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Expression and clinical implication of Beclin1, HMGB1, p62, survivin, BRCA1 and ERCC1 in epithelial ovarian tumor tissues

L.-L. JU[1], C.Y. ZHAO[2], K.-F. YE[2], H. YANG[3], J. ZHANG[2]

[1]Medical Faculty of Kunming University of Science and Technology, Chenggong District, Kunming City, Yunnan, China
[2]Gynaecology Department of First People's Hospital of Yunnan Province, Xishan District, Kunming City, Yunnan, China
[3]Pathology Department of First People's Hospital of Yunnan Province, Xishan District, Kunming City, Yunnan, China

Abstract. – OBJECTIVE: The aim of the present study is to investigate the differential expression of Beclin1, HMGB1, p62, survivin, ERCC1 and BRCA1 protein in epithelial ovarian cancer (EOC) and to evaluate the relationship between autophagy and platinum resistance of EOC patients during platinum-based chemotherapy with the protein expression.

PATIENTS AND METHODS: Expression of Beclin1, HMGB1, p62, survivin, ERCC1 and BRCA1 were detected with immunohistochemistry in 60 patients, including 39 with epithelial ovarian cancer (EOC), 13 benign epithelial ovarian tumor tissue (BET) and 8 borderline ovarian tumor tissue.

RESULTS: Beclin, p62 and ERCC1 expression was significantly higher in the EOC than the BET ($p<0.05$). No statistical significance was detected with HMGB1 or survivin expression among BET, borderline tumor and EOC ($p>0.05$). BRCA1 expression was lower in EOC than BET ($p<0.05$). The expression of Beclin, p62 and survivin significantly correlated with FIGO stage ($p<0.05$), while the expression of HMGB1 correlated with pathological type. For platinum-sensitive EOC patients, positive expression of Beclin1 and BRCA1 was lower, and positive P62 expression was higher than in platinum-resistant patients ($p<0.05$). BRCA1 expression was negatively correlated with Beclin1 and p62 expression ($p<0.05$)

CONCLUSIONS: Inhibition of expression of beclin1 may suppress autophagy to enhance the efficiency of platinum-sensitive ovarian cancer. HMGB1, survivin and p62 are implicated in the development of ovarian cancer. ERCC1 might be a potential predictive marker for neoadjuvant treatment in the early stage of ovarian cancer, and BRCA1, Beclin1 and p62 as a biomarker to predict platinum resistance and prognosis of epithelial ovarian cancer.

Key words:
Epithelial ovarian tumor tissues, Autophagy, Beclin1, HMGB1, p62, Survivin, ERCC1, BRCA1, Platinum-sensitive, Platinum-resistant.

Introduction

Ovarian cancer ranks fifth in cancer deaths among women in the world, accounting for more deaths than any other cancer of the female reproductive system. A woman's risk of getting ovarian cancer during her lifetime is about 1 in 75. This cancer mainly develops in older women. In China, the morbidity of ovarian cancer is the third next to cervical cancer and carcinoma of the corpus uteri, yet its mortality is the first of all gynecological tumors. The symptoms of ovarian cancer are frequently absent in early stages. In most cases, symptoms exist for several months before being recognized and diagnosed, or they may initially be misdiagnosed as a condition such as irritable bowel syndrome. The early stages of ovarian cancer tend to be painless unless the growing mass causes ovarian torsion. The subtle progress of condition makes 70%-80% ovarian cancers already in an advanced stage when first diagnosed.

Currently, the routine treatment for ovarian cancer includes the cytoreductive surgery (CRS) with taxol and platinum (cisplatin or carboplatin) combined with chemotherapy. However, poor prognosis is not rare due to the development of drug-resistance, and studies about drug resistance provided us critical information in order to improve therapy and prolong survival rate.

Autophagy, hallmarked by the formation of double-membrane bound organelles known as autophagosomes, is a lysosome-dependent pathway for protein degradation[1]. Data from recent studies are beginning to unveil the paradoxical nature of autophagy in cell-fate decision machinery. On one hand, autophagy contributes to cell death and inhibits inflammation and promotes genomic stability; on the other hand, autophagy may also act as a

Corresponding Author: Jie Zhang, MD; e-mail: zjzhangjie@yeah.net

pro-survival mechanism to protect cancer cells from various forms of cellular stress, such as hypoxia and malnutrition. Previous studies have confirmed that autophagy serves as critical link contributing to the development of drug resistance.

Beclin1 is a protein encoded by ì the BECN1 gene[2] and is required in the process of autophagosome formation and, thus, contribute to tumor formation through deregulation of autophagy[3]. Beclin1 expression levels tend to rise in the process of autophagy. Further studies inferred that BECN1 expression loss weakens autophagy and promotes the occurrence of the tumor.

