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Regular paper

The expression levels of *NF-κB* and *IKKβ* in epithelial ovarian cancer and their correlation with drug resistance-related genes *MDR1*, *TOPOII*, and *ERCC1*

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Objectives: To explore the expression levels of nuclear factor kappa B (*NF-κB*) and inhibitor of nuclear factor kappa B kinase (*IKKβ*) in epithelial ovarian cancer and the correlation analysis with multi-drug resistance-related genes 1 (*MDR1*), topoisomerase II (*TOPOII*) and nucleotide excision repair cross complementary group 1 (*ERCC1*). **Methods:** Immunohistochemical methods were used to detect the expression levels of *NF-κB* and *IKKβ* in epithelial ovarian cancer group (50 cases), ovarian benign tumor group (30 cases), and normal ovary group (10 cases). The expression levels of *NF-κB*, *IKKβ*, *MDR1*, *TOPOII* and *ERCC1* messenger ribonucleic acid (mRNA) and protein were analyzed using real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot. Student's *t*-test and one-way ANOVA were used for comparison of numerical data. Pearson's chi-squared and Fisher's exact tests were carried out for analysis of non-numerical data. **Results:** The levels of *NF-κB*, *IKKβ*, *MDR1* and *ERCC1* mRNA and protein were increased ($P<0.05$), and the expression levels of *TOPOII* were decreased ($P<0.05$) in the epithelial ovarian cancer group compared to the normal ovary and benign ovarian tumor groups. The expression of *NF-κB* and *IKKβ* in epithelial ovarian cancer was significantly increased in patients with higher tumor stage, lower differentiation and presence of lymph node metastasis and positively correlated with *MDR1* expression. *NF-κB* and *IKKβ* were negatively correlated with the expression of *TOPOII* and antagonized each other with *TOPOII*. **Conclusions:** The expression of *NF-κB* and *IKKβ* was positively correlated with the expression of *MDR1*, and negatively correlated with the expression of *TOPOII*. The correlation of *NF-κB*, *IKKβ* and resistance related genes, including *MDR1, TOPOII, ERCC1*, can predict the resistance of chemotherapy individuals to chemotherapy.

Keywords: epithelial ovarian cancer, nuclear factor kappa B, inhibitor of nuclear factor kappa B kinase, resistance-related genes, correlation

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Abbreviations: *ERCC1*, nucleotide excision repair cross complementary group 1; *IKKβ*, inhibitor of nuclear factor kappa B kinase; *MDR1*, multi-drug resistance-related genes 1; *NF-κB*, nuclear factor kappa B; RT-qPCR, real-time quantitative polymerase chain reaction

INTRODUCTION

Ovarian cancer is both the fifth most common cancer among women in China and the deadliest gynecological cancer in China, with mortality of over 15 000 deaths a year (Kim *et al.*, 2017). Most deaths are attributed to serous carcinoma, usually diagnosed at advanced stage, which is extremely difficult to cure. The goals in this field include improving screening, early diagnosis, initial treatment and relapse rescue (Deb *et al.*, 2018; Kotsopoulos *et al.*, 2018). Previous studies identified a subgroup of patients with aggressive diseases, who have different responses to standard surgery and chemotherapy (Liang *et al.*, 2018).

Sen and Baltimore discovered a nuclear protein factor in mature B lymphocytes in mice, which specifically binds to *κB* sequence of immunoglobulin *κ* light chain and named it nuclear factor kappa B (*NF-κB*). *NF-κB* participates in tumor invasion as *IKKα* target (Hayashi *et al.*, 2018). Transcription factors of the *NF-κB* family are widely expressed in solid tumors, which is related to their proliferation (Nadiminty *et al.*, 2013). Constitutive *NF-κB* activation has been found in epithelial tumors, including breast cancer, colon cancer, lung cancer and ovarian cancer (Liang *et al.*, 2018).

