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# DNA Repair Genes ERCC1 and BRCA1 Expression in Non-Small Cell Lung Cancer Chemotherapy Drug Resistance

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**Background:** Surgery combined with chemotherapy is an important therapy for non-small cell lung cancer (NSCLC). However, chemotherapy drug resistance seriously hinders the curative effect. Studies show that DNA repair genes ERCC1 and BRCA1 are associated with NSCLC chemotherapy, but their expression and mechanism in NSCLC chemotherapy drug-resistant cells has not been elucidated.

**Material/Methods:** NSCLC cell line A549 and drug resistance cell line A549/DDP were cultured. Real-time PCR and Western blot analyses were used to detect ERCC1 and BRCA1 mRNA expression. A549/DDP cells were randomly divided into 3 groups: the control group; the siRNA-negative control group (scramble group); and the siRNA ERCC1 and BRCA1siRNA transfection group. Real-time PCR and Western blot analyses were used to determine ERCC1 and BRCA1 mRNA and protein expression. MTT was used to detect cell proliferation activity. Caspase 3 activity was tested by use of a kit. Western blot analysis was performed to detect PI3K, AKT, phosphorylated PI3K, and phosphorylated AKT protein expression.

**Results:** ERCC1 and BRCA1 were overexpressed in A549/DDP compared with A549 ( $P < 0.05$ ). ERCC1 and BRCA1siRNA transfection can significantly reduce ERCC1 and BRCA1 mRNA and protein expression ( $P < 0.05$ ). Downregulating ERCC1 and BRCA1 expression obviously inhibited cell proliferation and increased caspase 3 activity ( $P < 0.05$ ). Downregulating ERCC1 and BRCA1 significantly decreased PI3K and AKT phosphorylation levels ( $P < 0.05$ ).

**Conclusions:** ERCC1 and BRCA1 were overexpressed in NSCLC drug-resistant cells, and they regulated lung cancer occurrence and development through the phosphorylating PI3K/AKT signaling pathway.

**MeSH Keywords:** 1-Naphthylamine • Abnormalities, Drug-Induced • Genes, BRCA1

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/896606>

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## Background

Millions of people worldwide die of lung cancer every year. Lung cancer has the highest mortality rate among all malignant tumors. Its incidence and mortality in China are the highest in the world. In the past 20 years, lung cancer in China has become increasingly common in younger people, with a trend toward higher morbidity and mortality [1,2]. Lung cancer mainly includes small cell lung cancer (SCL) and non-small cell lung cancer (NSCLC), according to pathology, and the incidence of NSCLC accounts for more than 80% of all cases [3]. Lung cancer treatment has greatly advanced, including improved surgical technique and a variety of new chemotherapy drugs. Chemotherapy is an indispensable adjuvant therapy for lung cancer [4]. During the process of chemotherapy, however, lung cancer patients often develop secondary resistance after 10–14 months, which seriously limits the clinical effectiveness of chemotherapy. Overall, lung cancer patient survival and prognosis are still poor [5,6]. The mechanism of NSCLC drug resistance has not been fully defined. Research shows it may be related to the increasing ability of the body to inactivate chemotherapy drugs, compound formation, reducing drug accumulation, or DNA repair ability enhancement in the treatment process. DNA repair ability enhancement plays a key role in drug resistance [7,8].

Excision repair cross-complementary gene 1 (ERCC1) and breast cancer susceptibility gene 1 (BRCA1) are important members of the DNA repair-related gene system [9,10]. Studies showed that they can reflect the sensitivity to chemotherapy drugs, and their overexpression can affect the clinical curative effect of chemotherapy drugs in NSCLC [11,12]. However, ERCC1 and BRCA1 expression and related mechanism in NSCLC chemotherapy drug-resistant cell lines have not been fully elucidated. This study intended to provide an experimental basis for clinical lung cancer drug resistance through analyzing the expression and function of ERCC1 and BRCA1 in NSCLC drug-resistant cell lines.