High-mobility group box 1 (HMGB1), a nuclear and extracellular protein, is implicated in the development and progression of some types of cancers. Its expression can be enhanced by stimuli such as poor nutrition, hypoxia, and chemotherapy drugs[4]. HMGB1 moves from nuclear to the cytoplasm to regulate cell autophagy pathway under a specific condition such as high level of reactive oxygen species and inhibit apoptosis. HMGB1 is a newly identified gene overexpressed in ovarian cancer and associated with poor clinicopathologic features[5].

p62, a multifunctional protein, plays a vital role in starting cell survival signals, including cell proliferation, differentiation and inducing apoptosis resistance gene. p62 acts as a receptor or adaptor for autophagic degradation of ubiquitinated proteins, and plays an important role in preventing ER stress-induced apoptosis, leading to cisplatin resistance. p62 upregulation is commonly observed in human tumors and contributes directly to tumorigenesis likely by perturbing the signal transduction adaptor function of p62-controlling pathways critical for oncogenesis[6].

Survivin is a member of the inhibitor of apoptosis proteins family with the double function of inhibiting cell apoptosis and regulating cell proliferation[7,8]. Abnormal expression of survivin in the tumor is closely related to the tumor chemotherapy drug resistance and relapse rate. High survivin expression is observed in tumor tissues resistant to chemotherapy and is associated with higher recurrence rate and poor prognosis.

Breast cancer susceptibility gene 1 (BRCA1) is related to both platinum and taxane resistance[9]. As a tumor suppressor gene, it is mainly responsible for double-stranded DNA damage repair[10]. Normal BRCA1 gene encodes the BRCA1 protein, which inhibits tumor development, while congenital or inherited mutation impedes such effect and thus contributes to tumorigenesis.

Excision repair cross-complementation group1 enzyme (ERCC1) is a component in nucleotide excision repair (NER) pathway and reflects NER repair activity level[11]. ERCC1 is human DNA repair gene that is associated with *in vitro* resistance to selected DNA-damaging agents, and ERCC1 expression levels in human tumor tissue may have a role in clinical resistance to platinum compounds. ERCC1 overexpression can identify and cut DNA damage parts, making the damaged DNA repair rapidly and, thus, leading to tumor cells cisplatin resistance[12].

In this study, several regulatory proteins implicated in autophagy, specifically Beclin1, HMGB1, p62 and cisplatin-resistant associated proteins Survivin, ERCC1 and BRCA1, were comparatively analyzed with ovarian cancer tissue samples collected from platinum drug–sensitive and drug-resistant tumor patients. The differential expression of Beclin1, HMGB1, p62, survivin, ERCC1 and BRCA1 in epithelial ovarian cancer (EOC) were evaluated as well as the relationship between autophagy and platinum resistance in ovarian cancer during platinum-based chemotherapy.

Patients and Methods

Patients

In this study, a total of 60 patients diagnosed with epithelial ovarian tumor were recruited between June 2011 and June 2014 and tumor tissue sample were collected by investigators in pathology department of Yunnan First People's Hospital. The age of the patients ranged from 25 to 72 years, with the median age of 48 years. All patients were native to radiotherapy and chemotherapy before study and necessary medical history were collected. The diagnosis was based on clinical symptoms and histopathological examination. Among the 60 subjects, 39 were diagnosed with epithelial ovarian cancer (EOC), 13 with benign epithelial ovarian tumor tissue (BET), and 8 with borderline ovarian tumor tissue. The 39 cases of EOC included 23 serous cystadenocarcinoma, 4 mucinous cystadenocarcinoma, 5 endometrioid carcinoma, 5 clear cell carcinoma, and 2 mixed carcinoma. According to the histological grading, 8 cases were of high differentiation and 31 cases were of moderate or low differentiation. According to Federation International of Gynecology and Obstetrics (FIGO, 2012), 19 cases were of Stage I-II and 20 cases

were of Stage III-IV. Based on the Response Evaluation Criteria in Solid Tumors (RECIST) and National Comprehensive Cancer Network (NCCN, 2011) Clinical Practice Guidelines in Oncology-Ovarian Cancer Guideline, 26 cases were categorized as platinum-sensitive and 13 as platinum-resistant among the 39 epithelial ovarian carcinoma patients.

Immunohistochemical Analysis

Paraffin-embedded tissues were cut into 4-μm sections, fixed, dewaxed and rehydrated. For antigen retrieval, slides for p62, Survivin and beclin-1 were immersed in citrate buffer (pH 6.0) and heated in a pressure cooker for 3 min. Similarly, slides for BRCA1, ERCC1 and HMGB1 were immersed with EDTA (pH 9.0) and heated in a pressure cooker for 3 min. Then, all slides were cooled to room temperature. Endogenous peroxidase was blocked with 3% H_2O_2 for 10 min, followed with rinsing for three times with phosphate buffered saline (PBS). Sections were incubated with a primary antibody against p62, survivin, beclin-1, BRCA1, ERCC1 or HMGB1 overnight at 4°C. After incubated with a labeled polymer for 30 min at 37°C, slides were rinsed three times with PBS. Moreover, 3, 3'-diaminobenzidine (DAB) was used as the chromogen, and sections were counterstained with hematoxylin for 3 s. Finally, the sections were mounted with coverslips.