A systematic approach integrating three global screening techniques showed the involvement of the inhibitor of *NF-κB* kinase (*IKKε*), a cytoplasmic kinase involved in triggering *NF-κB* signal transduction, in esophageal squamous cell carcinoma (Yang *et al.*, 2018). It has been proved that *IKK* is necessary for activating *NF-κB* in response to proinflammatory factors (Chantemargue *et al.*, 2018). Studies have shown that increased expression of multidrug resistance gene 1 (*MDR1*) is closely related to generation of drug resistance in tumor cells and blocking the expression of *MDRl* can effectively inhibit tumor multidrug resistance (Vogt *et al.*, 2018). It has been found that the expression of topoisomerase II gene (*TOPOII*) in ovarian cancer is related to histological grade and cancer cell proliferation (Cottenden *et al.*, 2018). Excision Repair Cross-Complementation Group 1 (*ERCC1*) is an important part of nucleotide excision repair system. and ovarian cancer patients with high *ERCC1* expression have lower survival rate (de Almeida *et al.*, 2017).

Present study was to explore the expression levels of *NF-κB* and *IKKβ* in epithelial ovarian cancer and their correlation with drug resistance related gene *MDR1*, *TOPOII* and *ERCC1*.

MATERIALS AND METHODS

This study was approved by the Ethic Committee of the Fourth Hospital of Shijiazhuang (No. SJZ4-05-08). Informed consent was waived for its retrospective nature and data anonymization.

Case selection

In this study, we analyzed 90 specimens collected in our hospital form the ovarian cancer patients. . They included 50 cases of epithelial ovarian cancer (41 cases of serous cancer and 9 others), which constituted experimental group in this study. Within this group, 19 specimens originated form patients younger than 50 and 31 from patients that were older than 50. According to FIGO standard of International Association of Obstetrics and Gynecology, 27 cases were classified stage I–II and 23 stage III–IV. Twenty seven specimens had high cell differentiation and 22 specimens had poor cell differentiation. Thirty-four patients had lymph node metastasis and 16 were metastasis free. As a control group, thirty cases of ovarian epithelial benign tumors and additional 10 cases of normal ovarian tissue were used. In this group, none of the patients received radiotherapy, chemotherapy or hormone replacement therapy. and all the patients were diagnosed as epithelial ovarian cancer without metastasis. The characteristics of experimental groups is shown in Table 1.

Instruments

The main instruments used in this experiment were paraffin embedding machine (Microm, Germany), electrothermal incubator (Chongqing Experimental Equipment Factory), vortex mixer (Shanghai Jingke Industrial Co., Ltd.), ultra-low temperature refrigerator (SANYO, Japan), water bath oscillator (Harbin Scientific Instrument Factory), slicer (BM2135, Leica company, Germany), digital display electrothermal moisturizing box (303-4A, Shanghai Sunshine Experimental Instrument Co., Ltd), image acquisition system (Qcapture, Qimaging, Canada), optical microscope (BX41, Olympus, Japan) and electronic balance.

APES (3-aminoprolyletriethoxysilane) coating of glass slides

Slides were soaked in concentrated sulfuric acid for 24 h, and then washed repeatedly in running water and air dried. Next, they were soaked in distilled water for 0.5 h twice. Next, they were soaked in 95% ethanol for 2 h, and dried in an incubator at 65°C. Then they were passed through APES solution (diluted 1:49 with acetone) for 2 minutes. Next, they were briefly soaked in acetone and air dried.

Paraffin embedding

During the embedding process, the tissue samples were fixed with 10% formalin overnight, then fixed with AF solution (95% ethanol 90 ml + 40% formaldehyde 10 ml) for 2 h. After soaking in 95% ethanol overnight, they were dehydrated gradually in anhydrous ethanol for 2 h. Next, the samples were soaked in xylene for 2 consecutive washes of 10 and 15 minutes.. Next, the samples were soaked paraffin at 65°C for 1 h and wax for 2 h. The ideal paraffin block was made by automatic embedding machine. A 4 μm thick continuous paraffin tissue sections were taken and mounted on the APES treated white glass slices, incubated in 65°C oven for 4 h, and kept at 4°C.

Hematoxylin-Eosin staining

The slides were put into an oven for 2 h incubation at 67°C; next, they were immersed twice in xylene for 15 minutes, twice in absolute ethanol for 10 minutes, then in 95% ethanol for 5 minutes and 80% ethanol for 5 minutes. Next, they were washed with tap water, and distilled water and stained with hematoxylin for 2–4 minutes, after which they were washed with tap water 3 times. Hydrochloric acid – ethanol solution was used for differentiation of tissue until the tissue dyed light pink, and then the solution was washed away with tap water 3 times. Next, the slices were put into ammonia water until the tissue turned light blue, and washed with water 3 times. Next, the slices were stained with eosin for about 40 s, and washed 3 times with tap water. Next, the slices were soaked in 70% ethanol, followed with 85% ethanol for 2 minutes, 95% ethanol for 5 minutes, anhydrous ethanol (I, II) for 5 minutes, and xylene (I and II) for 5 minutes, respectively. Then the slices were sealed with neutral gum and observed under a microscope.