## Material and Methods

### Main instruments and reagents

A549 and A549/DDP cell lines were purchased from the ATCC Cell Bank (USA). DMEM medium, fetal bovine serum (FBS), EDTA, and penicillin-streptomycin were bought from Hyclone (USA). Dimethyl sulfoxide and MTT powder were from Gibco. Tyresin-EDTA was from Sigma (USA). PVDF membrane was from Pall Life Sciences. Western blot-related chemical reagents were obtained from Beyotime. ECL reagent was from Amersham Biosciences. ERCC1, BRCA1, PI3K, AKT, phosphorylated PI3K, and phosphorylated AKT monoclonal antibodies, and

HRP-tagged IgG secondary antibody were from Cell Signaling. Caspase 3 activity kits were purchased from Pall Life Sciences. RNA extraction kits and reverse transcription kits were from Axygen (USA). Other common reagents were purchased from Sangon. The Labsystem version 1.3.1 microplate reader was purchased from Bio-Rad (USA).

### Methods

#### A549 and A549/DDP cell culture and grouping

A549 and A549/DDP cell lines were preserved in liquid nitrogen and thawed in a 37°C water bath. After centrifugation at 1000 rpm for 3 min, the cells were cultured in fresh medium and maintained at 37°C and 5% CO<sub>2</sub>. A549 and A549/DDP cells were seeded into dishes at 1×10<sup>6</sup>/cm<sup>2</sup> and maintained in high-glucose DMEM containing 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin. The 3<sup>rd</sup> to 8<sup>th</sup> generation cells in logarithmic phase were used for experiments. A549/DDP cells were randomly divided into 3 groups: the control group, the siRNA-negative control group (scramble group), and the siRNA group.

#### ERCC1 and BRCA1 siRNA transfection

ERCC1 siRNA and BRCA1 siRNA were transfected to A549/DDP cells, respectively. ERCC1 siRNA primers: forward, 5'-ATATCTGCCAACGTGCTACGT-3'; reverse, 5'-CGTACTCGAATTCAGCTACGT-3'. ERCC1 siRNA negative control primers: 5'-ACTTCCGTAATGGACCTACGT-3', 5'-ACCCTTCAATGGACACGGTTT-3'. BRCA1 siRNA primers: forward, 5'-CTGGA TTTACGTCGACCTCAA -3'; reverse, 5'-CTTTGGCGCATAAGACCGT -3'. BRCA1 siRNA negative control primers: 5'-CTCTATGAACACCTAGCT-3', 5'-ACCTTGAACCTAGCT-3'. After cell density reached 70–80%, ERCC1 siRNA and BRCA1 siRNA together with liposome were added to 200 µl serum-free medium, while negative control liposome was added to another medium for 15 min incubation at room temperature. Lipo2000 was then mixed with ERCC1 and BRCA1 siRNA for 30 min at room temperature. After changing cell medium to 1.6 ml serum-free medium, the mixture was added to each group and incubated in 37°C and 5% CO<sub>2</sub> for 6 h. After changing the medium, the cells were then cultured for 48 h for the following experiment.

#### Real time PCR

Total RNA was extracted using Trizol and reverse transcribed to DNA according to the manufacturer's instructions. Primers were designed by Primer 6.0 and synthesized by Invitrogen (Table 1). Real-time PCR was used to test target genes. The reaction was performed at 55°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. GAPDH was selected as the internal reference. The results were calculated by 2<sup>-ΔCt</sup> method.

**Table 1.** Primer sequence.

Gene	Forward 5'-3'	Reverse 5'-3'
GADPH	AGTACCAGTCTGTTGCTGG	TAATAGACCCGGATGTCTGGT
ERCC1	ATGAACCTTCTCTGTCTTGG	TCACCG CCTCGGCTTGTCACA
BRCA1	CTCCCACAGACTCTGTAAG	GCATTACCTGGGGCTGTAATT

### MTT assay

A549/DDP cells in logarithmic phase were seeded in 96-well plates at 3000/well and divided into control group, scramble group, and siRNA group, with 5 replicates each. After 48 h, 20  $\mu$ l MTT solution at 5 g/L was added to each well for 4 h in the incubator. After removing the supernatant, 150  $\mu$ l DMSO was added and the plate was vibrated for 10 min. The plate was read on a microplate reader at 570 nm to calculate the cell proliferation rate.