Immunohistochemical staining in the cytoplasm or nucleus, cytoplasm or nuclei appearing brown are judged as positive cells. 10 views were selected for each slide at high magnification and 200 tumor cells were counted within each view for scoring. The positive expression percentage on each slide was scored as: 1 ≤ 25%, 2 = 25 to <50%, 3 = 50 to < 75% and 4 ≥ 75%. Staining intensity on each slide was scored as: 0 = no staining, 1 = light brown yellow, 2 = brown and 3 = dark brown staining. The positive expression percentage and degree of staining scores provided a final score as follows: 0 to 3 point was negative (−), and >4 points was positive (+). These results were judged by two pathologists blind to diagnosis.

Statistical Analysis

All statistical analyses were performed using the IBM SPSS Statistics, version 19.0. (SPSS Inc., Chicago, IL, USA) Protein expression in different tissues was assessed using the Pearson $\chi 2$ test of association. Correlation between immunostaining and clinicopathological characteristics was assessed using the Fisher's exact test. Protein expression in the platinum-sensitive and platinum-resistant of EOC patients was also assessed using the Fisher's exact test. The correlation analysis was performed using Spearman's correlation test. All *p*-values were 2-sided and $p<0.05$ was considered statistically significant.

RESULTS

Expression of Proteins in the Tissues of BET, Borderline Ovarian Tumors and the EOC

Beclin1 was predominantly observed in cytoplasm and nuclei (Figure 1). The positive rates of Beclin1 in the tissues of the BET, borderline ovarian tumors and the EOC were 15.4%, 50.0%, and 56.4%, respectively (Table I), among which the detection rate of EOC was significantly higher than BET ($p<0.05$). HMGB1 was predominantly stained in the nuclei and a weakly stained in the cytoplasm (Figure 2). The positive rates of HMGB1 in the tissues of the BET, borderline ovarian tumors and the EOC were 92.3%, 100.0%, and 97.4%, respectively (Table I), yet no statistical significance was detected when comparing among groups. p62 was predominantly stained in the cytoplasm and nuclei (Figure 3).

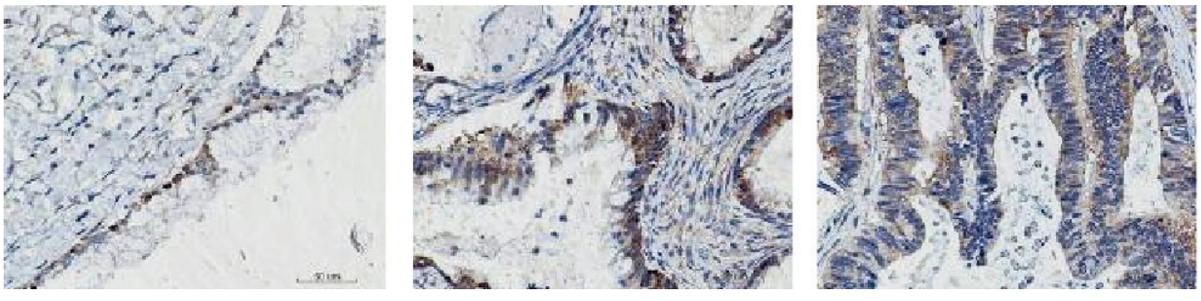

Figure 1. Beclin1 immunoreactivity was predominantly observed in cytoplasm and nuclei (Magnification x 400).

Table I. Exspression of Beclin-1 and HMGB1 protein in the tissues of the BET, the borderline ovarian tumors and the EOC.

Pathological type	N	Beclin-1 expression				HMGB1 expression			
		Negative N (%)	Positive N (%)	χ^2	*p*-value	Negative N (%)	Positive N (%)	χ^2	*p*-value
Benign epithelial ovarian tumor tissues	13	11 (84.6%)	2 (15.4%)	6.635	0.036	1 (7.7%)	12 (92.3%)	1.114	0.573
Borderline ovarian tumors tissue	8	4 (50.0%)	4 (50.0%)			0 (0.0%)	8 (100.0%)		
Epithelial ovarian carcinoma	39	17 (43.6%)	22 (56.4%)			1 (2.6%)	38 (97.4%)		

