P53, Bcl-2, Ki-67 Li (Labeling Index) Status in Benign, Proliferative, And Malignant Ovarian Surface Epithelial Neoplasms

Benign, Proliferatif ve Malign Over Yüzey Epitel Tümörlerinde P53, Bcl-2, Ki-67 Li (Labelıng Index)

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Abstract

Objective. Approximately 50% of human malignancies present with mutations in p53, which is the most common tumor suppressor gene involved with human malignancies. Bcl-2 is a protooncogene, and expression of its protein product is associated with a better prognosis in several malignancies. Ki-67 is a marker of cellular proliferation. The purpose of this study was to determine whether simultaneous detection of p53, bcl-2 and Ki-67 using immunohistochemical staining can be used as a diagnostic factor in the assessment of human ovarian epithelial tumors.

Materials and Methods. The study was performed on formalin-fixed, paraffin-embedded tissue samples from 75 epithelial ovarian tumors, 15 serous cystadenomas, 15 mucinous cystadenomas, 5 borderline serous cystadenomas, 5 borderline mucinous cystadenomas, 15 serous cystadenocarcinomas, 15 mucinous cystadenocarcinomas and 5 endometrioid carcinomas. Immunohistochemical staining was performed using monoclonal antibodies against p53, bcl-2, and Ki-67(MIB1).

Results. Anti-p53 reactivity was observed in 14 tumors, all of which were malignant tumors, and no reactivity was observed in borderline or benign tumors. Overexpression of bcl-2 was observed in 12 benign neoplasms (40%), 5 of which were borderline (50%), but was not observed in any of the malignant tumors. There was a statistically significantly higher level of Ki-67 LI positivity in the malignant tumors than in the benign and borderline tumors (p<0.005).

Conclusion. These data show significant differences in the expression of these markers in ovarian tumors and suggest a possible role for these tumor-associated genes as supplemental tools in diagnostic pathology. Furthermore, our findings support the redesignation of low malignant potential tumors (current nomenclature) to benign ovarian carcinoma.

Keywords: Ovarian cancer, p53, bcl-2, Ki-67

Özet

Amaç. *p53 insan malignesilerinde mutasyonu en sık izlenen tümör baskılayıcı gendir. Bütün kanserlerin %50 sinde izlenir. Bcl-2 ise çeşitli kanserlerde görülen, protein ürünü iyi prognozla ilişkili olan bir protoonkogendir. Ki-67 ise hücre proliferasyon markırıdır. Bu çalışmanın amacı overin epitelyal tümörlerinde immunohistokimyasal çalışma yaparak p53, bcl-2 ve Ki-67'nin tanıda yerini değerlendirmektir.*

Gereç ve Yöntem. *Bu çalışa; 15 seröz ve müsinöz kistadenom, 15 seröz ve müsinöz kistadenokarsinom, 5 borderline seröz ve müsinöz kistadenom ve 5 endometroid karsinomalı vakalara ait formalin ile fikse parafin bloklarda yapıldı. İmmunohistokimyasal olarak anti-p53, the anti-bcl-2, the anti-Ki-67(MIB) uygulandı.*

Bulgular. *Anti-p53 aktivesi bütün malign tümörlerin 14'ünde pozitif olup borderline ve benign tümörlerde boyanma izlenmedi. Bcl-2 overekspresyonu 12 (%40) benign tümörde, 5 (%50) borderline tümörde izlendi fakat malign tümörlerde gözlenmedi. Ki-67 LI ise malign tümörlerde benign ve borderline tümörlere göre istatistik olarak anlamlı daha yüksekti (p<0.005).*

Sonuç. *Bu veriler over tümörlerinde bu markırların tanıda kullanılabileceğini, özellikle düşük malign potansiyelli tümörler ile benign vakaların ayırımında faydalı olabileceğini göstermektedir.*

Anahtar Kelimeler: *Over tümörleri; p53; bcl-2; Ki-67*

Introduction

O varian surface epithelial tumors represent the most common lethal gynecologic neoplasms for women of reproductive age and older [1-5] and continue to present a challenge despite advances in our knowledge of the disease over the past 20 years [6]. These tumors display biological behaviors that follow their histopathological grading of malignant, borderline or low malignant potential (LMP), or benign. Of particular interest are those classified as borderline or LMP because the pathologist must rely on somewhat vague and poorly reproducible morphological criteria. These include architectural criteria, such as the increased complexity of papillary excrescences with the stratification of epithelial nuclei and epithelial budding or tufting in the absence of stromal invasion, and cytological criteria, such as nuclear atypia and mitosis. Clinically, these LMP lesions display a more indolent behavior, with an overall 10-year survival rate of 80-90% [7].

