JBUON 2018; 23(5): 1390-1395
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues and their clinical significance

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Summary

Purpose: *To investigate the expressions of class III β-tubulin (TUBB3), nucleotide excision repair cross-complementary gene 1 (ERCC1) and P-glycoprotein (P-gp) in ovarian cancer tissues and adjacent normal tissues and their clinical significance.*

Methods: *Ovarian cancer patients undergoing surgical resection at the Department of Oncology of the Affiliated Hospital of Shandong Medical College from March 2012 to May 2016 were enrolled in this study, from which 166 cases of pathologically confirmed cancer tissues and 50 cases of adjacent normal tissues were collected. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the messenger RNA (mRNA) expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues, and their relationships with ovarian cancer clinical stage and grade of pathological differentiation were analyzed.*

Results: *The expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues were significantly higher than those in adjacent normal tissues ($p<0.05$). The later the clinical stage of ovarian cancer was, the higher the expression levels of TUBB3, ERCC1 and P-gp were ($p<0.05$). The lower the pathological differentiation grade of ovarian cancer was, the higher the expression levels of TUBB3, ERCC1 and P-gp were ($p<0.05$). TUBB3, ERCC1 and P-gp were positively correlated with clinical stage and pathological differentiation grade.*

Conclusion: *TUBB3, ERCC1 and P-gp are involved in the occurrence and development of ovarian cancer and can be used as important indexes judging the severity of ovarian cancer, providing a reference for the occurrence and development of the disease in ovarian cancer patients in clinical practice.*

Key words: *ovarian cancer, TUBB3, ERCC1, P-gp, clinical stage*

Introduction

Ovarian cancer is one of the frequently-occurring malignancies in women, which has increasingly high incidence among gynecological tumors. Its incidence and death rates rank third and first among gynecological cancers, respectively. Moreover, the 5-year survival rate of patients with ovarian cancer is often not more than 50% [1]. The pathogenesis of ovarian cancer is unknown, and it is difficult to identify a benign ovarian tumor from a malignant ovarian tumor in clinical practice. Patients often show no symptoms in the early stage. When a definite diagnosis is made, the disease of most patients is the middle or advanced stages, and tumor cells have spread to the uterus, pelvis and bilateral adnexae [2]. Therefore, exploring the occurrence and development of ovarian cancer and

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Received: 15/04/2018; Accepted: 12/05/2018

how to reduce its incidence rate has been the focus of much clinical research. Many studies have indicated that TUBB3, ERCC1 and P-gp are closely related to chemoresistance [3]. However, there are only few studies on the expression characteristics of TUBB3, ERCC1 and P-gp in ovarian cancer patients. TUBB3 is an important part of the cytoskeleton and a subtype of tubulin, which mainly plays roles in cell growth, motility and apoptosis and can maintain cell morphology [4]. ERCC1 plays a role of damage recognition and acts as a nucleic acid repair endonuclease in the body [5]. P-gp is a molecular pump protecting cells from entrance of damaging molecules [6]. This study aimed at analyzing the expression features of TUBB3, ERCC1 and P-gp in ovarian cancer patients and their clinical significance by investigating the expressions of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues.

Methods

General data

Ovarian cancer patients subjected to surgical disease resection in the Department of Oncology of the Affiliated Hospital of Shandong Medical College from March 2012 to May 2016 were selected, from which 166 cases of pathologically confirmed cancer tissues and 50 cases of adjacent normal tissues (3 cm away from cancer tissues). Patients were aged 30-62 years. According to 2013 International Federation of Gynecology and Obstetrics (FIGO) classification for ovarian tumors [7], there were 41 patients with mucous ovarian cancer, 95 with serous ovarian cancer, 17 with endometrioid ovarian cancer and 13 patients with ovarian clear cell cancer. Based on surgical staging, 21 patients had stage I disease, 48 had stage II, 76 had stage III, and 21 patients had stage IV disease. In terms of tumor grade differentiation, there were 22 cases with well-differentiated tumors, 50 with moderately-well differentiated tumors and 94 with poorly differentiated tumors. General clinical information of ovarian cancer patients from which tissue specimens were collected are shown in Table 1.

Inclusion criteria

Patients pathologically diagnosed with ovarian cancer operated for the first time; patients with no history of previous gynecological surgery; patients without any radiotherapy and chemotherapy before admission; whose cancer tissue and adjacent normal tissue specimens taken were quickly stored in a refrigerator at -80°C for later use; patients with complete clinical data.