S-P immunohistochemistry

S-P staining procedure was used for paraffin specimens, which were routinely sectioned in 4 μm, and placed on slides coated with APES. The slides were incubated at 67°C overnight. Then I paraffin samples were dewaxed for 10 minutes with xylene I and 10 minutes with xylene II. Next, they were soaked in absolute ethanol I, absolute ethanol II, 95% ethanol, and 80% ethanol for 5 minutes each, washed three times with tap water, and soaked in PBS buffer twice for 5 minutes each time. The heat repair was carried out in and high-pressure pot. The slides were heat-repaired for 20 minutes in methanol – hydrogen peroxide solution (freshly prepared: methanol 188 ml + hydrogen peroxide 12 ml), and soaked twice in PBS solution, 5 minutes each time. After removing the excess liquid from around the tissue, the slides were put in a wet chamber, sealed with serum (liquid A), and put in the incubator at 37°C for 45 minutes. Next, the serum was removed without washing and the primary antibody *NF-κB* or *IKKβ*, diluted at 1:100, was added, and incubated overnight at 4°C. Next, the slides were washed in PBS 2 times for 5 minutes, and incubated with the secondary antibody. The slides were put in a wet chamber and incubated at 37°C for

Table 1. Experimental grouping

Experimental grouping	Normal ovary	Benign ovarian tumor	Epithelial ovarian cancer									
			age		by stages		pattern of organization		Histopathological grading		Lymph node metastasis	
			<50	≥50	I–II	III–IV	Serous mucinous carcinoma	other	High differentiation	poorly differentiated	have	without
Cases (n)	10	30	19	31	27	23	41	9	28	22	34	16

30 minutes, then washed in PBS twice for 5 minutes.. Then tertiary antibody was added by dropping, and the sections were placed in a wet chamber and incubated at 37°C for 30 minutes. Next, they were twice washed in PBS for 5 minutes. DAB (3,3'-diaminobenzidine) solution (850 μl distilled water + 50 μl of a, b, c solution each) was used for color development; the process was stopped with washing in distilled water. The slides were additionally stained with hematoxylin as described above. Then the slides were sealed with gum and observed under microscope.

S-P staining control

PBS solution was used to replace the primary antibody, and other experimental conditions and steps did not change.

Result judgment

According to the Fromowitz score standard, 5 high power visual fields (×400 times magnification) were randomly selected to count the total number of tumor cells, count the number of positive cells, and calculate the percentage of positive cells. The positive cell rate ≤ 25% was assigned 0 points, 26–50% 1 point, 51–75% 2 points, and ≥76% was 3 points. Then, according to the degree of S-P staining of the majority of positive cells, the score was 0 for no color, 1 point for light brown-yellow, 2 points for brown-yellow and 3 points for brown. The above two scores were added and categorized as: "+" for 0 points, "+" for 1–2 points, "++" for 3–4 points, and "++" for 5–6 points. In addition, 1-6 points were considered positive results, and 0 points was considered negative result. *NF-κB/p65* staining in ovarian tumor tissue was located in the nucleus, and the positive expression was brown granules. *IKKβ* staining located mainly in the cytoplasm and to a much lesser extent in the nucleus. Therefore, quantification of *IKKβ* staining was based on the cytoplasmic staining. *TOPOII* and *ERCC1* staining was granular and located to the nucleus. *MDR1* staining was located to cell membrane.

Real time quantitative polymerase chain reaction (RT-qPCR)

RNA isolation Kit (Beijing Kangwei Biotechnology Co., Ltd., China) was used to extract total RNA from the tissue sections. cDNA was synthesized using super RT cDNA Synthesis Kit (antivirus gene of China Biotechnology Co., Ltd.). RT-qPCR was performed on the qPCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR Green PCR kit. The optimized PCR parameters were as follows: 95°C for 2 minutes, 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 40 seconds (40 cycles). The average relative expression of *NF-κB*, *IKKβ, MDR1*, *TOPOII* and *ERCC1* in triplicate samples were calculated according to $2^{-\Delta\Delta Ct}$ method.

