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Protein expressions of AIB1, p53 and Bcl-2 in epithelial ovarian cancer and their correlations with the clinical pathological features and prognosis

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Abstract. – OBJECTIVE: To investigate the protein expressions of steroid receptor coactivator amplified in breast cancer 1 (AIB1), apoptosis-related protein p53 and B-cell lymphoma-2 (Bcl-2) in epithelial ovarian cancer; to analyze the correlations among the expressions of these three proteins; to explore their correlations with the clinical pathological features and prognosis.

MATERIALS AND METHODS: Immunohistochemistry streptavidin-peroxidase (IHC-SP) method was performed to detect the positive protein expressions of AIB1, Bcl-2, and p53 in the pathological sections of normal ovarian tissues, benign ovarian epithelial tumor, and epithelial ovarian cancer, thereby analyzing the protein expression rates of these genes in different pathological stages, lymphatic metastasis and postoperative recurrent ovarian cancer, and carrying out the correlation analysis of these three proteins.

RESULTS: Positive protein expressions of AIB1, Bcl-2, and p53 were identified in the epithelial ovarian cancer tissues, and with an increase in the tumor staging of ovarian cancer, we found that the positive protein expression rate was gradually augmented. Particularly in ovarian cancer with lymphatic metastasis and postoperative recurrence, the positive expression rate was almost 100%. The protein expression of AIB1 was positively correlated with those of the p53 and Bcl-2.

CONCLUSIONS: We showed that positive expressions of AIB1, Bcl-2, and p53 in ovarian cancer are closely correlated with the pathological staging, metastasis, and recurrence, and the positive protein expression suggests a poor prognosis.

Key Words

Ovarian cancer, AIB1, Bcl-2, p53, Lymphatic metastasis, Clinical staging.

Introduction

Ovarian cancer, one of the frequent malignant tumors in gynecology, accounts for 3% of the incidence in all gynecological tumors. It mainly occurs in the postmenopausal women with a low 5-year survival rate of only 30%[1]. Among the ovarian cancer patients, over 90% of the cases originate from the malignant transformation of ovarian epithelial cells, which, according to typical histological heterogeneity, can be divided into 5 subtypes, i.e., serous type, mucous type, endometrioid type, clear-cell type, and transitional-cell type. These subtypes can be further divided into benign, junctional, and malignant types[2].

Various oncogenes and tumor suppressors are involved in the development and progression of ovarian cancer. AIB1, a steroid receptor coactivator amplified in breast cancer 1, is a kind of transcriptional coactivator acting as an oncogene[3]. Research[4-8] has shown that AIB1 is associated with the development and progression of various tumors, including breast cancer, colorectal cancer, gastric, pancreatic and esophageal squamous cancer. Anzick et al[9] detected AIB1 expression in ovarian cancer and found that AIB1 may be involved in the development and progression of this cancer through the signal pathway of the estrogen receptor. Genes modulating cell apoptosis are involved in the development of ovarian cancer, e.g., Bcl-2 and p53[10,11]. B-cell lymphoma-2 (Bcl-2) can inhibit cell apoptosis. The overexpression of Bcl-2 will lead to a decrease in cell apoptosis, but has no effects on cell proliferation, resulting in the proliferative lesion or tumorigenesis[12,13]. Besides, Bcl-2 can block the apoptosis of tumor cells caused by various chemotherapeutics or radiotherapy, and give rise to the drug-resistance of ovarian cancer cells through inhibiting the variations in nucleotide caused by chemotherapeutics in tumor cells[14]. However, metformin can be used in the treatment of ovarian cancer through inhibiting the expression of Bcl-2, thereby inducing cell apoptosis[15]. p53, a classic tumor suppressor, can initiate the mitochondrial apoptotic pathway through regulating the expressions of apoptosis-related genes to upregulate the pro-apoptosis

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genes like Bcl-2-associated X protein (Bax) and apoptotic protease activating factor 1 (Apaf-1), and inhibit the expression of Bcl-2, thereby facilitating the apoptosis of tumor cells[16]. However, the mutated p53, commonly found in the carcinoma cells, is one of the indicators of malignancy and poor prognosis of tumors[17]. Bali et al[18] showed that the high expression of p53 in epithelial ovarian cancer represents a poor clinical outcome.

