Expression of p53, p27 and Jab1 protein in epithelial ovarian tumors

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Summary

Objective: The study aimed to investigate expression of p53, p27 and Jun activation domain-binding protein 1 (Jab1) proteins in epithelial ovarian tumors and the values of these factors as discriminating markers for the transformation of borderline tumors to cancers. *Methods:* Forty-seven cases of paraffin-embedded tissues of epithelial ovarian tumors including 22 cases of benign ovarian tumors, nine cases of borderline tumors, and 16 cases of invasive cancers were used to evaluate expression of p53, p27 and Jab1 proteins by immunohistochemical methods. *Results:* p53 protein was expressed in 13.6% of the benign tumors, 44.4% of the borderline tumors and 62.5% of the malignant tumors and p27 protein was expressed in 95.5% of the benign tumors, 66.7% of the borderline tumors, and 37.5% of the malignant tumors. Expression of Jab1 protein was observed in 22.7% of the benign tumors, 77.8% of the borderline tumors and 62.5% of the malignant tumors. Expressions of p53, p27 and Jab1 proteins in malignant tumors were all higher than in benign tumors and the expression of p27 protein in malignant tumors was lower than in benign tumors ($p < 0.05$). Expression of Jab1 protein in borderline tumors was significantly higher than in benign tumors ($p < 0.05$). *Conclusions:* Expression of p53, p27 and Jab1 proteins can be used to discriminate between benign and malignant tumors in epithelial ovarian tumors.

Key words: p53; p27; Jab1; Borderline tumor.

Introduction

Epithelial ovarian tumors are classified as benign tumors, borderline tumors, and malignant tumors according to the histological characteristics. Borderline ovarian tumors, which have the characteristics of the intermediate stage between benign and malignancy, were first described by the Taylor in 1929 [1] and named as 'borderline malignancy' by the World Health Organization (WHO) and International Federation of Gynecology and Obstetrics in the early 1970s [2].

If there are pushing borders, they are borderline tumors, and if destructive invasion, malignant tumors [3].

The prognoses of borderline tumors are known to be good. However, the prognoses of some borderline ovarian tumors are reported to be poor, and there have been enormous efforts for the last several decades to discriminate histologically diagnosed epithelial borderline ovarian tumors due to the poor prognosis [4].

The recent development of oncogene and tumor suppressor gene examination in molecular biology is being used to find out the cause of the cancers, to estimate the prognosis of the disease and to decide the treatment at a gene level. Among genes, p53 is a tumor suppressor gene which usually regulates cell growth and proliferation but when it is damaged by mutation, it loses its ability to suppress oncogenesis and plays as an oncogene [5, 6].

Another tumor suppressor gene, p27, halts cell divisions by combining with cyclin E/cyclin-dependent kinase (CDK)2 complex and suppressing them, and eventually blocking the progression from the G1 phase to S phase in a cell cycle [7]. The loss of p27 protein means the loss of ability to suppress mitosis of cancerous cells and it is thought to be related to rapid growth of cancer cells. p27 protein is also known to be involved in cellular adhesion and the loss of p27 protein is expected to weaken cellular adhesion and make the metastasis of cancerous cells easier.

In addition to p27 protein, Jun activation domain-binding protein 1 (Jab1) has recently been researched [8]. However, there is a lack of research about Jab1 in regards to epithelial ovarian cancer, especially borderline ovarian cancer.

For this reason, this study was carried out to investigate the outlook of the expressions of p53, p27 and Jab1 proteins in epithelial ovarian tumors through immunohistochemical staining, and the value of these factors as discriminating markers to predict the degeneration of malignancy in borderline ovarian cancers.

Materials and Methods

Forty-seven well preserved tissues from patients who had been diagnosed pathologically as having benign, borderline and malignant ovarian tumors and who underwent surgery in the University Hospital between August 2003 and December 2004.

Clinical records and histopathological investigations

Ages of each patient were recorded and each histological slide was reviewed and then classified as benign, borderline or malignant.