Previous studies have shown that the p53 gene is mutated in 30-80% of ovarian carcinomas [1,8]. The role of p53 in ovarian cancer is contentious, as there are a number of contradictory studies. Several studies have identified p53 protein expression, detected by immunohistochemistry, as an adverse prognostic factor for survival in human ovarian cancer [9-11]. Other studies have suggested that alterations in p53 expression in ovarian cancer affect sensitivity to chemotherapy [12]. In contrast, there are a number of studies that suggest that p53 expression has no prognostic value in epithelial ovarian cancer [5,13-15].

The role of bcl-2 in gynecological malignancies has been investigated [5]. Expression of bcl-2 has been correlated with improved survival in ovarian cancer [16,17].

The proliferation index has been correlated with prognosis and other clinicopathological features in a number of human malignancies [18-20]. Expression of the Ki-67 proliferation marker, which detects all phases of the cell cycle except G0, is known to predict disease outcome in many human malignancies [20]. The tumor proliferative fraction of ovarian carcinomas has been investigated immunohistochemically by means of antibodies that recognize the nuclear antigen Ki-67 expressed in proliferating cells. A number of studies on ovarian cancer have reported an association between high proliferation indexes and reduced overall survival or reduced disease-free survival [21,22]. Multivariate analysis revealed a significant relationship between high Ki-67 immunostaining in ovarian neoplasms and disease-free survival and also found that high proliferation was associated with poor prognosis for ovarian cancer in both univariate and multivariate analyses [23,24].

The aim of this study was to better define the biology of surface ovarian neoplasms using monoclonal antibodies (MoAbs) that recognize P-53, Ki-67 and Bcl-2.

Patients and Methods

Case Selection
A total of 75 cases of ovarian surface epithelial neoplasms, including 15 serous cystadenomas, 15 mucinous cystadenomas, 5 borderline serous cystadenomas, 5 borderline mucinous cystadenomas, 15 serous cystadenocarcinomas, 15 mucinous cystadenocarcinomas and 5 endometrioid carcinomas, were retrieved from the Surgical Pathology Archives, Medical Faculty Atatürk University between 1996-2001. The diagnoses were reviewed, and a representative block was selected from each case for immunohistochemical analysis.

Immunohistochemistry
Parallel 4-mm serial sections cut from paraffin-embedded tumor blocks were mounted on Superfrost glass slides, dewaxed and rehydrated in a graded alcohol series, followed by microwave antigen target retrieval. Immunostaining was performed using the rabbit or mouse DAKO EnVision(TM)+System, Peroxidase (DAB) Kit (DAKO). The sections were incubated for 35 min with the following primary antibodies at the dilutions specified: mouse monoclonal anti-bcl-2 antibody (Clone 124) (DAKO) diluted 1:40; mouse monoclonal anti-p53 antibody (DO-1) (Santa Cruz Biotechnology) diluted 1:200; and monoclonal mouse anti-Ki-67 antibody (Clone 7B11) diluted 1:100.

Each stained section and the controls were examined via light microscopy by two independent observers who were blinded to both the clinicopathological data and the results from the scoring for each section with each of the other primary antibodies. Tissues with defined expression or absence of expression of a particular antigen were used as positive and negative controls, respectively. Each section was examined for positive staining in the cytoplasm and nuclei of the malignant cells, the absolute numbers of malignant cells stained, as well as the pattern and `intensity' of the staining pattern in the sections.

Statistical analysis
The Chi-square test for contingency tables or the Fisher's exact test were performed to determine if there was a difference in the Ki-67 staining between proliferative and malignant lesions, with the hypothesis that P-53 and bcl-2 are expressed to a significantly greater degree in malignant compared to proliferative tumors. All statistical analyses were performed using the SAS statistical software package.
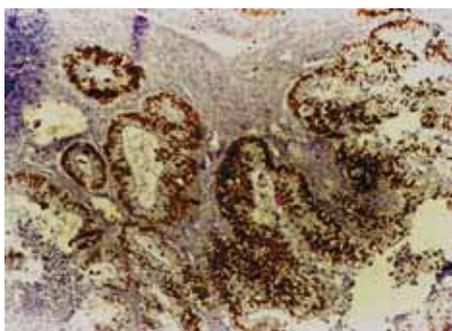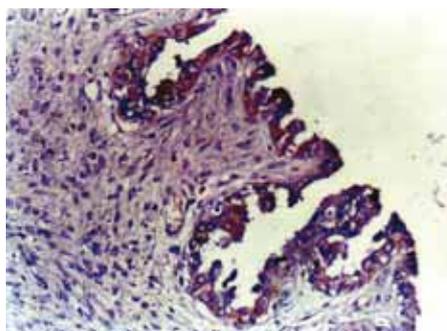
Fig. 1 — Positive p53 immunostaining.