Western blotting

Total protein was extracted with RIPA lysis buffer (Beijing Biotech Corporation, Beijing, China) and protein concentration was determined using Bradford method. Protein (20 μg) was separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membrane by semi dry transfer method. The membrane was blocked with 5% skimmed milk and incubated with primary antibody (1:3000) at 4°C overnight. After washing three times with TBST, the membrane was incubated with secondary antibody (1:1000) at room temperature for 1 hour. Protein was detected with enhanced chemiluminescence kit and visualized under ultraviolet light.

Statistical analysis

Each experiment was repeated at least three times and SPSS software (version 22.0; SPSS, Inc., Chicago, Illinois, USA) was used for statistical analysis. Normally distributed measurement data were expressed as mean ± standard deviation (S.D.), and the comparisons were examined by Student-t test and one-way ANOVA test (for more than 2 groups). The categorical data were expressed as *n* (%), and the differences between the two groups were examined using chi-squared or Fisher's exact test. $P<0.05$ was considered statistically significant.

RESULTS

The expression of NF-κ B in epithelial ovarian cancer

As shown in Fig. 1, the expression of *NF-κB* in epithelial ovarian cancer was positive. Staining was in the form of brown-yellow particles located mainly in the nucleus No brown-yellow particles were observed in benign ovarian tumors *NF-κB* staining in normal ovaries did not show color. In 10 cases of normal ovary, there was no expression of *NF-κB*, making the overall the expression rate 0%. In 30 cases of ovarian benign tumors, 6 cases were positive, making the expression rate 20%. In 50 cases of epithelial ovarian cancer, 38 cases were positive, making the expression rate 76%. There was a significant difference between normal ovary and epithelial ovarian cancer ($P<0.05$), and between benign ovarian tumor and epithelial ovarian cancer ($P<0.05$). The results are shown in Table 2.

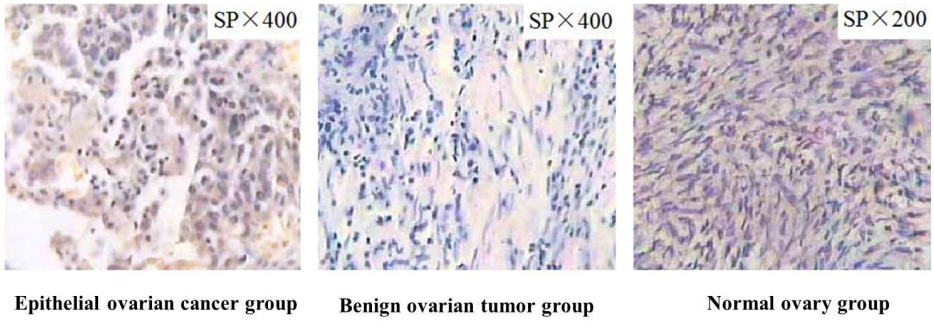

Figure 1. Immunohistochemical staining of *NF-κB* in various tissues.
NF-κB: nuclear factor kappa B.

Table 2. Positive patients, quantitive analysis and protein expression level of different variables in patients included

Variables	Normal ovary (n=10)	Benign ovarian tumor (n=30)	Epithelial ovarian cancer (n=50)	*P*-value
Positive number of patients				
NF-κB	0	6	38	<0.001
IKKβ	1	4	30	<0.001
mRNA quantitive analysis				
NF-κB	0.64±0.13	1.23±0.57	4.86±0.32	<0.001
IKKβ	0.87±0.14	1.89±0.25	5.53±0.37	<0.001
MDR1	0.72±0.14	1.59±0.23	4.86±0.27	<0.001
TOPOII	4.28±0.24	2.06±0.15	0.53±0.14	<0.001
ERCC1	0.85±0.16	1.73±0.26	5.88±0.19	<0.001
Protein expression level				
NF-κB	0.54±0.12	1.33±0.16	3.53±0.14	<0.001
IKKβ	0.63±0.11	1.87±0.21	3.56±0.18	<0.001
MDR1	0.87±0.55	1.76±0.24	3.95±0.13	<0.001
TOPOII	3.96±0.24	1.55±0.18	0.63±0.24	<0.001
ERCC1	0.43±0.15	1.37±0.15	5.58±0.21	<0.001

Abbreviations: *NF-κB*: nuclear factor kappa B; *IKKβ*: inhibitor of nuclear factor kappa B kinase; *MDR1*: multidrug resistance gene 1; *TOPOII*: topoisomerase II; *ERCC1*: nucleotide excision repair cross complementary group 1.