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Fig. 1

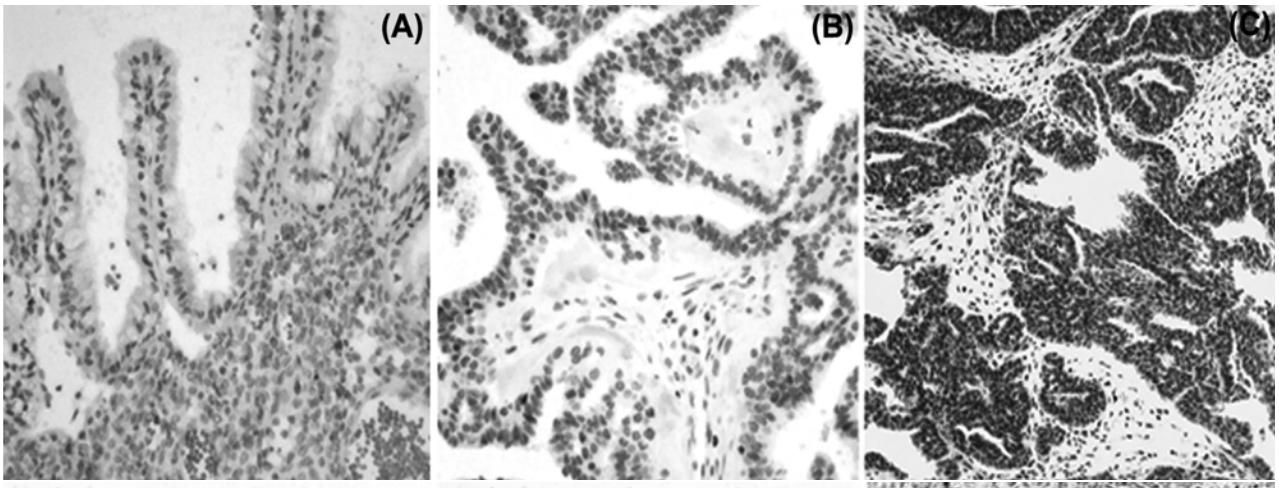

Fig. 2

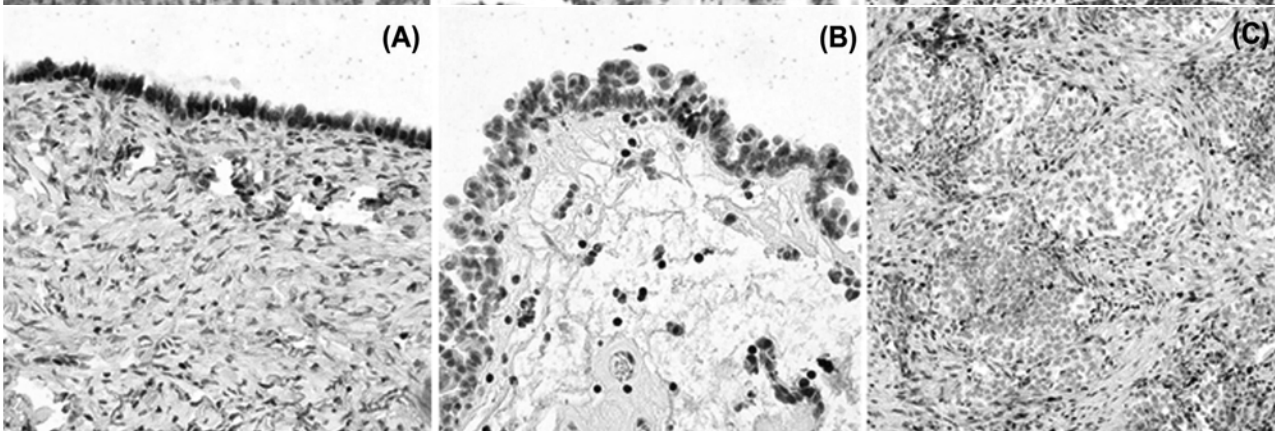

Fig. 3

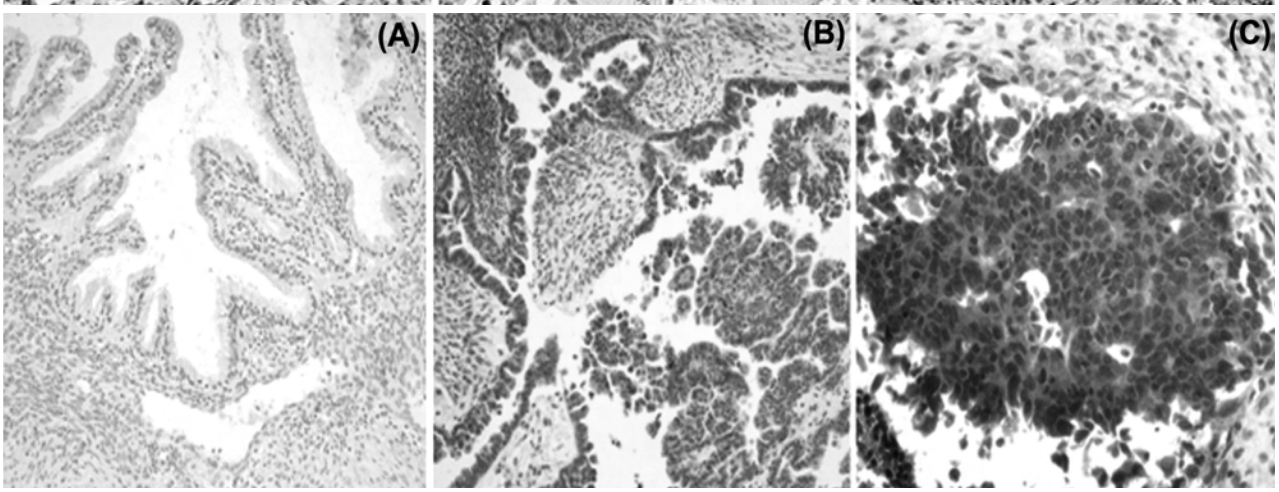

Figure 1. — Expression of p53 in immunoreactivity of benign (A), borderline (B) and malignant (C) ovarian epithelial tumors.
Figure 2. — Expression of p27 in immunoreactivity of benign (A), borderline (B) and malignant (C) ovarian epithelial tumors.
Figure 3. — Expression of Jab1 in immunoreactivity of benign (A), borderline (B) and malignant (C) ovarian epithelial tumors.

Immunohistochemical staining

Paraffin-embedded tissues of the selected patients were cut into 4-5 μm sections, dehydrated three times for 5 min, rehydrated with 100% alcohol, 90%, 75% and 50% of ethanol for 2 min. and rinsed. Afterwards, the sections were processed with 0.3% of hydrogen peroxide-methanol for 10 min to block endogenous peroxidase within the sections, washed with water, washed again with 50 mM Tris of buffered saline (TBS, pH 7.5), processed with goat serum for 30 min to block non-specific binding sites of the tissue and the remaining solution was removed. Then, the primary antibodies, p53 (DAKO, Carpinteria, CA, USA), p27 (DAKO, Carpinteria, CA, USA) and Jab1 (Spring Bioscience, Fremont, CA, USA) were applied for two hours at room temperature. After the primary antibody application, the tissue was washed with TBS for 5 min three