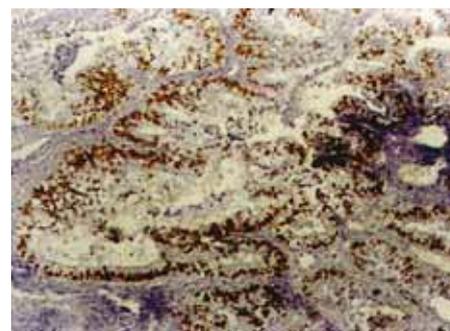**Fig. 2 —** Positive bcl-2 immunostaining.

Fig. 3 — Positive Ki-67 immunostaining.

Results

Tumor suppressor protein (p53)

None of the benign tumors and ten of the proliferative neoplasms were observed to have immunoreactivity with anti-p53. However, expression of p53 protein, which was found exclusively in the nucleus of the epithelial cells, was frequently found in 40% of the malignant ovarian cells. Nine out of 14 positive patients had a serous cystadenocarcinomas. There is a statistically significant difference in the expression of p53 between malignant and benign tumors (p< 0.005, chi square test).

bcl-2

Bcl-2 immunoreactive products appeared as brown granules that were predominantly localized in the perinuclear locations and cytoplasmic regions of epithelial cells, which presumably are locations for mitochondria and endoplasmic reticulum. Bcl-2 cytoplasmic staining was only observed in 17 of the 75 (22.7%) patient tumors. Twelve of the 18 benign tumors and 5 of the 10 borderline tumors demonstrated bcl-2 expression. Overexpression of bcl-2 was most frequently observed in serous cystadenomas and this association was high-

ly significant (p<0.001). Malignant tumors showed no bcl-2 reactivity (Table 1, Figure 2).

Ki-67

The staining pattern of the Ki-67 antigen is shown in figure 3 and Ki-67 LI is shown in table 2. The mean Ki-67 LI in benign tumors was 14.9% (0.7-32.5%); in borderline tumors it was 22.8% (0.2-61.2%); in malign tumors it was 42.8% (0.4-84.2%). When compared with the benign tumors, Ki-67 LI was found to be significantly increased in the malign tumors. For example, Ki-67 LI was 52.7% in serous cystadenocarcinoma, 39.7% in endometrioid carcinoma and 33.2% in mucinous cystadenocarcinoma.

For all of the malignant ovarian tumors examined, high grade tumors were associated with a high mean Ki-67 LI. Among the malignant ovarian tumors examined, the mean Ki-67 LI for grade III tumors (52.7%) was the highest, whereas the Ki-67 LI was the lowest for grade I tumors (25.9%) and the index for grade II tumors was an intermediate value (49.8%).

Expression of p53 and Ki-67 LI were significantly higher in the bcl-2 negative tumors than in the bcl-2 positive ones (p<0.005).

Table 1. P53, bcl-2, Ki-67 Li (labeling index) status in benign, proliferative, and malignant ovarian surface epithelial neoplasms

Tumor type (n)	P53		Bcl-2		Ki-67
	Negative	Positive	Negative	Positive	Labeling Index
Benign (30)	30	0	18	12	14.9%
Borderline (10)	10	0	5	5	22,8%
Malign (35)	21	14	35	0	42,8%
Grade I (14)	13	1	14	0	25.9%
Grade II (8)	4	4	8	0	49.8%
Grade III (13)	4	9	13	0	52.7%

Discussion

More than 100 cancer related genes have been discovered, several of which have been implicated in the natural history of human cancer because they consistently are found to be mutated in tumors [1,3,4]. The p53 tumor suppressor gene is the most striking example because it is mutated in approximately half of a wide spectrum of tissues [6,25,26]. The two main methods for studying p53 are sequence analysis and immunohistochemical staining. Immunohistochemistry is used to detect approximately 90% of the p53 mutations [1]. Positive p53 staining has been associated with more frequent cancer relapse and aggressive tumors [1,6,27]. Marks et al. [28] analyzed p53 staining in ovarian tissue and found positive staining in 50% of malignant tumors but no staining in benign tissue. The presence of mutant p53 protein has been significantly associated with high histological grade. p53 gene mutations are most typically observed in ovarian cancer patients with stage III or IV disease and appear at a lower frequency in the early stages of the disease [1]. Wen [29] noted that positive staining was related to stage. Michael et al. [3] noted that the rate of p53 overexpression was 5%, 17%, 57%, 67% for stage I-IV, respectively (p<0.002).