We aimed to detect the expressions of AIB1, Bcl-2, and p53 in pathological sections of tissues collected from the epithelial ovarian cancer patients, and to investigate the correlations among the expressions of these three proteins in the epithelial ovarian cancer. Moreover, our objective was to compare the expression rate of the three proteins in tissue sections collected from menopausal or menstrual women, tumors in different clinical stages and tissue types, metastatic lymphatic tissues and postoperative recurrent tissues, in order to evaluate the prognosis of these patients.

Patients and Methods

Patients

We enrolled the female patients who were admitted to Lanzhou General Petrochemical Hospital for treatment between June 2013 and December 2016. There were A): 35 patients with diagnosis of ovarian epithelial benign tumor aged between 21 and 75 years old with an average of (43.6±21.5) years old, including 9 with serous type, 18 with mucous type, and 8 with endometrioid type, and B): 83 patients with diagnosis of epithelial ovarian cancer aged between 24 and 81 years old with an average of (41.5±26.9) years old, including 45 with serous type, 23 with mucous type, 9 with endometrioid type, and 6 with clear-cell type. Pathological sections were re-examined by two pathologists independently. All patients had not received chemotherapy or radiotherapy before surgery. Patients with epithelial ovarian cancer received cytoreductive surgery of ovarian cancer followed by cisplatin-based chemotherapy. According to the Guidelines for Staging of Gynecologic Malignancies and Clinical Practice (2001 Edition) by International Federation of Gynecology and Obstetrics (FIGO)[19], there were 16 epithelial ovarian cancer patients in Stage I, 9 in Stage II, 52 in Stage III, and 6 in Stage IV; 25 patients were in early stage of epithelial ovarian cancer (Stage I or II), and 58 in advanced stage (Stage III or IV). Additionally, normal ovarian tissues collected from 15 patients with ovarian cyst served as control. General materials of patients in the three groups were comparable. This study was approved by the Ethics Committee of Lanzhou General Petrochemical Hospital, and all participants signed the written informed consent.

Detection of Pathological Sections Via Immunohistochemistry Method

Immunohistochemistry streptavidin-peroxidase (IHC-SP) method was applied to stain the pathological sections of patients. During the surgical resection, the ovarian cancer tissues were collected from patients. For patients in the control group, the normal ovarian tissues during the resection of the cyst were collected. All tissues were embedded in paraffin and sliced into 5 μm-thick sections, then dewaxed in xylene, dehydrated in ethanol, and treated with 3% hydrogen peroxide. Thereafter, sections were blocked in serum supplemented with mouse anti-human primary antibodies of AIB1, p53, and Bcl-2 (Invitrogen, Carlsbad, CA, USA), and then incubated at 37°C for 1 h. After sections were washed using phosphate-buffered saline (PBS) three times, biotin-labeled rabbit anti-mouse secondary antibodies of immunoglobulin G (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd, Beijing, China) was added on the sections for incubation at 37°C for 1 h. Thereafter, sections were rinsed three times with PBS, and the horseradish peroxidase-conjugated streptavidin (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd, Beijing, China) was added to bind to the biotin-labeled secondary antibody. Then, diaminobenzidine (DAB; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd, Beijing, China), the substrate of the enzyme, was added on the section for 15 min color development, followed by re-dyeing with hematoxylin, dehydration, decoloring, and mounting with neutral balsam.

Observation Indexes

Evaluation criteria for positive protein expressions of AIB1, p53, and Bcl-2: Among the cells with positive expressions exhibiting pale brown particles in the cytoplasm or nucleus in epithelial cells, we selected 100 cells in each section randomly, and if there were 5 or more positive cells in one section, the section would be categorized with the percentage of positive cells, in which section with positive cells less than 5% was regarded as negative (-), 5 to 50% as +, 50 to 75% as ++, and 75 to 100% as +++; sections with the percentage of positive cells not less than 5% were considered as positive.