times and the biotin-binding secondary antibody (1:300; Zymed Co., San Francisco, CA, USA) was applied for 20 min and then stained by the avidin-biotin complex method. Three-amino-9-ethylcarbazole (AEC), the color coupler, was used and counter staining was done with Mayer's hematoxylin.

The results of p27, Jab1 and p53 protein immunohistochemical staining were determened through zoomed microscopy images (200 x). If dark brown color stained nuclei were more than 10%, the result was considered to be positive and if less than 10%, it was considered to be negative.

Statistical analysis

SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL., USA) was used for statistical analysis and the chi-square and Fisher's exact tests were carried out afterwards; a p value less than 0.05, was considered as statistically significant.

Results

Cliniopathological findings

There were 22 cases of benign tumors, nine cases of borderline tumors and 16 cases of malignant tumors among 47 epithelial ovarian tumors. There were eight cases of serous tumors, 13 cases of mucinous tumors and a case of serous fibroma in benign tumors, and there were six cases of serous tumors and three cases of mucinous tumors in borderline tumors. Among malignant tumors, serous tumors were the highest in number with 13 cases and there was also one case of mucinous tumor and one case of clear cell metastatic adenocarcinoma. In terms of age distribution, the mean age of patients with benign tumors was 42.3 ± 5.5 years old, patients with borderline tumor 51.1 ± 6.8 years old, and for patients with malignant tumors it was 56.2 ± 2.1 years old.