The expression of IKK β in epithelial ovarian cancer

As shown in Fig. 2, *IKKβ* was expressed in in epithelial ovarian cancer, and the staining in the form of brown-yellow particles was located mainly in the cytoplasm. Conversely, staining of the benign ovarian tumors did not show any brown-yellow particles. In normal ovaries, staining did not show *IKKβ* expression in general. In 10 cases of normal ovary, 1 case was qualified as *IKKβ* positive, accounting for the expression rate of 10%. In 30 cases of ovarian benign tumor, 4 cases were positive, accounting for the expression rate of 13.33%. In 50 cases of epithelial ovarian cancer, 30 cases were qualified as *IKKβ* positive, making the *IKKβ* expression rate in this group 60%. There was significant difference between normal ovary and epithelial ovarian cancer ($P<0.05$), and also between benign ovarian tumor and epithelial ovarian cancer. The results are shown in Table 2.

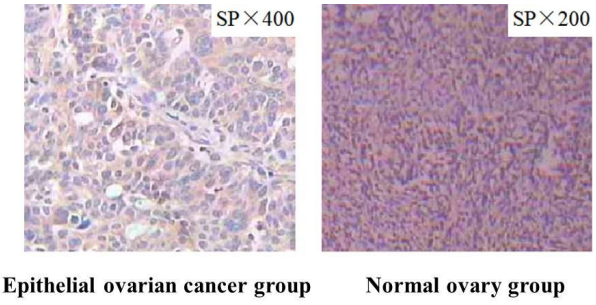

Figure 2. Immunohistochemical staining of *IKKβ* in various tissues.
IKKβ: inhibitor of nuclear factor kappa B kinase subunit β.

The mRNA expression levels of *NF-κB*, *IKKβ*, *MDR1*, *TOPOII* and *ERCC1* in epithelial ovarian cancer

RT-qPCR was used to analyze the mRNA levels of *NF-κB, IKKβ, MDR1, TOPOII* and *ERCC1*. Compared to normal ovary group and ovarian benign tumor group, mRNA expression levels of *NF-κB, IKKβ, MDR1 and ERCC1* in epithelial ovarian cancer group were significantly increased ($P<0.05$), while *TOPOII* mRNA level was decreased ($P<0.05$). The results are shown in Fig. 3 and Table 2.

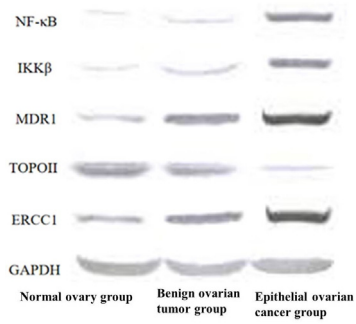

Figure 3. Real-time quantitative polymerase chain reaction analysis of *NF-κB*, *IKKβ*, *MDR1*, *TOPOII* and *ERCC1* mRNA in various tissues.
NF-κB: nuclear factor kappa B; *IKKβ*: inhibitor of nuclear factor kappa B kinase subunit β; *MDR1*: Multi-drug resistance protein 1; *TOPOII*: topoisomerase II; *ERCC1*: Excision Repair Cross-Complementation Group 1.