The relationship between p53 mutation or p53 protein expression and the histopathological subtype in ovarian carcinomas is still controversial [6]. The highest incidence of p53 positivity was reported in serous adenocarcinomas (61%) by Milner et al [30] and by Gotlier and Berek [31]; whereas in other studies, no significant relationship between histopathological subtype and p53 expression could be found [1,6,26]. The lowest rate of p53 expression was found in endometroid carcinoma. In our series, no significant relationship between histopathological subtype and p53 expression could be found. The P53 gene is not important in the pathogenesis of borderline ovarian tumors [2]. In a study of 79 LMP ovarian tumors, Kupryjanczyk et al. [32] 40 of the tumors were observed to be immunoreactive for anti-p53 in 14% of the tumors with no significant differences among various histological variants. Michael [1] demonstrated that 10.5% of borderline ovarian tumors and 1 of 48 ovarian cancers presented in a study by Wertheim et al. [29] showed anti-p53 reactivity. In this series, none of the ten proliferative tumors showed reactivity. Therefore, immunohistochemistry is a good screening method that can be used to predict malignant versus proliferative tumors. Expression of bcl-2 has been correlated with improved survival in ovarian cancer [8]. The strongest bcl-2 immunoreactivity was detected in benign and borderline tumors, whereas malignant tumors showed moderate immunoreactivity [2,33,34]. Bcl-2 expression has been associated with the histopathological subtype, and positive staining has most frequently been observed in serous (77%) and endometroid (56%) carcinomas, but was less common in mucinous carcinomas (20%)

[6]. Diebold et al.[16] demonstrated that intense bcl-2 expression is associated with a low tumor grade in a study of 118 patients with ovarian cancer in FIGO stages I-IV and it is seen most often in endometroid carcinomas [32]. Among the malignant tumors, the strongest bcl-2 immunoreactivity was detected in grade I tumors, whereas grade II and III malignant tumors showed moderate immunoreactivities [2]. The protein expression patterns of p53 and bcl-2 negatively correlated during ovarian carcinogenesis. Chan et al.[2] identified a p53-regulating domain present in the 5′untranslated region of bcl-2 gene that was able to inhibit bcl-2 expression and showed that downregulation of bcl-2 expression may be a result of the inhibitory effect of p53 expression on bcl-2 during ovarian carcinogenesis [2]. In our series, a significantly negative relationship between bcl-2 and p53 expression was found (p<0.005).

The proliferation index has been correlated with prognosis and other clinicopathological features in a number of human malignancies [18-20]. The tumor proliferative fraction of ovarian carcinomas has been investigated immunohistochemically by means of antibodies that recognize the nuclear antigen Ki-67 expressed in proliferating cells. Expression of the Ki-67 proliferation marker, which detects all phases of the cell cycle except G0, is known to predict disease outcome in many human malignancies [20]. Multivariate analysis revealed a significant relationship between high Ki-67 immunostaining in ovarian neoplasms and disease-free survival and also found that high proliferation was associated with poor prognosis for ovarian cancer in both univariate and multivariate analyses [5,23]. However, no association was found with age, FIGO stage, or tumor grade [21]. At the same time, Ki-67 and p53 have been reported to be used in a parallel manner [35]. We also confirmed this finding. In our study, there was a statistically significant correlation between the expression of p53 and Ki-67 LI (P<0.005).

Although the staining pattern for p53, bcl-2, and Ki-67 in predicting malignant versus borderline tumors is statistically significant, light microscopy remains the best method to evaluate these types of lesions. In difficult cases, such a panel of markers (or one similar to this) may be helpful in the diagnosis and may further define the biologic potential of a specific tumor. Also, additional studies of borderline lesions using multiple tumor markers with follow-up data and histopathological correlation are warranted to further define the true biologic behavior of these tumors.been shown [16,17]. For smaller vessels (e.g., coronary or cerebral arteries) imaging of the lumen, neointimal hyperplasia or in-stent re-stenosis is more challenging.

Conflict interest statement The authors declare that they have no conflict of interest to the publication of this article.

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