Statistical Analysis

Statistical Product and Service Solutions (SPSS; IBM Inc, Armonk, NY, USA) was used for statistical analysis, in which the positive expression rates of AIB1, p53, and Bcl-2 in different pathological sections were compared via chi-square test, and the correlations among the protein expressions of AIB1, p53, and Bcl-2 were analyzed with Pearson correlation analysis. $p<0.05$ suggested that the difference had statistical significance.

Results

Expressions of AIB1 in Ovarian Tissues

Among 15 normal ovarian tissues, there was 1 case (6.7%) with positive expression of AIB1; while in 35 cases of epithelial ovarian benign tumor, there were 8 (22.9%) with positive expression of AIB1. In 83 cases of epithelial ovarian cancer, there were 57 (68.7%) with positive expression of AIB1. In chi-square test of expressions in the above three groups, the results showed $\chi^2= 42.63$ and $p=0.000$, suggesting that the positive expression rate of AIB1 in epithelial ovarian cancer tissues was significantly higher than those in the epithelial ovarian benign tumors and normal ovarian tissues. However, the expression rate in the epithelial ovarian benign tumors was significantly higher than that in the normal ovarian tissues ($p<0.01$). We did not identify any positive expression of Bcl-2 and p53 in normal ovarian tissues, and the expression rates of these genes in the epithelial ovarian cancer tissues were significantly higher than those in the epithelial ovarian benign tumors ($p<0.01$). See Table I for details.

Protein Expressions of AIB1, p53, and Bcl-2 in Epithelial Ovarian Cancer

In the epithelial ovarian cancer tissues of menopause patients, we found that the positive expression rates of p53 and Bcl-2 proteins were significantly higher than those in the non-menopausal patients ($p<0.05$). Moreover, the positive protein expression rate of AIB1 was higher than the menopause patients but without any statistically significant difference. With an increase in the clinical staging of epithelial ovarian cancer, the positive protein expressions of AIB1, p53, and Bcl-2 were also gradually augmented. Due to the limited number of cases of ovarian cancer Stage I, II, and IV, we could hardly identify the statistically significant differences in positive expression rates in different stages. Among patients with lymphatic metastasis and postoperative recurrence, we observed that the positive expression rates of these three proteins were significantly higher than those in the patients without lymphatic metastasis or postoperative recurrence ($p<0.05$; Table II).

Analysis of Correlations of AIB1 Protein Expression With Those of p53 and Bcl-2

Among 83 epithelial ovarian cancer patients, there were 57 patients with AIB1 positively correlated AIB1 expression, in which 38 patients with p53 and Bcl-2 expressions (p53: r=0.457, $p=0.006$; Bcl-2: r=0.416, $p=0.005$). Among 26 patients with lymphatic metastasis, there were 24 patients together with positive expressions of AIB1, p53, and Bcl-2. Besides, 13 epithelial ovarian cancer patients with postoperative recurrence exhibited positive expressions of AIB1, p53, and Bcl-2.

Table I. Expression rates of Bcl-2 and p53 in ovarian tissues.

	AIB1		Bcl-2		p53	
	Positive expression (n)	Positive expression rate	Positive expression (n)	Positive expression rate	Positive expression (n)	Positive expression rate
Normal ovarian tissue (n=15)	1	6.7%	0	0%	0	0%
Epithelial ovarian benign tumor (n=35)	8	22.9%*	3	8.6%	2	5.7%
Epithelial ovarian cancer (n=83)	57	68.7%*,a	52	62.7%a	45	54.2%a
		χ^2=33.15 p=0.000		χ^2=41.61 p=0.000		χ^2=34.59 p=0.000

*, $p<0.01$ vs. normal ovarian tissues. a, $p<0.01$ vs. epithelial ovarian benign tumors.

Table II. Protein expressions of AIB1, Bcl-2 and p53 in epithelial ovarian cancer and their correlations with the clinical pathological features and clinical outcome.