The protein levels of *NF-κB*, *IKKβ*, *MDR1*, *TOPOII* and *ERCC1* in epithelial ovarian cancer

Western blot was used to analyze protein expression of *NF-κB, IKKβ, MDR1, TOPOII* and *ERCC1*. Compared to normal ovary group and ovarian benign tumor

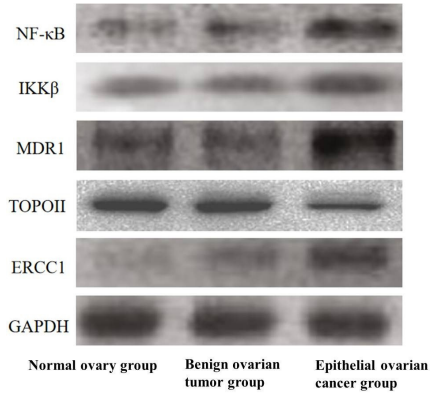

Figure 4. Western blot analysis of *NF-κB*, *IKKβ*, *MDR1*, *TOPOII* and *ERCC1* in various tissues.
NF-κB: nuclear factor kappa B; *IKKβ*: inhibitor of nuclear factor kappa B kinase subunit β; *MDR1*: Multi-drug resistance protein 1; *TOPOII*: topoisomerase II; *ERCC1*: Excision Repair Cross-Complementation Group 1.

group, protein expression of *NF-κB, IKKβ, MDR1 and ERCC1* in epithelial ovarian cancer group were increased ($P<0.05$), while protein expression of *TOPOII* was decreased ($P<0.05$). The results are shown in Fig. 4 and Table 2.

Relationship between *NF-κB* expression and clinicopathological factors in epithelial ovarian cancer

Among 19 samples of epithelial ovarian cancer originating from the patients younger than 50, 13 were positive for *NF-κB*, and the expression rate was 68.42%. Among the 31 samples from the patients older than 50, 23 cases were positive for *NF-κB*, and the expression rate was 74.19%. The difference in expression rate between the two age groups was not significant ($P>0.05$). Among 27 cases of epithelial ovarian cancer in stage I–II, 20 cases were positive for *NF-κB*, and the expression rate was 74.07%. In 23 cases of stage III–IV, 16 cases were positive for *NF-κB*, and the expression rate was 69.57%. The difference in expression rate between the two groups was not significant ($P>0.05$). Among 41 cases of serous mucinous carcinoma, 29 cases were positive for *NF-κB*, and the expression rate was 70.73%. Among 9 cases with other types, 7 cases were positive for *NF-κB*, the expression rate was 77.78%. The difference in expression rate between the two groups was not significant ($P>0.05$). Among 28 cases of epithelial ovarian cancer with high differentiation, 15 cases were positive for *NF-κB*, and the expression rate was 53.57%. Among 22 cases with low differentiation, 21 cases showed positive expression of *NF-κB*, the expression rate was 95.45%. The observed difference in expression rate between the two groups was statistically significant ($P<0.05$). Among 34 cases of epithelial ovarian cancer with lymph node metastasis, 29 cases were positive for *NF-κB*, and the expression rate was 85.29%. Among 16 cases without lymph node metastasis, 7 cases were positive for *NF-κB*, and the expression rate was 43.75%. The observed difference in expression rate between the two groups was statistically significant ($P<0.05$). The results are shown in Table 3.

The relationship between *IKKβ* expression and clinicopathological factors in epithelial ovarian cancer

In 19 samples of epithelial ovarian cancer derived from the patients younger than 50, 11 cases were *IKKβ* positive, and the expression rate was 57.89%. Among the 31 cases of epithelial ovarian cancer that occurred in women older than 50, 18 were *IKKβ* positive and the expression rate was 58.06%. The difference in expression rate between the two groups was not significant ($P>0.05$). Among 27 cases of epithelial ovarian cancer in stage I–II, 15 were *IKKβ* positive, and the expression rate was 55.56%. In 23 cases of stage III–IV, 14 were *IKKβ* positive and the expression rate was 60.87%. The difference in expression rate between the two groups was not significant ($P>0.05$). Among 41 cases of serous

Table 3 Comparison of NF-κB and IKKβ in different subgroups of epithelial ovarian cancer patients

Variables	Total number	Positive number	P-value
NF-κB			
Age			0.693
<50	19	13	
≥50	31	23	
Stages			0.736
I–II	27	20	
III–IV	23	16	
Histology			0.879
Serous mucinous carcinoma	41	29	
Other	9	7	
Grade			0.001
High differentiation	28	15	
Poor differentiation	22	21	
Lymph node metastasis			0.009
Yes	34	29	
No	16	7	
IKKβ			
Age			0.964
<50	19	11	
≥50	31	18	
Stages			0.695
I–II	27	15	
III–IV	23	14	
Histology			1.000
Serous mucinous carcinoma	41	24	
Other	9	5	
Grade			0.003
High differentiation	28	11	
Poor differentiation	22	18	
Lymph node metastasis			0.009
Yes	34	25	
No	16	4	

Abbreviations: NF-κB: nuclear factor kappa B; IKK β: inhibitor of nuclear factor kappa B kinase.