### Caspase-3 activity detection

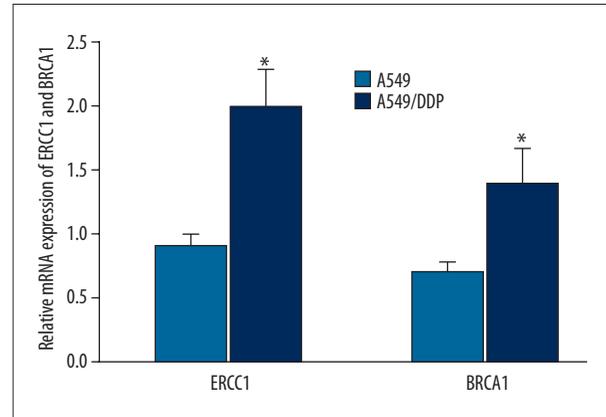
Caspase 3 activity was determined according to the manufacturer's instructions. After enzyme digestion, the cells were centrifuged at 4°C and 600 g for 5 min. After being cracked on ice for 15 min, the cells were centrifuged at 4°C and 20 000 g for 5 min. The cells were detected at 405 nm to calculate caspase 3 activity after adding 2 mM Ac-DEVD-pNA. All experiments were repeated 3 times.

### Western blot analysis

A549/DDP cells were cracked on ice for 15–30 min after adding cell lysis. After ultrasonication at 5 s  $\times$  4, the cells were centrifuged at 4°C and 10 000 g for 5 min and the supernatant was moved to a new EP tube. The protein was stored at –20°C. Total protein was separated by 10% SDS-PAGE electrophoresis and transferred to PVDF membrane by half-dry method. After blocking with 5% skim milk for 2 h, the membrane was incubated in various concentrations of ERCC1, BRCA1, PI3K, AKT, phosphorylated PI3K, and phosphorylated AKT antibody at 4°C overnight. After being incubated with secondary antibody at 1:2000, the membrane was exposure-imaged on X-ray film. Quantity One software was used to determine binding density. All experiments were repeated 4 times.

### Statistical analysis

All statistical analyses were performed on SPSS 16.0 software. Measurement data are presented as mean  $\pm$  standard deviation ( $\bar{x} \pm SD$ ) and compared using one-way ANOVA.  $P < 0.05$  was considered as statistically significant.



**Figure 1.** ERCC1 and BRCA1 mRNA expression in A549 and A549/DDP \*  $P < 0.05$ , compared with A549.

## Results

### ERCC1 and BRCA1 mRNA expression in A549 and A549/DDP

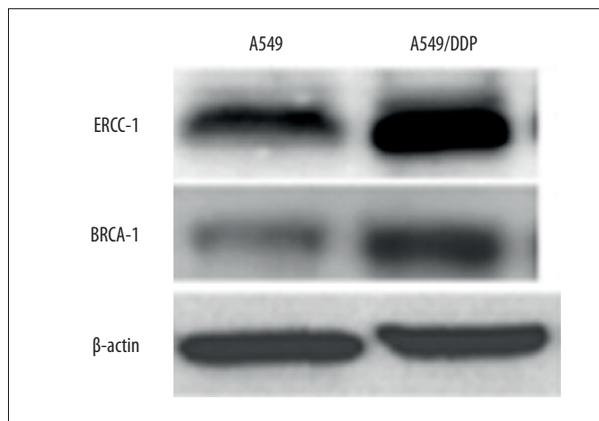
Real-time PCR results showed that ERCC1 and BRCA1 mRNA were overexpressed in A549/DDP, compared with A549 ( $P < 0.05$ ) (Figure 1).