Exclusion criteria

Patients pathologically diagnosed with severe heart, lung, renal or hematopoietic disorders; patients with previous family history of mental illness and psychosis.

This study was approved by the ethics committee of Affiliated Hospital of Shandong Medical College and all of the patients signed informed consent.

Main experimental equipment and reagents

Used were the following equipment and reagents: Applied Biosystems fluorescence quantitative polymerase chain reaction (PCR) instrument (purchased from ABI, Waltham, USA), total ribonucleic acid (RNA) extraction kit (TRIzol assay) (purchased from ABI, USA), Moloney Murine Leukemia Virus (M-MLV) reverse transcription kit [purchased from Promega (Beijing) Biotech

Table 1. General clinicopathological characteristics of ovarian cancer patients from which tissue specimens were collected

Characteristics	*Cases n (%)*
Age, years	
<50	70 (42.17)
≥50	96 (57.83)
Menopause	
Yes	49 (29.52)
No	117 (70.48)
Results of tumor markers before operation	
Positive	124 (74.70)
Negative	42 (25.30)
Tumor size, cm	
≤3	98 (59.04)
>3	68 (40.96)
Ascites	
Yes	67 (40.36)
No	99 (59.64)
Pathological type	
Mucous carcinoma	41 (24.70)
Serous carcinoma	95 (57.23)
Endometrioid carcinoma	17 (10.24)
Clear cell carcinoma	13 (7.83)
Clinical stage	
I	21 (12.65)
II	48 (28.92)
III	76 (45.78)
IV	21 (12.65)
Pathological grade of differentiation	
Well differentiated	22 (13.25)
Moderately differentiated	50 (30.12)
Poorly differentiated	94 (56.63)
Tumor location	
Unilateral	53 (31.93)
Bilateral	113 (68.07)
Lymph node metastasis	
Yes	86 (51.81)
No	80 (48.19)

Table 2. TUBB3, ERCC1, P-gp and β-actin gene sequence primers

Gene	*Forward primer*	*Reverse primer*	*Fragment length (bp)*
TUBB3	5'-CGGATCAGCGTCTACTAC-3'	5'-CACATCCAGGACCGAATC-3'	222
ERCC1	5'-CCCTGGGAATTTGGCGACGTAA-3'	5'-CTCCAGGTACCGCCCAGCTTCC-3'	273
P-gp	5'-CACCTGGACGTTACCAAAGAAGATATA-3'	5'-TCACCAACCAGCGTCTCATATTT-3'	253
β-actin	5'-ACACTGTGCCCATCTACGAGG-3'	5'-AGGGGCCGGACTCGTCATACT-3'	621

Co., Ltd.], and TUBB3, ERCC1 and P-gp PCR kits (purchased from Biomega). TUBB3, ERCC1, P-gp and β-actin internal reference primers were synthesized by TaKaRa Biotechnology (Dalian Co., Ltd, China). The sequences of required primers are shown in Table 2.

Detection methods

Standby tissues that were stored in liquid hydrogen were added with TRIzol reagent, shocked and left to stand at room temperature for 30 min for complete lysis. Then, total RNA was extracted in strict accordance with the instructions of the manufacturer and the concentration and purity of extracted RNA were determined using an ultraviolet spectrophotometer and protein electrophoresis. Following this, extracted total RNA was reversely transcribed according to the instructions of the manufacturer, and extracted complementary deoxyribonucleic acid (cDNA) samples were stored at -20°C. TUBB3, ERCC1 and P-gp primers were designed and synthesized by TaKaRa Biotechnology (Dalian Co., Ltd). PCR system was prepared according to the instructions, with a total volume of 12.62 μL that was made up to 20 μL using diethylpyrocarbonate (DEPC). PCR conditions: pre-denaturation at 94°C for 10 min, and 40 cycles of 94°C for 45 s, 60°C for 45 s and 72°C for 45 s. Amplification data were analyzed according to the manufacturer's software, with β-actin as an internal reference gene. The experiments were repeated 3 times, and the average was taken as the amplification result that was treated using $2^{-\Delta\Delta CT}$.

Statistics

SPSS 18.0 software (Guangzhou Pomine Info. Tech. Co., Ltd.) was used for statistical analyses. Quantitative data were expressed as mean ± standard deviation. Students *t*-test was employed for comparisons of measurement data, and chi-square test was utilized for comparisons of qualitative data. The correlations of TUBB3, ERCC1 and P-gp with clinical stage and pathological grade of differentiation were analyzed using Cox logistic regression analysis. P<0.05 suggested that the difference was statistically significant.