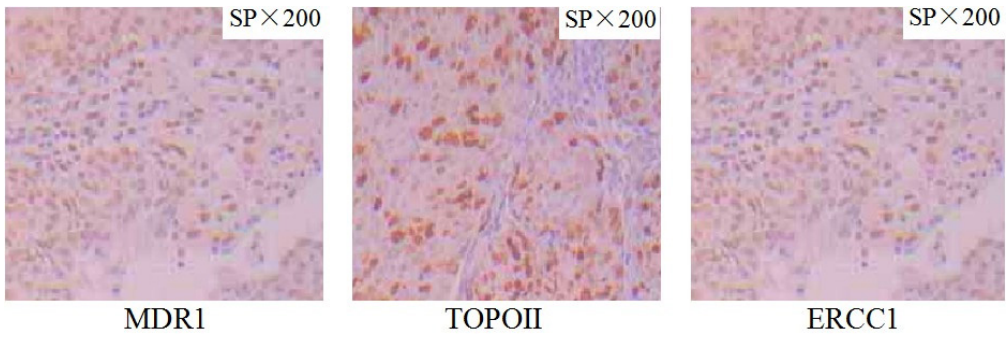

Figure 5. Immunohistochemical staining of *TOPOII*, *ERCC1* and *MDR1* in epithelial ovarian cancer.
NF-κB: nuclear factor kappa B; *IKKβ*: inhibitor of nuclear factor kappa B kinase subunit β; *MDR1*: Multi-drug resistance protein 1; *TOPOII*: topoisomerase II; *ERCC1*: Excision Repair Cross-Complementation Group 1.

mucinous carcinoma, 24 cases were *IKKβ* positive, and the expression rate was 58.54%. Among 9 other cancer types, 5 cases were *IKKβ* positive and the expression rate was 55.56%. The difference in expression rate between the two groups was not significant ($P>0.05$). Among 28 cases of epithelial ovarian cancer with high differentiation, 11 cases were positive for *IKKβ* and the expression rate was 39.29%. Among 22 cases with poor differentiation, 18 cases were positive for *IKKβ* and the expression rate was 81.82%. The difference in expression rate between the two groups was statistically significant ($P<0.05$). Among 34 cases of epithelial ovarian cancer with lymph node metastasis, 25 cases were positive for *IKKβ* and the expression rate was 73.53%. Among the 16 cases without lymph node metastasis, there were 4 cases with positive expression of *IKKβ* and the expression rate was 25%., The difference in expression rate between the two groups was statistically significant ($P<0.05$). The results are shown in Table 3.

Expression of *TOPOII*, *ERCC1* and *MDR1* in epithelial ovarian cancer

In epithelial ovarian cancer, *TOPOII*, *ERCC1* and *MDR1* stained positive. In addition, *NF-κB*, *IKKβ* and *TOPOII*, *ERCC1* and *MDR1* mRNAs were co-expressed in epithelial ovarian cancer. The results are shown in Fig. 5.

The relationship between the expression of *NF-KB* and *IKKβ* and *MDR1*, *TOPOII*, *ERCC1* genes

There was a positive correlation between the expression of *NF-κB, IKKβ* and *MDR1* based on (r=0.322; 0.329, $P<0.001$). There was a negative correlation between the expression of *NF-κB*, *IKKβ* and *TOPOII* (r=–0.506; –0.784, $P<0.001$). There was no correlation between the expression of *NF-κB, IKKβ* and *ERCC1* (r=–0.037; 0.135, $P=0.636$; 0.158). The results are shown in Table 4.