Pearson χ^2 test

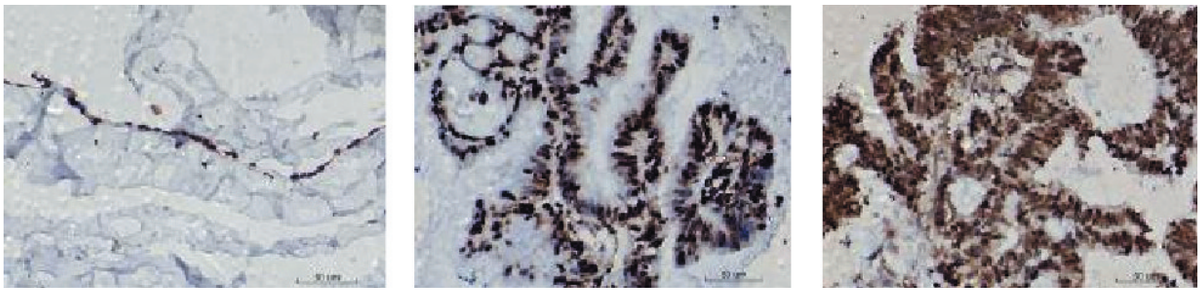

Figure 2. HMGB1 was predominantly stained in the nuclei and a weakly stained in cytoplasm (Magnification x 400).

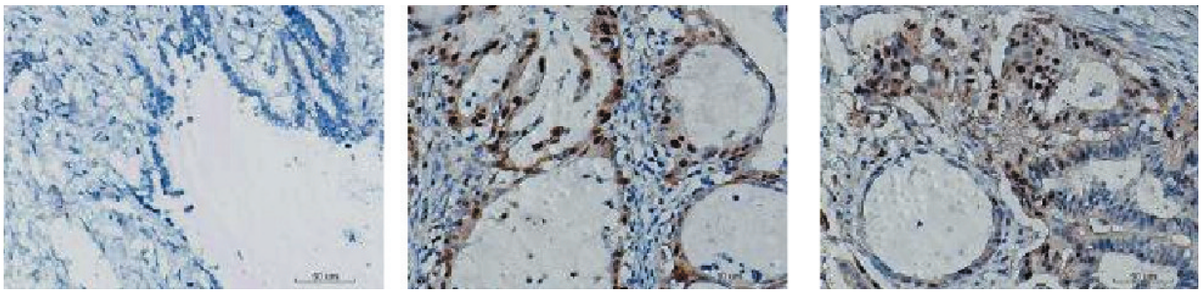

Figure 3. p62 immunoreactivity was predominantly found in the cytoplasm and nuclei (Magnification x 400).

The positive rates of p62 in tissues of the BET, borderline ovarian tumors and the EOC were 0.0%, 50.0%, and 38.5%, respectively (Table II), among which the detection rate of EOC and borderline tumor were significantly higher than BET ($p<0.05$). Survivin was detected in the nuclei and cytoplasm (Figure 4). The positive rates of survivin in tissues of the BET, borderline ovarian tumors and the EOC were 7.7%, 37.5%, 20.5%, respectively (Table II). ERCC1 staining was observed mainly in the nuclei (Figure 5). The positive rates of ERCC1 in the tissues of BET, borderline ovarian tumors and EOC were 7.7%, 50.0%, and 15.4%, respectively (Table III, $p<0.05$). Staining of BRCA1 located in both the nuclei and cytoplasm (Figure 6). The positive rates of BRCA1 in the tissues of BET, borderline ovarian tumors and EOC were 53.8%, 50.0%, and 20.5%, respectively (Table III, $p<0.05$).

Correlation among Protein Expression and Clinicopathological Characteristics of EOC Patients

The positive rate of Beclin1 in I/II stage and III/IV stage were 36.8% and 75.0% (Table IV, $p<0.05$). However, the expression of Beclin1 was independent of age, pathological type and grade of differentiation.

Table II. Exspression of Beclin-1 and HMGB1 protein in the tissues of the BET, the borderline ovarian tumors and the EOC.

Pathological type	N	P62 expression				Survivin expression			
		Negative N (%)	Positive N (%)	χ^2	*p*-value	Negative N (%)	Positive N (%)	χ^2	*p*-value
Benign epithelial ovarian tumor tissues	13	13 (100.0%)	0 (00.0%)	8.099	0.017	12 (92.3%)	1 (7.7%)	2.768	0.251
Borderline ovarian tumors tissue	8	4 (50.0%)	4 (50.0%)			5 (62.5%)	3 (37.5%)		
Epithelial ovarian carcinoma	39	24 (61.5%)	15 (38.5%)			31 (79.5%)	8 (20.5%)		

Pearson χ^2 test

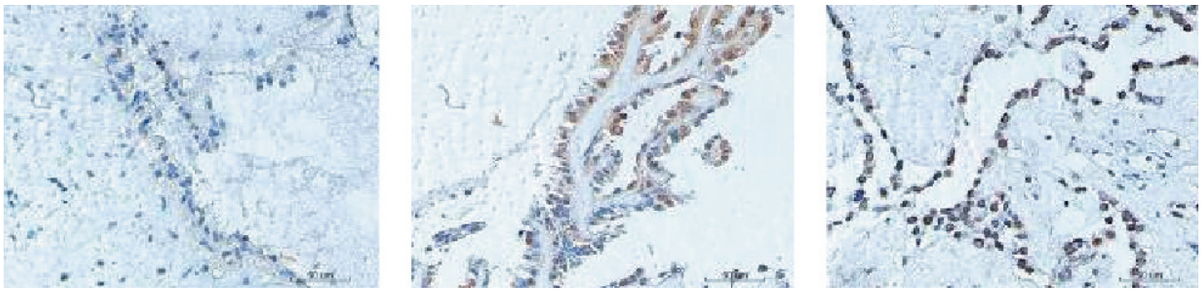

Figure 4. Survivin was detected in the nuclei and cytoplasm (Magnification x 400).