### ERCC1 and BRCA1 protein expression in A549 and A549/DDP cells

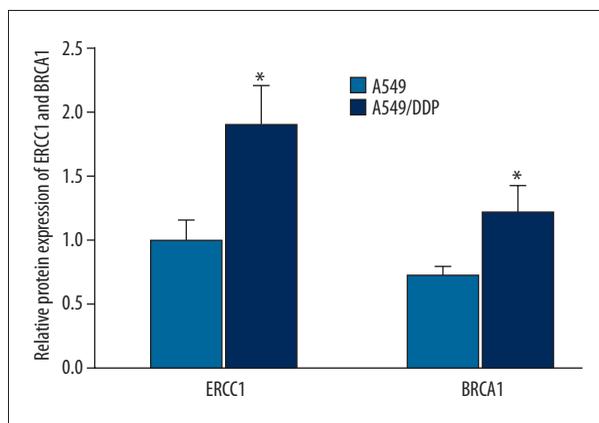
Western blot analysis was used to compare ERCC1 and BRCA1 protein expression in A549 and drug-resistant cell line A549/DDP. We found that, similar to mRNA results, ERCC1 and BRCA1 protein expression was clearly elevated in A549/DDP compared with A549 ( $P < 0.05$ ) (Figures 2, 3), suggesting that ERCC1 and BRCA1 overexpression was closely related to NSCLC drug resistance.

### siRNA impact on ERCC1 and BRCA1 expression in A549/DDP

ERCC1 and BRCA1 siRNA were co-transfected to A549/DDP cells for 48 h. The results showed that ERCC1 and BRCA1 siRNA can significantly suppress ERCC1 and BRCA1 mRNA and protein expression in A549/DDP cells ( $P < 0.05$ ) (Figures 4, 5).



**Figure 2.** ERCC1 and BRCA1 protein expression in A549 and A549/DDP.



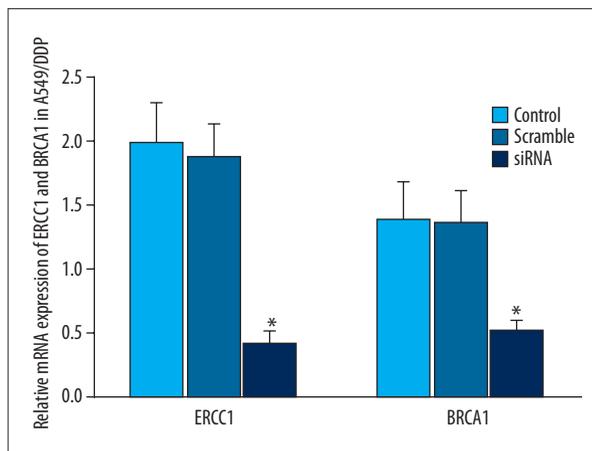
**Figure 3.** ERCC1 and BRCA1 protein expression analysis in A549 and A549/DDP cell. \*  $P < 0.05$ , compared with A549.

