASI for Kİ-67 increased with increasing CIN grade, but there was no statistically significant difference among the five groups ($p = 0.144$). Although there was no statistically significant difference, ASI for Kİ-67 was greater in the normal squamous epithelium group than in CIN I.

There was no statistically significant difference among the five groups for the ASI of Bcl-2 ($p = 0.383$).

Table 1. — *Average p53, Bcl-2 and KI-67 staining index in normal cervical epithelium, cervical intraepithelial neoplasia (CIN) and squamous cell cervical cancer.*

	Case number	Average staining index	Standard deviation	Minimum	Maximum
p53					
Normal epithelium	22	1.27	0.63	0	2
CIN I	14	1.93	1.73	0	6
CIN II	5	0.60	0.89	0	2
CIN III	8	3.13	2.75	0	9
Cancer	14	4.21	2.12	1	8
Total	63	2.25	2.04	0	9
Bcl-2					
Normal epithelium	22	1.68	1.52	0	4
CIN I	14	2.71	4.08	0	15
CIN II	5	3.20	3.56	0	8
CIN III	8	0.88	2.10	0	6
Cancer	14	2.43	4.24	0	15
Total	63	2.10	3.14	0	15
Ki-67					
Normal epithelium	22	4.14	2.47	1	9
CIN I	14	3.86	3.13	0	9
CIN II	5	2.80	4.02	1	10
CIN III	8	5.38	3.66	1	12
Cancer	14	6.86	5.05	1	15
Total	63	4.73	3.70	0	15

Discussion

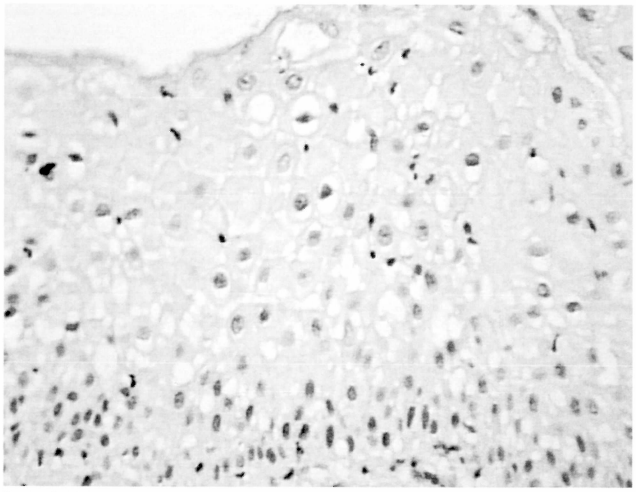
The pathogenesis of carcinoma of the uterine cervix is thought to occur through a multistep process. In this process some premalignant cervical lesions progress and some regress spontaneously however we do not know the determinant.

Attention has been focused on molecular markers that may be associated with the process of carcinogenesis. In our study, we examined the expression of p53, Bcl-2, and Kİ-67 in cervical carcinomas and their premalignant lesions in order to assess its role in the carcinogenesis of cervical cancer.

p53 protein, which encoded on chromosome 17p13, has been shown to have a central role in regulation of the cell cycle and apoptosis [9]. Mutation of the p53 gene has been quoted as one of the most common in human cancers [10]. There have also been multiple trials investigating the role of the p53 gene in the carcinogenesis of cervical cancer. Some trials have found a correlation between the expression of p53 and progress of CIN to cervix cancer [4-6], but others did not find the same relationship [11, 12]. These conflicting reports may be due to evaluation of positive staining by different cut-off values taken for the percentage of stained cells. What percentage constitutes a 'majority' is nonetheless debatable. Cut-off values varied widely in different studies; for example 5%, 10%, 30%, 75% in the study of Cheah *et al.*, Giarnieri *et al.*, Tjalma *et al.*, Chen *et al.*, respectively [5, 6, 13, 14]. In these studies staining intensity was not evaluated for immunoquantification. Nygan *et al.* [12] evaluated p53 expression by using an immunohistochemical score which was calculated by totaling the staining intensity and staining percentage scores. We also evaluated p53 expression with a staining index which was calculated by multiplying the staining intensity and staining ratio for immunoquantification. With this method of evaluation we found that p53 expression has a significant role in progression to cervical cancer ($p = 0.002$). p53 expression was detected in CIN I cases and increased gradually as CIN grade increased, so it might play a role in early stages of carcinogenesis. In CIN II cases p53 expression was less than CIN I cases. This could be due to the small number of cases in the CIN II group. It is not expected to detect p53 expression in benign cervical tissue but we did also detect p53 expression in normal squamous epithelium. It is thought that the p53 protein detected by immunohistochemistry is a product of a mutated gene [15]. However in recent studies it was found that wild type p53 protein can also be detected immunohistochemically as a result of cellular damage [16, 17]. In our study in the normal squamous epithelium group all the cases had a diagnosis of chronic cervicitis. This may explain the expression of p53 in this group.