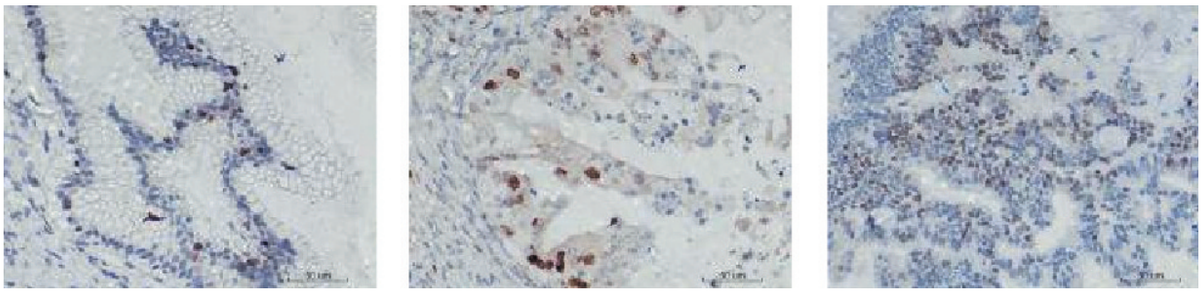

Figure 5. ERCC1 immunoreactivity was detected in the nuclei (Magnification x 400).

The positive rate of HMGB1 in serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometrioid carcinoma, clear cell carcinoma and mixed carcinoma was 100.0%, 100.0%, 100.0%, 100.0% and 50.0%, respectively (Table IV, p=0.001). The expression of HMGB1 was independent of age, grade of differentiation and FIGO stage (p>0.05).

The positive expression rates of p62 in I/II stage and III/IV stage were 21.1% and 55.0%, and positive expression rates of survivin in I/II stage and III/IV stage were 5.3% and 35.0% (Table V, p<0.05). However, expression of p62 and survivin were both independent of age, pathological type and grade of differentiation.

The expression of BRCA1 was independent of age, pathological type, differentiation and FIGO stage (Table VI).

Protein Expression in Platinum-Sensitive and Platinum-Resistant EOC Patients

The positive Beclin1 expression in the platinum-sensitive of EOC patients was 15.4%, which was significantly lower than 53.8% in the platinum-resistant (Table VII). While expressions of HMGB1 were comparable between the platinum-sensitive and platinum-resistant patients.

The positive rate of p62 in the platinum-sensitive patients (53.8%) was significantly higher than platinum-resistant (7.7%) (Table VIII, p<0.05).

Table III. Exspression of BRCA1 and ERCC1 protein in the tissues of the BET, the borderline ovarian tumors and the EOC.

Pathological type	N	BRCA1 expression				ERCC1 expression			
		Negative N (%)	Positive N (%)	χ^2	*p*-value	Negative N (%)	Positive N (%)	χ^2	*p*-value
Benign epithelial ovarian tumor tissues	13	6 (46.2%)	7 (53.8%)	6.44	0.04	12 (92.3%)	1 (7.7%)	6.568	0.037
Borderline ovarian tumors tissue	8	4 (50.0%)	4 (50.0%)			4 (50.0%)	4 (50.0%)		
Epithelial ovarian carcinoma	39	31 (79.5%)	8 (20.5%)			33 (84.6%)	6 (15.4%)		

Pearson χ^2 test

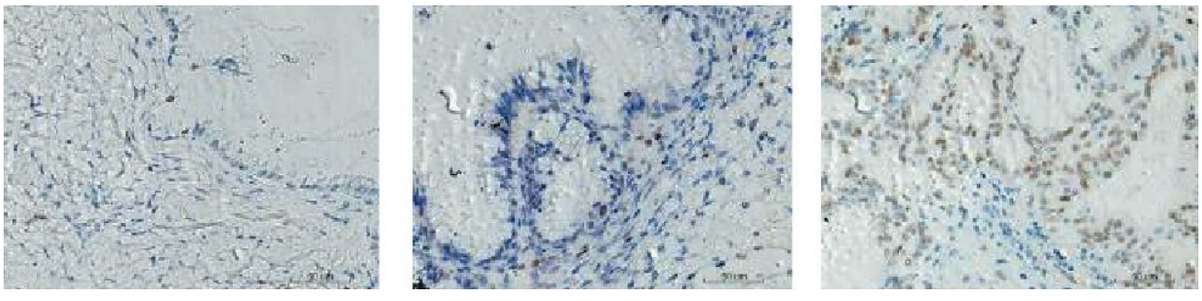

Figure 6. Staining of BRCA1 located in both the nuclei and cytoplasm (Magnification x 400).