### Downregulating ERCC1 and BRCA1 expression impact on A549/DDP cell proliferation

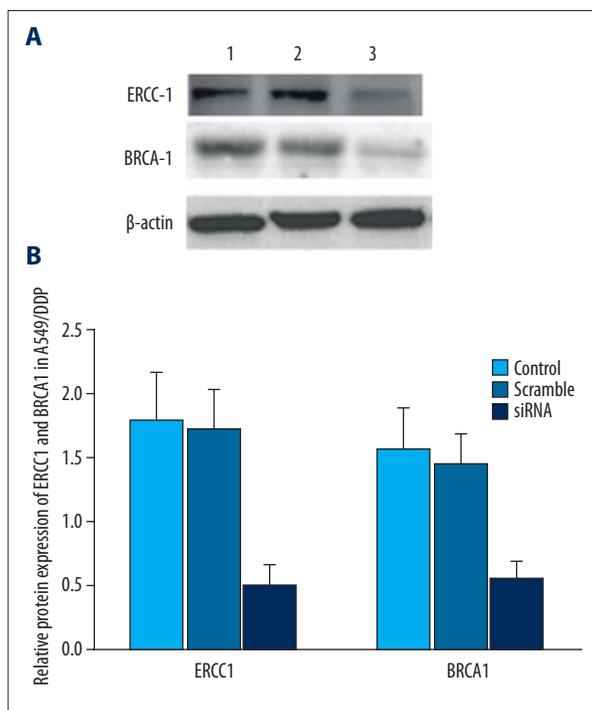
MTT assay was used to determine the effect of downregulating ERCC1 and BRCA1 on A549/DDP cell proliferation. The results revealed that ERCC1 and BRCA1 siRNA co-transfection reduced ERCC1 and BRCA1 expression and obviously suppressed A549/DDP cell proliferation ( $P < 0.05$ ) (Figure 6), indicating that downregulating ERCC1 and BRCA1 in NSCLC facilitated suppressing drug-resistant cell proliferation.

### Effect of downregulating ERCC1 and BRCA1 expression on caspase 3 activity in A549/DDP

Downregulating ERCC1 and BRCA1 expression markedly increased caspase 3 activity in A549/DDP ( $P < 0.05$ ) (Figure 7), suggesting that downregulating ERCC1 and BRCA1 can mediate cell apoptosis and promote drug-resistant cell death.



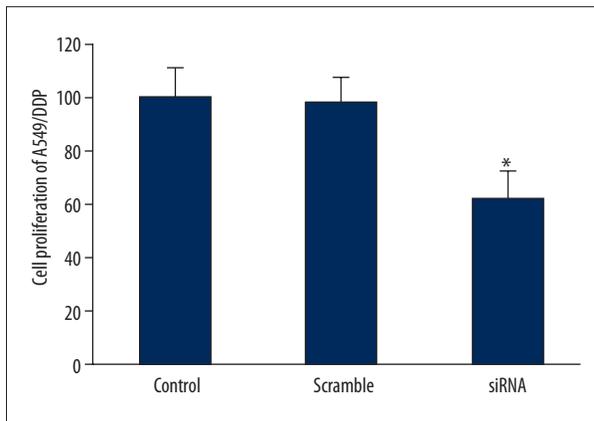
**Figure 4.** Effect of siRNA on ERCC1 and BRCA1 expression in A549/DDP. \*  $P < 0.05$ , compared with control.



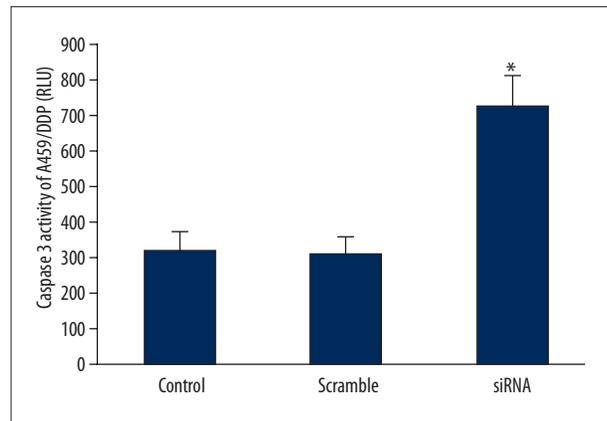
**Figure 5.** Effect of siRNA on ERCC1 and BRCA1 protein expression in A549/DDP. (A) ERCC1 and BRCA1 protein expression in A549/DDP. (B) ERCC1 and BRCA1 protein expression analysis in A549/DDP. 1 – control; 2 – scramble group; 3 – siRNA group. \*  $P < 0.05$ , compared with control.