Expression of p53 in epithelial ovarian tumors

Among 22 cases, the expression of p53 was shown in 13.6% of the benign tumors (3 cases), in 44.4% of the borderline tumors (4 cases) and in 62.5% of the malignant tumors (10 cases) which shows that the expression rate is significantly higher in malignant tumors ($p < 0.05$). There was no statistically significant difference between borderline tumors and malignant tumors or between borderline tumors and benign tumors (Table 1).

Expression of p27 in epithelial ovarian tumors

Expression of p27 protein was shown in 95.5% of the benign tumors (21 cases). In comparison, 66.7% and 37.5% were shown in the borderline tumors and malignant tumors, respectively, which demonstrates that the expression rate was significantly higher in benign tumors compared to malignant tumors ($p < 0.05$) but there was no statistically significant difference between borderline tumors and malignant tumors or between borderline tumors and benign tumors (Table 1).

Expression of Jab1 protein in epithelial ovarian tumors

Among 47 cases, the expression of Jab1 protein was shown in 77.8% of the borderline tumors (7 cases out of 9 cases) which was not significantly different from the expression rate of malignant tumors which was 62.5%. However it was significantly different from the expression rate of benign tumors which was 22.7% ($p < 0.05$). Also, the expression rate was significantly higher in malignant tumors compared to benign tumors ($p < 0.05$) (Table 1).

Correlation of the expressions of each protein

Among the 17 cases that expressed p53, 63.6% of them (14 cases) also expressed Jab1 protein and among the 30 cases that did not express p53 protein, 88.0% (22 cases) also did not express Jab1 protein either, which shows that there was a correlation between them ($p = 0.05$) (Table 2).

On the other hand, among 22 cases with expression of Jab1, 76.9% (10 cases) showed loss of p27 and among 24 cases without expression of Jab1, 63.6% (21 cases) still showed expression of p27, suggesting that there was a significant correlation between the expression of Jab1 protein and loss of p27 ($p < 0.05$) (Table 3). Also, among 33 cases that expressed p27 protein, 86.2% (25 cases)

Table 1. — *Expression of p53, p27, Jab1 in epithelial ovarian tumors.*

	p53		p27		Jab1	
	–	+	–	+	–	+
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Benign	86.4 (19)	13.6 (3)	4.5 (1)	95.5 (21)	77.3 (17)	22.7 (5)
Borderline	55.6 (5)	44.4 (4)	33.3 (3)	66.7 (6)	22.2 (2)	77.8 (7)
invasive	37.5 (6)	62.5 (10)	62.5 (10)	37.5 (6)	37.5 (6)	62.5 (10)
p value*	0.150		0.063		0.012	
p value**	0.434		0.400		0.661	
p value***	0.004		0.000		0.020	

* benign vs borderline
** borderline vs invasive
*** benign vs invasive

Table 2. — *Comparison of expression of p53 and Jab1 in epithelial ovarian tumors.*

		p53	
		(–) % (n = 30)	(+) % (n = 17)
Jab1	(–) (n = 25)	88.0 (22)	12.0 (3)
	(+) (n = 22)	36.4 (8)	63.6 (14)
p value		0.000	

Table 3. — *Comparison of expression of p53 and Jab1 in epithelial ovarian tumors.*

		Jab1	
		(–) % (n = 24)	(+) % (n = 22)
p27	(–) (n = 13)	23.1 (3)	76.9 (10)
	(+) (n = 33)	63.6 (21)	36.4 (12)
p value		0.021	

Table 4. — *Comparison of expression of p53 and p27 in epithelial ovarian tumors.*

		p27	
		(–) % (n = 13)	(+) % (n = 33)
p53	(–) (n = 29)	13.8 (4)	86.2 (25)
	(+) (n = 17)	52.9 (9)	47.1 (8)
p value		0.007	

showed loss of p53 protein and among 13 cases that did not express p27, 52.9% (9 cases) expressed p53, which again suggests that there was a significant correlation between the loss of p27 protein and expression of p53 ($p < 0.05$) (Table 4).

In other words, as the expression of p53 increased, the expression of Jab1 protein increased, but the expression of Jab1 was significantly related to loss of p27 and the expression of p53 was also significantly related to loss of p27.