DISCUSSION

Currently, postoperative chemotherapy in epithelial ovarian cancer patients is essential.. However, due to the chemotherapy resistance of tumor cells, it is difficult for many patients to be cured. Therefore, exploring new tumor markers, detecting the expression of *NF-κB, IKKβ* and *MDR1, TOPOII, ERCC1*, and exploring the correlation between *NF-κB, IKKβ* and *MDR1, TOPOII, ERCC1* expression in epithelial ovarian cancer have certain significance for predicting chemotherapy resistance and guiding clinical rational drug use. *MDR1* is the first confirmed multidrug resistance-related gene, which is highly expressed in ovarian cancer tissues (Hartz *et al.*, 2018). The expression level of *TOPOII* is directly proportional to the histology grade of ovarian cancer, and the content of *TOPOII* is the highest in the cell proliferation and stage. It decreases in the late stage and is the lowest in the quiescent stage (Cottenden *et al.*, 2018). Moreover, chemotherapy resistance of epithelial ovarian cancer is related to *ERCC1*.

NF-κB is involved in the onset, development, and metastasis of malignant tumors by regulating the transcription of many factors related to adhesion and invasion (Matelski *et al.*, 2021). The positive expression rate of *NF-κB* and *IKKβ* in epithelial ovarian cancer is related to its clinical stage, histopathological grade and lymph node metastasis. It can be seen that *NF-κB* and *IKKβ* both participate in the onset, growth, metastasis and infiltration of epithelial ovarian cancer. Co-expression of *NF-*

Table 4. Logistic regression analysis of NF-κB and IKK β expression with MDR1, TOPOII, ERCC1 Resistance Related Genes

Variables positive		*NF-κB* positive	*NF-κB* negative	*R*-value	*P*-value	*IKKβ* positive	*IKKβ* negative	*R*-value	*P*-value
MDR1	positive	33	2	0.322	0.001	26	3	0.329	0.001
	negative	5	10			4	17		
TOPOII	positive	1	11	–0.506	0.001	1	19	–0.784	0.001
	negative	37	1			29	1		
ERCC1	positive	22	4	–0.037	0.636	17	9	0.135	0.158
	negative	16	8			13	11		

Abbreviations: *NF-κB*: nuclear factor kappa B; *IKKβ*: inhibitor of nuclear factor kappa B kinase; *MDR1*: multidrug resistance gene 1; *TOPOII*: topoisomerase II; *ERCC1*: nucleotide excision repair cross complementary group 1.

κB and *IKKβ* with drug resistance related genes *MDR1, TOPOII* and *ERCC1* in epithelial ovarian cancer suggested that *NF-κB* and *IKKβ* are related to drug resistance of epithelial ovarian cancer.

It has been found that *IKKβ* of *NF-κB* inhibitors family had decreased expression in oral squamous epithelium close to tumor, and also has decreased expression in epithelial dysplasia tissues, but its expression is increased in oral squamous cell carcinoma (Song *et al.*, 2019). The abnormal expression of *NF-κB* is closely related to malignant tumors. Inactivation of tumor suppressor gene and increased activation of *NF-κB* caused by *IKKβ* downregulation can often be found in cancer cells (Al-Huseini *et al.*, 2018). In addition, *NF-κB* can exert different cellular effects. For example, *NF-κB* can promote apoptosis and activate tumor suppressor gene *P53* or enhance tumor progression by regulating the proliferation of tumor cells (Li *et al.*, 2018; Lkhagvasuren *et al.*, 2019; Zhang *et al.*, 2018). In addition, *IKKβ* also plays an important role in anti-apoptosis.

This study had several limitations. First, there was unavoidable confounders in this study due to its retrospective nature. Second, it was a single center study with small sample size, and studies with large number of samples should be conducted in the future. Third, the predicted prognostic value of *NF-κB* and *IKKβ* in epithelial ovarian cancer should be verified in animal study or clinical practice.

In conclusion, the expression of *NF-κB* and *IKKβ* was significantly increased in epithelial ovarian cancer samples originating from the patients with higher clinical stage, lower tissue differentiation and lymph node metastasis, which indicated that *NF-κB* and *IKKβ* were related to the ovarian cancer progression. The positive expression rates of *NF-κB* and *IKKβ* in epithelial ovarian cancer were significantly higher than those in benign ovarian tumors and normal ovaries. The expression of *NF-κB* and *IKKβ* was positively correlated with *MDR1* and negatively correlated with *TOPOII*. The correlation of *NF-κB* and *IKKβ* expression with *MDR1*, *TOPOII* and *ERCC1* expression could potentially predict the drug resistance in chemotherapy patients.

Declarations

Disclosure. None

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