### Effect of downregulating ERCC1 and BRCA1 expression on PI3K/AKT signaling pathway

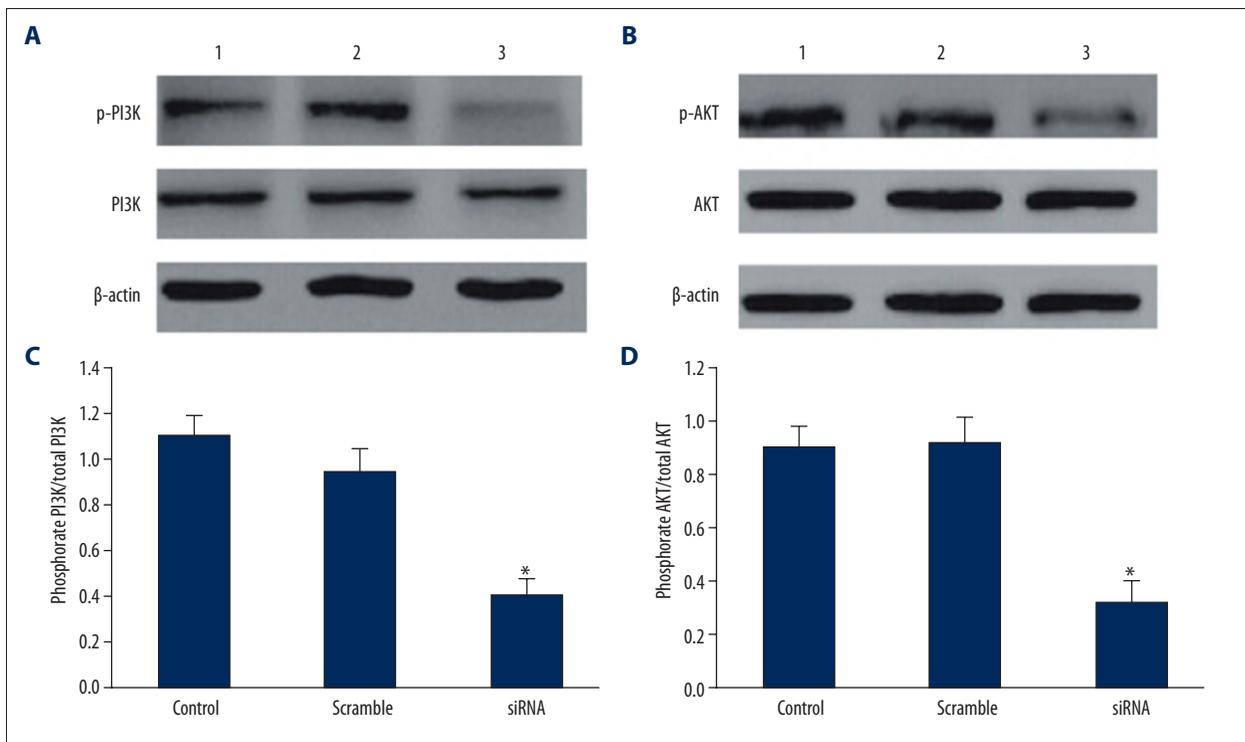
Downregulating ERCC1 and BRCA1 significantly decreased PI3K and AKT phosphorylation levels ( $P < 0.05$ ), but PI3K/AKT total protein level showed no statistical changes (Figure 8), suggesting that ERCC1 and BRCA1 can regulate drug-resistant lung



**Figure 6.** Effect of downregulating ERCC1 and BRCA1 expression on A549/DDP cell proliferation. \*  $P < 0.05$ , compared with control.



**Figure 7.** Effect of downregulating ERCC1 and BRCA1 expression on caspase 3 activity in A549/DDP. \*  $P < 0.05$ , compared with control.