Discussion

The histological diagnostic criteria of borderline ovarian cancer are the stroma, without invasion, which is differentiated from malignancy. Borderline ovarian cancer rarely progresses to malignancy. There is a lack of studies on the characteristics of prognostic factors in borderline ovarian tumors. It is important to discriminate between benign and malignancy in the progress of borderline ovarian cancer [4]. We intended to discriminate between borderline tumors and malignant tumors in epithelial ovarian cancers based on the genes p53, p27 and Jab1. The expression of p53 proteins and p27 proteins between benign tumors and borderline tumors was not significantly different but, the expression of Jab1 protein was significantly higher in borderline tumors than benign.

p53 is the most commonly found gene in genetic mutations of several human cancers and the frequency is known to be about 50% [9]. Therefore, it has been extensively researched and the normal form of p53 is known as a tumor suppressor gene. Abnormal mutated p53 protein accumulates in the nucleus, and can be detected on immunohistochemical staining. It is commonly known that the overexpression of p53 protein is related to prognosis of ovarian malignancies but, there are reports saying that expression of p53 is not high in borderline ovarian tumors [10] nor does it have characteristics of benign or early borderline ovarian tumors [11]. There are also findings that the expression of p53 is higher in borderline ovarian tumors than benign ovarian tumors, and it is claimed that the expression of abnormal p53 could be an early phenomenon that contributes to malignant changes in some borderline ovarian tumors, endometriosis and other precancerous lesions [12, 13].

The overexpression rate of abnormal p53 was significantly higher in ovarian malignancies than benign ones in this study, but there were no significant differences between benign tumors and borderline tumors or between malignant tumors and borderline tumors.

p27 protein is located at 12p13 as a powerful tumor suppressor gene and directly suppresses the enzyme of cyclin-CDK complexes and halts cell cycles by suppressing the progression from the G1 phase to S phase [14, 15].

There are reports suggesting that the lower the expression of p27 protein is, the higher the resistance against chemotherapy drugs becomes in epithelial ovarian cancers [16] and that the loss of p27 protein is related to malignant findings and poor prognosis in lung cancers, breast cancers, prostate cancers, oral cancers, brain tumors, lymphomas and colon cancers as well as ovarian cancers [17-24].

On the other hand, Jab1 is a protein that induces cell proliferation by strengthening the activation of c-Jun gene as an activator protein 1 coactivator [25].

There is a negative correlation between the expression of Jab1 protein and expression of p27 protein in epithelial ovarian cancers and it is estimated that it is related to the progress of the cancers and their prognosis [26]. This tendency, the loss of p27 increased significantly statistically as the expression of Jab1 protein increased, is also shown in this study. Moreover, the expression of p53 increased significantly when the expression of Jab1 was high ($p < 0.05$) and there was a statistically significant correlation between the loss of p27 and expression of p53 ($p < 0.05$). Whereas the expression of p27 was shown in 95.5% of benign tumors, it decreased to 66.7% in borderline tumors and 37.5% in malignant tumors, suggesting poorer prognosis as there was more loss of p27 protein expression. While the expression of Jab1 was shown in 22.7% of benign tumors, it was shown in 77.8% of borderline tumors and 62.5% of malignant tumors suggesting poorer prognosis as the expression of Jab1 protein increased. Thus the expression of p53, the loss of p27 expression and the expression of Jab1 protein suggest poor prognosis.

The expression of p53 and Jab1 protein and loss of p27 protein expression were shown significantly more in malignant tumors than benign tumors in this study. The expression of these proteins on immunohistochemical staining can be valuable in the judgment of prognosis as suggested in the studies of Sui *et al*. [26] or Patah *et al*. [15]. However, it is known that expression rates of p53 in epithelial ovarian cancers are high but are irrelevant to their prognosis [27, 28].

The expressions of these proteins were not significantly different between borderline tumors and malignant tumors, therefore, studies with bigger samples should be carried out.

p53, p27 and Jab1 are all thought to be valuable factors to discriminate between benign and malignant tumors and the expression of Jab1 protein is assumed to be involved in the malignant degeneration of ovarian tumors. The expression of p53, Jab1 protein and the loss of p25 protein expression were linked together. Borderline tumors are in continuity with malignant tumors, therefore, further research should be considered regarding the oncogenesis of ovarian tumors.

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