A balance between apoptosis and proliferation is thought to be important in tumor progression and apoptosis is an important determinant of this balance. The over-expression of the Bcl-2 protein can block apoptosis and prolong cell survival [18], thus it is thought to have an important role in carcinogenesis. However some studies support the role of Bcl-2 in the carcinogenesis of cervical cancer [6-8], while others do not [19-21]. In our study ASI for Bcl-2

Fig. 1



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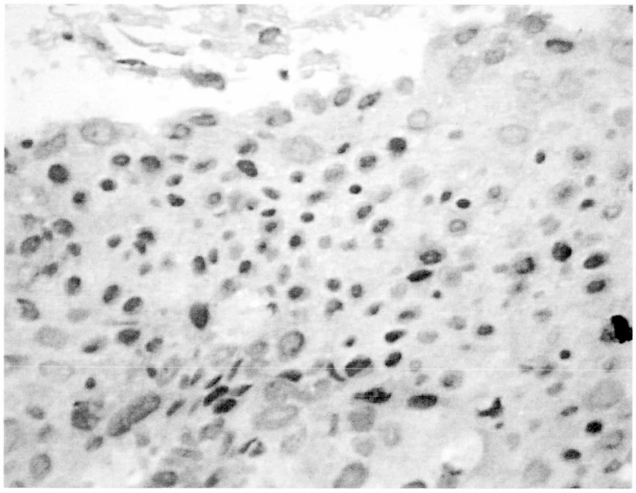
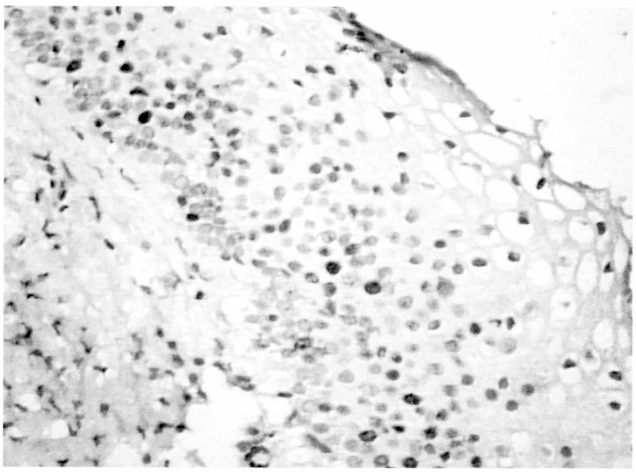


Fig. 3



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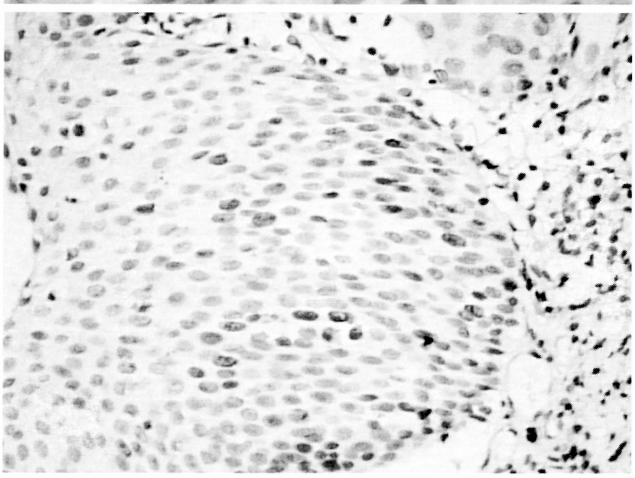
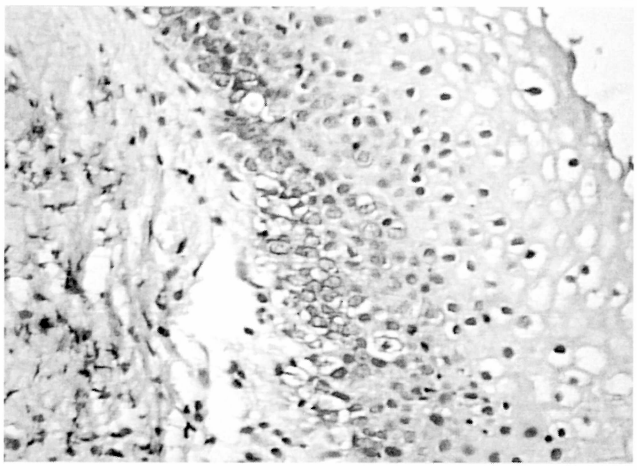