Positive staining of survivin in platinum-sensitive EOC patients (38.5%) tended to be higher than platinum-resistant patients (7.7%) (Table VIII). However, the difference did not reach statistical significance ($p>0.05$).

Significantly lower expression of BRCA1 was detected in platinum-sensitive patients (7.7%) than in platinum-resistant patients (46.2%) (Table IX, $p<0.05$).

Table IV. Correlation between Beclin-1 and HMGB1 expression and clinicopathological characteristics of EOC patients.

Pathological type	N	Beclin-1 expression			HMGB1 expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Age (years)							
< 50	20	7 (35.0%)	13 (65.0%)	0.341	0 (0.0%)	20 (100.0%)	0.487
≥50	19	10 (52.6%)	9 (47.4%)		1 (5.3%)	18 (94.7%)	
Pathological type							
Serous cystadenocarcinoma	23	7 (30.4%)	16 (69.6%)	0.051	0 (0.0%)	23 (100.0%)	0.001
Mucinous cystadenocarcinoma	4	4 (100.0%)	0 (0.0%)		0 (0.0%)	4 (100.0%)	
Endometrioid carcinoma	5	2 (40.0%)	3 (60.0%)		0 (0.0%)	5 (100.0%)	
Clear cell carcinoma	5	2 (40.0%)	3 (60.0%)		0 (0.0%)	5 (100.0%)	
Mixed carcinoma	2	2 (100.0%)	0 (0.0%)		1 (50.0%)	1 (50.0%)	
Differentiation							
Poor/Moderate	31	16 (51.6%)	15 (48.4%)	0.106	1 (3.2%)	30 (96.8%)	1.000
Well	8	1 (12.5%)	7 (87.5%)		0 (0.0%)	8 (100.0%)	
FIGO stage							
I/II	19	12 (63.2%)	7 (36.8%)	0.025	1 (5.3%)	18 (94.7%)	0.487
III/IV	20	5 (25.0%)	15 (75.0%)		0 (0.0%)	20 (100.0%)	

Fisher's exact test

Table V. Correlation between Beclin-1 and HMGB1 expression and clinicopathological characteristics of EOC patients.

Pathological type	N	P62 expression			Survivin expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Age (years)							
< 50	20	11 (55.0%)	9 (45.0%)	0.514	14 (70.0%)	6 (30.0%)	0.451
≥50	19	13 (68.4%)	6 (31.6%)		17 (85.0%)	3 (15.0%)	
Pathological type							
Serous cystadenocarcinoma	23	13 (56.5%)	10 (43.5%)	0.289	18 (78.3%)	5 (21.7%)	0.551
Mucinous cystadenocarcinoma	4	4 (100.0%)	0 (0.0%)		3 (75.0%)	1 (25.0%)	
Endometrioid carcinoma	5	3 (60.0%)	2 (40.0%)		3 (60.0%)	2 (40.0%)	
Clear cell carcinoma	5	2 (40.0%)	3 (60.0%)		5 (100.0%)	0 (0.0%)	
Mixed carcinoma	2	2 (100.0%)	0 (0.0%)		2 (100.0%)	0 (0.0%)	
Differentiation							
Poor／Moderate	31	20 (64.5%)	11 (35.5%)	0.686	27 (85.7%)	4 (14.3%)	0.054
Well	8	4 (50.0%)	4 (50.0%)		4 (50.0%)	4 (50.0%)	
FIGO stage							
I/II	19	15 (78.9%)	4 (21.1%)	0.048	18 (94.7%)	1 (5.3%)	0.044
III/IV	20	9 (45.0%)	11 (55.0%)		13 (65.0%)	7 (35.0%)	

Fisher's exact test

Table VI. Correlation between BRCA1 and ERCC1 expression and clinicopathological characteristics of EOC patients.