Results

Expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues

The expression level of TUBB3 in ovarian cancer tissues was higher than that in adjacent normal tissues (t=2.018, p=0.029). The expression level of ERCC1 was higher in ovarian cancer tissues than that in adjacent normal tissues (t=2.374, p=0.036). The expression level of P-gp in ovarian cancer tissues was increased compared with that in adjacent normal tissues (t=3.264, p=0.016) (Figure 1).

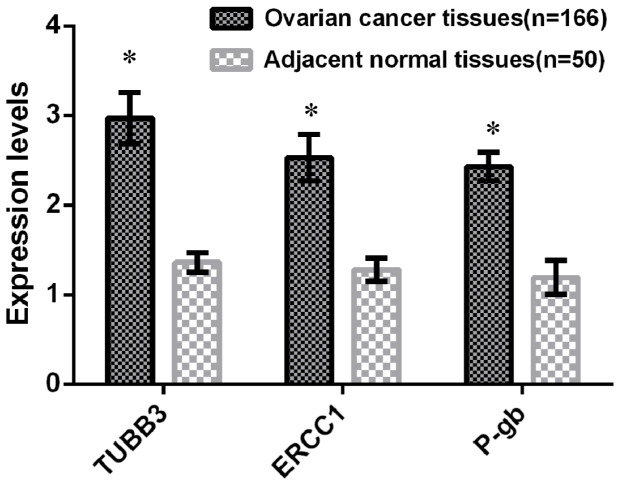

Figure 1. Comparisons of expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues. The expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues and adjacent normal tissues were detected using quantitative reverse transcription (QRT)-PCR, and the results were statistically analyzed. The expression level of TUBB3 in ovarian cancer tissues was higher than that in adjacent normal tissues (t=2.018, p=0.029). The expression level of ERCC1 in ovarian cancer tissues was higher than that in normal tissues (t=2.374, p=0.036). The expression level of P-gp in ovarian cancer tissues was higher than that in adjacent normal tissues (t=3.264, p=0.016). *p<0.05, compared with adjacent normal tissues.

Expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues with different surgical stages

Compared with those in clinical stage I cancer tissues, the expression levels of TUBB3, ERCC1 and P-gp in cancer tissues of stage II, III and IV ovarian cancer patients were significantly increased (p<0.05). The expression levels of TUBB3, ERCC1 and P-gp in cancer tissues of stage III and IV ovarian cancer patients were overtly higher than those in clinical stage II cancer tissues (p<0.05). In comparison with clinical stage III cancer tissues, the expression levels of TUBB3, ERCC1 and P-gp

Table 3. Comparisons of expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues with different clinical stages (mean±SD)

Clinical stage	*n*	*TUBB3*	*ERCC1*	*P-gp*
I	21	1.37±0.17	1.76±0.17	2.16±0.13
II	48	2.46±0.21[a]	2.43±0.23[a]	3.01±0.23[a]
III	76	3.07±0.19[a,b]	3.16±0.15[a,b]	3.74±0.33[a,b]
IV	21	3.98±0.23[a,b,c]	4.15±0.26[a,b,c]	4.54±0.29[a,b,c]
f	-	0.125	0.228	0.375
p	-	0.032	0.028	0.019

Compared with stage I, [a]p<0.05; compared with stage II, [b]p<0.05; compared with stage III, [c]p<0.05

Table 4. Comparisons of expression levels of TUBB3, ERCC1 and P-gp in ovarian cancer tissues with different degrees of pathological differentiation (mean±SD)

Grade of pathological differentiation	*n*	*TUBB3*	*ERCC1*	*P-gp*
Well differentiated	22	1.23±0.19	1.66±0.16	2.03±0.21
Moderately differentiated	50	2.36±0.26[a]	2.59±20[a]	3.16±0.25[a]
Poorly differentiated	94	3.87±0.31[a,b]	3.93±0.29[a,b]	4.33±0.31[a,b]
f	-	1.264	1.113	0.981
p	-	0.031	0.044	0.023

Compared with well differentiated, [a]p<0.05; compared with moderately differentiated, [b]p<0.05

were increased in stage IV cancer tissues (p<0.05) (Table 3).

Expression levels of TUBB3, ERCC1 and P-gp in cancer tissues with different grades of pathological differentiation

Compared with those with well-differentiated cancer tissues, the expression levels of TUBB3, ERCC1 and P-gp in cancer tissues of patients with moderately and poorly differentiated disease were significantly increased (p<0.05). The cancer tissues of patients with poorly differentiated ovarian cancer had elevated expression levels of TUBB3, ERCC1 and P-gp compared with those with moderately differentiated cancer tissues (p<0.05) (Table 4).