**Figure 8.** Effect of downregulating ERCC1 and BRCA1 expression on PI3K/AKT signaling pathway. (A) Effect of downregulating ERCC1 and BRCA1 expression on PI3K expression. (B) Effect of downregulating ERCC1 and BRCA1 expression on AKT expression. (C) Effect of downregulating ERCC1 and BRCA1 expression on PI3K expression analysis. (D) Effect of downregulating ERCC1 and BRCA1 expression on AKT expression analysis. 1 – control; 2 – scramble group; 3 – siRNA group. \*  $P < 0.05$ , compared with control.

cancer cell proliferation through inhibiting PI3K/AKT phosphorylation level.

## Discussion

First-line chemotherapy drugs for NSCLC include platinum, such as cisplatin and carboplatin, while platinum resistance often

leads to chemotherapy failure [13,14]. Platinum drugs can combine with nucleophilic DNA in tumor cells to form DNA-platinum adduct, thus inhibiting DNA replication, causing DNA damage, and ultimately inducing tumor cell death [15]. However, the DNA damage repair system can modify DNA damage to various extents, which affects the sensitivity of chemotherapy drugs. Therefore, the repair ability of DNA repair genes is closely related to the effectiveness of lung cancer chemotherapy. High

repair ability induces chemotherapy resistance formation and causes chemotherapy failure [16].

In the clinical trials, ERCC1 and BRCA1 expression increased significantly in NSCLC patients with chemotherapy resistance. Thus, they can reflect the sensitivity of patients to chemotherapy drugs, as well as indicating prognosis [11]. However, the mechanism of ERCC1 and BRCA1 in NSCLC chemotherapy drug-resistant cells has not been fully defined. This research analyzed ERCC1 and BRCA1 mRNA and protein expression in NSCLC chemotherapy drug-resistant cells and drug-sensitive cells, and discovered that ERCC1 and BRCA1 are overexpressed in cell line A549/DDP, which is consistent with clinical findings [11,12]. Furthermore, we co-transfected ERCC1 and BRCA1siRNA to the drug-resistant cell line, thus confirming that downregulating ERCC1 and BRCA1 expression in NSCLC can regulate apoptosis, inhibit cell proliferation, and promote drug-resistant lung cancer cell death. The cellular DNA repair system includes base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and double-strand break repair (DSBR). During DNA damage identification and removal, NER is the rate-determining step in the DNA repair pathway. ERCC1 and BRCA1 are important members of the NER pathway, which may reflect NER repair level through changing expression and activity reaction [17,18]. After removing the DNA-damaged part, ERCC1 and BRCA1 utilize ribonucleotide provided by nucleotide reductase-regulating subunits M1 to fill the gap, so as to complete DNA synthesis and repair. They are key materials in the DNA repair system [19]. Therefore,

targeting BRCA1 and ERCC1 can significantly inhibit DNA synthesis and repair, leading to tumor cell death.

This study discovered that downregulating BRCA1 and ERCC1 suppressed PI3K/AKT phosphorylation levels to regulate chemotherapy-resistant lung cancer cell proliferation. The PI3K/Akt signaling pathway plays an important role in physiological and pathological process of tumor occurrence and development. In microvascular endothelial cells of NSCLC, endothelin-1 mediates the PI3K/AKT signaling pathway through tyrosine kinase C-SRC. It then activates C-Jun/AP-1 protein coupling and combines with the corresponding locus of COX-2 promoter, thus promoting COX-2 transcription and expression, and facilitating prostaglandin E2 (PGE2) biosynthesis and release, thereby accelerating angiogenesis [20]. PI3K/AKT can promote tumor angiogenesis, induce downstream signaling molecule structural changes, and the activate bypass signaling pathway and epithelial-mesenchymal transformation, which are related to drug resistance [21,22].

## Conclusions

ERCC1 and BRCA1 are overexpressed in NSCLC drug-resistant cells, and regulate lung cancer occurrence and development through the PI3K/AKT pathway. Therefore, detecting ERCC1 and BRCA1 expression can not only help in NSCLC diagnosis and prognosis, but also provide a reference basis for individualized treatment in patients with advanced NSCLC.

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