Pathological type	N	BRCA1 expression			ERCC1 expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Age (years)							
< 50	20	16 (80.0%)	4 (20.0%)	1.000	17 (85.0%)	3 (15.0%)	1.000
≥50	19	15 (78.9%)	4 (21.1%)		16 (4.2%)	3 (15.8%)	
Pathological type							
Serous cystadenocarcinoma	23	18 (78.3%)	5 (21.7%)	0.214	19 (73.9%)	4 (26.1%)	0.533
Mucinous cystadenocarcinoma	4	2 (50.0%)	2 (50.0%)		4 (100.0%)	0 (0.0%)	
Endometrioid carcinoma	5	5 (100.0%)	0 (0.0%)		3 (60.0%)	2 (40.0%)	
Clear cell carcinoma	5	5 (100.0%)	0 (0.0%)		5 (100.0%)	0 (0.0%)	
Mixed carcinoma	2	1 (50.0%)	1 (50.0%)		2 (100.0%)	0 (0.0%)	
Differentiation							
Poor/Moderate	31	24 (77.4%)	7 (22.6%)	1.000	27 (87.1%)	4 (12.9%)	0.583
Well	8	7 (87.5%)	1 (12.5%)		6 (75.0%)	2 (25.0%)	
FIGO stage							
I/II	19	13 (68.4%)	6 (31.6%)	0.127	15 (78.9%)	4 (21.1%)	0.407
III/IV	20	18 (90.0%)	2 (10.0%)		18 (90.0%)	2 (10.0%)	

Fisher's exact test

Table VII. Exspression of Beclin-1 and HMGB1 protein in the platinum- sensitive and platinum-resistant EOC patients.

Pathological type	N	Beclin-1 expression			HMGB1 expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Platinum-sensitive	26	22 (84.6%)	4 (15.4%)	0.022	1 (3.8%)	25 (96.2%)	1
Platinum-resistant	13	6 (46.2%)	7 (53.8%)		0 (0.0%)	13 (100.0%)	

Fisher's exact test

Table VIII. Exspression of P62and survivin protein in the platinum-sensitive and platinum-resistant EOC patients.

Pathological type	N	P62 expression			Survivin expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Platinum-sensitive	26	12 (46.2%)	14 (53.8%)	0.006	16 (61.5%)	10 (38.5%)	0.063
Platinum-resistant	13	12 (92.3%)	1 (7.7%)		12 (92.3%)	1 (7.7%)	

Fisher's exact test

Table IX. Exspression of BRCA1 and ERCC1 protein in the platinum-sensitive and platinum-resistant EOC patients.

Pathological type	N	P62 expression			Survivin expression		
		Negative N (%)	Positive N (%)	*p*-value	Negative N (%)	Positive N (%)	*p*-value
Platinum-sensitive	26	24 (92.3%)	2 (7.7%)	0.01	22 (84.6%)	4 (15.4%)	1
Platinum-resistant	13	7 (53.8%)	6 (46.2%)		11 (84.6%)	2 (15.4%)	

Fisher's exact test

Correlation Among Protein Expression in Epithelial Ovarian Cancer

The expressions between Beclin1 and BRCA1, expressions p62 and BRCA1 were negatively correlated (Table X, $p<0.05$).

Discussion

Autophagy is a physiological phenomenon widely exists in eukaryotic cells and is essential to maintain the stability of the internal environment and adapt to external environment change. Studies show that autophagy can enhance the survival of normal cells and tumor cells, both *in vitro* and *in vivo*, under the condition of metabolic stress[13,14]. It has been confirmed that autophagy is implicated in drug resistance against tumor therapy, and decreased expression of autophagy proteins may contribute to the development or progression of breast and other human malignancies[5].

In this study, we examined the correlation between several autophagy-related proteins expression in epithelial ovarian cancer (EOC), benign epithelial ovarian tumor tissue (BET), and borderline ovarian tumor tissue, and found that the positive expression rates of Beclin1 in ovarian epithelial cancers (56.4%) were significantly higher than benign ovarian tumors (15.4%) and the borderline ovarian tumors (50.0%), which agreed with the results reported by Cai et al[16], that Beclin1 expression was higher in ovarian carcinomas. However, the result is contrary to

Table X. The expression of correlation among Beclin-1, HMGB1, P62 and survivin, ERCC1, BRCA1 in the epithelial ovarian cancers.

		Survivin				ERCC1				BRCA1			
		Neg. N	Pos. N	r	*p*-value	Neg. N	Pos. N	r	*p*-value	Neg. N	Pos. N	r	*p*-value
Beclin-1	Negative N	15	2	0.19	0.246	14	3	-0.055	0.739	11	6	-0.322	0.046
	Positive N	16	6			19	3			20	2		
HMGB1	Negative N	1	0	0.082	0.618	1	0	0.069	0.676	1	0	0.082	0.618
	Positive N	30	8			32	6			30	8		
P62	Negative N	19	5	-0.1	0.952	19	5	-0.191	0.244	16	8	-0.402	0.011
	Positive N	12	3			14	1			15	0		

Spearman's correlation test

the report by Duan et al[17,18], in which stated that expression of Beclin1 was positively correlated with better prognosis and with the accomplishment of an optimal postoperative therapy. The difference may be attributed to the fact that Beclin1 is upregulated in gastric cancer, colorectal cancer and intrahepatic cholangiocellular carcinoma[15,19], while it is downregulated in breast cancer, nasopharyngeal carcinoma, high-grade gliomas and esophageal squamous cell carcinoma[20,21]. The positive expressions of beclin1 in I/II stage were lower than in more advanced (III/IV) stages, and the expression in platinum-sensitive EOC patients was lower than in the platinum-resistant patients. Therefore, higher Beclin 1 expression may suggest worse prognosis in ovarian cancer and may be associated with the development of ovarian cancer platinum resistance.