	No.	AIB1			Bcl-2			p53		
		Positive case (no.)	Positive rate	p	Positive case (no.)	Positive rate	p	Positive case (no.)	Positive rate	p
Menstruation										
Non-menopausal	34	20	58.8%	χ^2=2.598	15	44.1%	χ^2=16.14	19	55.9%	χ^2=4.38
Menopause	49	37	75.5%	p=0.107	42	85.7%	p=0.000	38	77.6%	p=0.036
Tissue type										
Serous	45	34	75.6%	χ^2= 6.94	30	66.7%	χ^2=0.71	31	68.9%	χ^2= 1.60
Mucous	23	15	65.2%		16	69.6%		14	60.9%	
Endometrioid	9	3	33.3%	p=0.074	6	66.7%	p=0.871	7	77.8%	p=0.660
Clear-cell	6	5	83.3%		5	83.3%		5	83.3%	
Clinical staging										
I	16	4	25.0%	χ^2=18.34	4	25.0%	χ^2=19.44	3	18.8%	χ^2=20.57
II	9	6	66.7%		6	66.7%		5	55.6%	
III	52	42	80.8%	p=0.000	41	78.8%	p=0.000	39	75.0%	p=0.000
IV	6	5	83.3%		6	100%		6	100%	
Lymphatic metastasis										
No	57	33	57.9%	χ^2=9.83	27	47.4%	χ^2=18.16	19	33.3%	χ^2=31.97
Yes	26	24	92.3%	p=0.002	25	96.2%	p=0.000	26	100%	p=0.000
Postoperative recurrence										
No	70	44	62.9%	χ^2=7.03	39	55.7%	χ^2=9.19	32	45.7%	χ^2=13.02
Yes	13	13	100%	p=0.008	13	100%	p=0.002	13	100%	p=0.000

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Discussion

Without any evident symptoms in early stage or effective diagnostic methods for early diagnosis, patients with ovarian cancer have progressed into the advanced stage at the first time of diagnosis with metastatic lesions outside the ovary[11]. Currently, surgical resection, despite its promising efficacy, can frequently lead to recurrence and metastasis in short time[20,21]. Research has shown that signal pathways mediated by estrogen and its receptors are involved in the development of ovarian cancer. AIB1, a steroid receptor coactivator and transcriptional coactivator, can participate in the estrogen-dependent transcriptional activation through the estrogen receptor[22]. AIB1 is scarcely expressed in the normal ovarian tissues, and from the benign epithelial tumor in the ovary to ovarian epithelial cancer, the expression rate of AIB1 is gradually increased. In ovarian cancer with lymphatic metastasis and postoperative recurrence, the positive expression rate of AIB1 is higher than 90%, suggesting that AIB1 expression is correlated with the poor prognosis.

In this study, we found that the expression rates of Bcl-2 and p53 in the menopause women were significantly higher than those in the non-menopausal women, which may be correlated with the age of menopause women[22]. With an elevation in clinical staging of epithelial ovarian cancer, the expression rates of Bcl-2 and p53 were also increased, and particularly in ovarian cancer with lymphatic metastasis and postoperative recurrence, could be nearly 100%, indicating that Bcl-2 and p53 proteins are closely associated with the malignancy and poor prognosis of ovarian cancer. In 24 AIB1-positive patients with lymphatic metastasis, they were also positive to Bcl-2 and p53. Furthermore, in 13 AIB1-positive patients with postoperative recurrence, they were also positive to Bcl-2 and p53, revealing that the protein expression of AIB1 is significantly correlated with those of p53 and Bcl-2. Therefore, the positive expressions of AIB1, Bcl-2, and p53 can be used to predict the poor clinical outcome of ovarian cancer patients.

Conclusions

We showed that epithelial ovarian cancer was accompanied by positive expressions of AIB1, Bcl-2, and p53, which were increased with an elevation in clinical staging of ovarian cancer. Moreover, in ovarian cancer with lymphatic metastasis or postoperative recurrence, the positive expression rates of AIB1, Bcl-2, and p53 approximated 100%. This suggests 1) that there are significant correlations among the protein expressions of AIB1, Bcl-2, and p53 in ovarian cancer, and 2) that the positive expressions of these genes achieve a poor prognosis.

Conflict of Interests:

The authors have no conflicts of interest to declare.

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