Correlations of TUBB3, ERCC1 and P-gp with clinical stage and grade of differentiation

Cox logistic regression analyses of clinical stage and pathological grade of differentiation with TUBB3, ERCC1 and P-gp in ovarian cancer tissues were carried out, and the results suggested that TUBB3 was positively correlated with clinical stage and pathological grade of differentiation (r=0.367, p<0.05); ERCC1 was positively associated with clinical stage and pathological grade of differentiation (r=0.553, p<0.05), and P-gp was positively related to clinical stage and pathological grade of differentiation (r=0.667, p<0.05).

Discussion

Ovarian cancer, short for ovarian malignant tumor, has the widest onset age range among gynecological tumors and can occur at any time in women's life [8]. The pathogenesis of ovarian cancer is not yet clear, and the main risk factors include living environment, race, endocrine disorders and eating habits [9,10]. Ovarian cancer is characterized by rapid tumor growth and generally has no symptoms in the early stages. Moreover, most ovarian cancer patients are already in advanced stage when diagnosed, and tumor cells have spread to various organs and sites. In addition, ovarian cancer can induce a series of complications such as tumor rupture, hemorrhage and anemia, seriously threatening the lives of patients [11].

This study explored the relationships of TUBB3, ERCC1 and P-gp with ovarian cancer through clinical stage of disease and tumor pathological grade of differentiation. The results indicated that the later the clinical stage was, and the lower the grade of tumor pathological differentiation was, the higher the expression levels of TUBB3, ERCC1 and P-gp were, and TUBB3, ERCC1 and P-gp were positively correlated with clinical stage and grade of differentiation, suggesting that TUBB3, ERCC1 and P-gp are involved in the occurrence and development of ovarian cancer. TUBB3 is an important part of the cytoskeleton and a subtype of tubulin [12]. Based on analyses of the results, TUBB3 is involved in

the tumorigeness of ovarian cancer, and in the key step, the increased TUBB3 expression level and elevated proteins in tubulin protein pool lead to the formation of multipolar spindle, resulting in the loss of cancer suppressor genes in the body and altering the phenotype of cells, thereby promoting the occurrence and development of tumor cells [13-15]. ERCC1 has the function of damage recognition and acts as a nucleic acid repair endonuclease, which is an important part of the process of nucleotide excision and repair. The highly expressed ERCC1 repairs the DNA damaging tumor cells in the body's immune system, leading to reproduction of cancer cells in the body and resulting in the occurrence and development of ovarian cancer [16]. P-gp is a transmembrane glycoprotein with a molecular weight of 170 kD, which binds to both drugs and adenosine triphosphate (ATP) and has an energy-dependent function [17]. This study found that P-gp was highly expressed in ovarian cancer patients, which was speculated to be caused because P-gp could excrete energy-dependent chemotherapeutic drugs out of the cells, resulting in reduced drug concentration in cells and decreased effect of chemotherapeutic drugs, thus facilitating the reproduction of tumor cells in the body [18]. Studies by Ganta et al. [19] and Herzog et al. [20] have suggested that TUBB3, ERCC1 and P-gp are involved in the initiation and development of ovarian cancer, which are similar to the findings of this study. The difference is that the above studies have indicated that TUBB3, ERCC1 and P-gp are potential therapeutic targets for chemotherapy in ovarian cancer, which was not studied in depth in this study. Therefore, it is hoped that the effects of TUBB3, ERCC1 and P-gp in ovarian cancer will be further studied in our next study.

This study used rigorous inclusion criteria for experimental subjects, and tissue samples of ovarian cancer were strictly screened, so as to ensure the reliability of the experiments. This study was to analyze cancer tissues. Surgical resection is the main therapeutic approach for ovarian cancer patients and also the main method to collect cancer tissue specimens. It was relatively difficult to collect specimens from patients who were unable to be operated due to their physical conditions. Moreover, mRNA was stored at -20°C, which might affect the detection results. It is hoped to find a better detection method in our next study.

In conclusion, TUBB3, ERCC1 and P-gp participate in the occurrence and development of ovarian cancer and can be used as important detection indexes to determine the severity of the disease in ovarian cancer patients, providing a reference for the occurrence and development of the disease in patients with ovarian cancer.

Authors' contributions

YJ wrote the manuscript and extracted total RNA. SS and XG helped synthesizing cDNA. XC performed and analyzed PCR. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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