It is widely accepted that HMGB1 is a critical regulator of autophagy by acting as an inducer of autophagy, and closely related to the development and progression of cancer[22]. Our results also indicated that the expression of HMGB1 was elevated in selected tumor samples. HMGB1 also upregulated in serous cystadenocarcinoma, mucinous cystadenocarcinoma, endometrioid carcinoma, clear cell carcinoma and mixed carcinoma. Zhang et al[22] reported that silencing HMGB1 could inhibit autophagy in ovarian cancer cells subjected to cisplatin treatment. However from our results the expression of HMGB1 in platinum-sensitive and platinum-resistant ovarian epithelial cancers patients were comparable.

p62 binds the autophagy regulator Atg8/LC3 through a region termed the LC3-interacting region (LIR)[23]. Suppressing p62 accumulation prevented the damage from autophagy defects[6]. Modulation of p62 by autophagy is a key factor in tumorigenesis. In our study, the positive expression rate of p62 in BET was significantly higher than EOC and is correlated with FIGO stage that lower expression of p62 was detected in I/II stages than in III/IV stage. In addition, the expression in the platinum-sensitive patients was higher than platinum-resistant patients. Enhanced expression of p62 in ovarian cancer decreases the formation of autophagosome and weakens the function of autophagy, which may be associated with the occurrence, development and prognosis of ovarian cancer platinum resistance.

Antiapoptotic mechanisms play an important role in chemotherapy resistance of tumor cells. Survivin induced by chemotherapy inhibits proteins against apoptosis. As presented in the results, the positive expression rate of survivin was connected with FIGO stage. Besides, according to Cohen et al[24], positive expressions of survivin also connected with pathological type and grade of differentiation and majority (74%) of ovarian carcinoma show survivin expression, which correlates with poor prognostic parameters (high grade, histologic type, p53 mutation) but not with survival. Therapeutic targeting of survivin in ovarian carcinoma is a future possibility. A previous study[25] also found reducing survivin expression can reverse the platinum resistance, which was contrasted by our findings that survivin expression was higher in platinum-sensitive EOC patients.

BRCA1 gene is one of the breast and ovarian cancer susceptibility genes and plays an important role in transcription activation and inhibition, DNA damage repair, cell cycle regulation[26]. The mechanism of platinum drugs in the tumor is combining with DNA in the tumor cell nucleus, making DNA chain crosslinking, fracturing the DNA single or double chain, inhibiting tumor cell fission, and finally leading to cell death. Nucleotide excision repair (NER) plays an important role in DNA damage repair causing by platinum drugs in which BRCA1 is involved in the damage repair pathways[27]. Enhanced BRCA1 expression improves the ability of DNA damage repair and thus generates resistance to platinum drugs. From our results, the positive expression of BRCA1 in BET tissue was significantly lower than EOC, while the expression in platinum-sensitive patients was lower than platinum-resistant patients. The results were consistent with Wang at el[28], that BRCA1 was negatively correlated with the sensitivity of tumor cells to cisplatin.

ERCC1 plays a key role in NER. This research showed that positive expression rate of ERCC1 in borderline ovarian tumors was significantly higher than BET and EOC, but not associated with age, pathological type, differentiation and FIGO stage of EOC patients. ERCC1 might be a potential predictive marker used to neoadjuvant treatment in early ovarian cancer. Previous studies had pointed out that the positive expression rate of ERCC1 in the platinum resistant group was higher than the platinum sensitive group, but our data failed to reach the same conclusion.

When comparing among proteins expression in epithelial ovarian cancer, the expression of BRCA1 was negatively correlated with Beclinl and p62 expression ($p<0.05$). The positive expression

rates of Beclinl and p62 were both higher than the expression rate of BRCA1. The enhanced expression may be suggestive of development of ovarian cancer and poor prognosis. It could guide the postoperative treatment of ovarian cancer.

Conclusions

We compared the expression of Beclin1, HMGB1, ERCC1, p62 and surviving in tumor samples from platinum-sensitive and -resistant ovarian cancer patients and analyze the correlation among their expression. From the immunohistochemical test, we can see that Beclin 1 inhibits autophagy to enhance the platinum-sensitive ovarian cancer. HMGB1 might be involved in the occurrence of ovarian cancer. We also showed that survivin and p62 might play a significant role in the development of ovarian cancer. ERCC1 might be a potential predictive marker used to neoadjuvant treatment in early ovarian cancer. BRCA1, Beclinl and p62 could provide a further reference for development and prognosis of epithelial ovarian cancer and platinum resistance during chemotherapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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