Fig. 5



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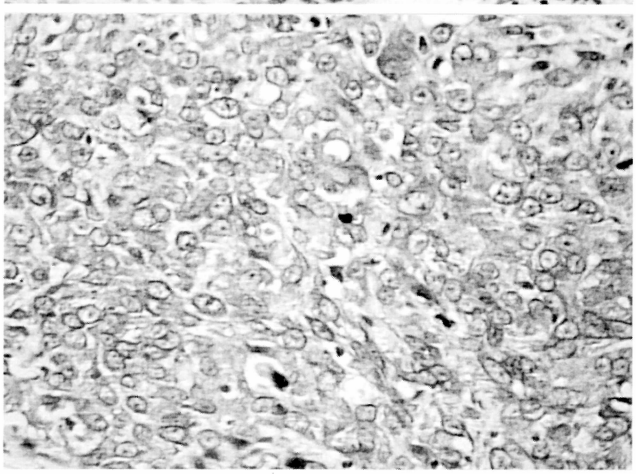

Figure 1. — Nuclear staining for p53 in the basal layer of a CIN I case (x400).
Figure 2. — Nuclear staining for p53 in all layers of the epithelium in a CIN III case (x400).
Figure 3. — Nuclear staining for KI-67 in the basal layer of a CIN I case (x400).
Figure 4. — Nuclear staining for KI-67 in all layers of the epithelium in a CIN III case (x400).
Figure 5. — Perinuclear staining for Bcl-2 in the basal layer of a CIN I case (x400).
Figure 6. — Diffuse perinuclear staining for Bcl-2 in a cervix cancer case (x400).

increased as the grade of CIN rose, but it decreased in CIN III and cervical cancer cases. Dimitrakakis *et al.* found a similar result in their study and concluded that proliferation is more important than apoptosis in maintenance of the tumor [7]. Kokawa *et al.* found a decrease in Bcl-2 expression in cervical cancer cases but could not find an increase in apoptosis. This implies changes in other factors regulating apoptosis [20]. Cheung *et al.* could not find a relationship between Bcl-2 expression and cervical cancer progression, but found a decrease in Bak, caspase 3 and caspase 6 expression which increase apoptosis [21]. Chung *et al.* found Bcl-2, Mcl 1, Bcl XL, Bax, Bak, caspase 3 and caspase 6 expression in different ratios in all cases of cervical cancer. Finding these markers in different ratios in the same case shows that the control of apoptosis is a complex event [22]. Our results do not support a possible role of Bcl-2 in the carcinogenesis of cervical cancer. It is known that there are several mechanisms regulating the process of apoptosis; a reduction in Bcl-2 therefore does not necessarily translate into an increase in apoptosis because other factors may also be altered. Other Bcl-2 family proteins must also be investigated for a possible role in the carcinogenesis of cervical cancer.

Kİ-67 immunoquantification is used for evaluating the proliferative activity in CIN and cervical cancer. Kİ-67 expression was found to be high in some low-grade squamous intraepithelial lesions (SIL) and low in some high-grade SIL cases. It has been stated that Kİ-67 immunoquantification in SIL can be used to determine the progression potential [3, 23]. In our study ASI for Kİ-67 increased as the grade of CIN increased. Moreover, we detected Kİ-67 protein in the basal layer in CIN I cases and in the intermediate and superficial layers in CIN II and CIN III cases. The location and the amount of Kİ-67 protein correlated with the pathological diagnosis of the cases but we could not find a statistically significant difference among the groups. More extensive series of samples are required to establish the prognostic significance of Kİ-67 in CIN lesions. We also detected Kİ-67 expression in normal cervical epithelium in the basal layer. It has been stated that Kİ-67 expression can be detected in the parabasal layer due to the presence of cells with high proliferation potential [23].

Conclusion

p53 expression was detected in early stages in cervical cancer carcinogenesis and has a role in progression to cervix cancer. Bcl-2 has no role in cervical cancer carcinogenesis and Kİ-67 expression can not be used in determining the progression potential of CIN lesions. More studies on molecular markers, associated with carcinogenesis, may help to better understand the clinical outcome of premalignant cervical lesions.

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