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ERCC5 abnormal expression influences the biological behaviour of ovarian cancer by Wnt pathway

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Nucleotide excision repair (NER) is the most important repair pathway of DNA, and a variety of cancers are closely related to the gene abnormal expression and the deletion or downregulation of gene function in NER pathway. In this study, we investigated the differential expression of ERCC5 in different ovarian cancer cells and its potential mechanism. By detecting the expression of ovarian in both SKOV3 and Caov4 cell lines, we found that the expression of ERCC5 in SKOV3 was significantly higher than that in Caov4. We further constructed the SKOV3 cell line with low ERCC5 expression by RNA interference and Caov4 cell line with high ERCC5 expression by transient transfection, then comparing the effect of ERCC5 abnormal expression on the biological behaviour of ovarian cancer cells. The results showed that high expression of ERCC5 could significantly promote proliferation and migration of ovarian cancer but inhibit apoptosis. However, low expression of ERCC5 could significantly promote apoptosis but inhibited proliferation and migration. We further observed the effect of ERCC5 abnormal expression on potential regulation pathway by Western blot and noticed that ERCC5 abnormal expression could activate the Wnt pathway. Overall, the results revealed that ERCC5 abnormal expression could influence the proliferation, apoptosis and migration ability of ovarian cancer cells by activating the Wnt pathway.

Keywords: Nucleic acid repair pathway, Tumor

Epithelial ovarian cancer, referred to as ovarian cancer, is one of the most common gynecological malignant tumors in women with high mortality, rapid transfer, strong invasion ability and high recurrence rate. World over, with 3,13,959 new cases and 207 252 fatality in 2020, ovarian cancer ranks 18th in incidence and 14th in mortality among all types of cancers. Over all, Asia accounted for 54.4% of total cases with 54.5% mortality[1,2]. USA is reported to have an estimated ovarian cancer incidence of 19,880 with 2810 fatalities in 2022[3]. Five-year survival rate of advanced ovarian cancer patients is less than 30%. Because there is no specific symptom of early ovarian cancer, and the lack of high specificity and high sensitivity diagnostic methods in clinical, more than 70% of patients are in advanced stage when definite diagnosed[2].

The maintenance of DNA repair ability (DRC) depends on the sufficient expression of DNA repair gene, and the lack of expression could not activate the repair pathway, resulting in the decrease of DRC and the increase of tumor susceptibility[4,5]. Nucleotide excision repair (NER) is the most important DNA repair pathway that can repair DNA damage in a broad spectrum, including UV-induced damage, DNA adducts and some forms of oxidative damage, and at least 30 kinds of proteins were involved[5,6]. The ERCC5 gene, also known as the XPG gene, is one of the eight key genes of the NER pathway[7]. There are five major DNA repair pathways in the human body. Among them, NER pathway mainly repairs endogenous and exogenous DNA damage affecting regional chromosome structure to maintain the stability of DNA, and its genetic alteration will affect DNA repair ability, resulting in abnormal DNA replication and abnormal cell proliferation and differentiation[7]. Therefore, the occurrence of many tumors is closely related to the changes of gene expression and the deletion or downregulation of gene function in NER pathway. The role of the ERCC5 gene in the NER pathway is to recognize and cleave the 3' end of the damaged DNA strand and to function as a non-enzymatic role for ERCC1 gene which is responsible for cleaving the 5' end in the NER pathway[8].

Many studies have found that the abnormal expression of NER pathway gene plays an important role in the development and prognosis of many kinds of tumors. For example, Zhao *et al.*[9] found that elevated expression of ERCC6 in colorectal cancer can cause resistance to 5-fluorouracil and was associated with poor prognosis. Jeong YH *et al.*[10] found that the survival time of non-small cell lung cancer patients with ERCC1 high expression was significantly shortened. As an important member of the NER pathway gene, ERCC5 has also been found

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to be abnormally expressed in a variety of tumors, including ovarian cancer. Walsh CS *et al*.[11] found that ERCC5 was abnormally expressed in ovarian cancer tissues, and the survival of patients with low expression of ERCC5 was significantly prolonged. Here, we studied the impact of ERCC5 abnormal expression on the biological behaviour of ovarian cancer and the potential mechanism which has not yet been reported.

Materials and Methods

Ovarian cancer cell lines SKOV3 and Caov4 were selected in this study. SK-OV-3 is a human ovarian cancer cell line with epithelial-like morphology. These cells are resistant to tumor necrosis factor and to other cytotoxic drugs such as diphtheria toxin, cisplatin, and adriamycin[12,13]. The Caov-4 cell line is an ovarian cancer cell line with epithelial morphology that grows in adherent culture. These cells harbor a loss-of-function mutation in the *p53* gene and are sensitive to cisplatin[14]. Real-time PCR and western blot were carried out to compare the expression of ERCC5 in this two cell lines. We further observed the effect of low expression or overexpression of ERCC5 on biology behaviour of colorectal cancer cells by real-time PCR and western blot. Finally, we explored the effect of ERCC5 abnormal expression on Wnt pathway by Western blot.

Cell lines and cell culture

Ovarian cancer cell lines SKOV3 and Caov4 were both purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). SKOV3 silence cell line (SKOV3 KD) and Caov4 over-expression cell line (Caov4 OE) were all constructed by transient transfection with lipo2000 according to the manufacturer's specification. Interference RNA was purchased from Guangzhou Geneseed Company and plasmid was purchased from Shanghai Genechem Company. SKOV3 was cultured with McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher), while Caov4 was cultured with L15 medium supplemented with 20% FBS (Hyclon). The cell incubator was set to 5% carbon dioxide and the temperature was 37°C. In addition, Ki-67, PNCA, caspase-3, TFAR-19, MMP-9 and NM23 which can indirectly reflect the ability of proliferation, apoptosis and invasion were selected in this study and detected by real-time PCR and Western blot.

RNA extraction and real-time PCR

Trizol buffer was used to extract total RNA (Thermo Fisher Scientific, Massachusetts, USA). RNA was reverse transcribed to cDNA by PrimeScript RT Master Mix (Takara). Real-time PCR was performed on Eppendorf equipment using SYBR Premix Ex Taq (Takara, Liaoning, China) based on the manufacturer's protocols.

Protein extraction and western blot analysis

RIPA buffer was used to extract total proteins (Aidlab Biotechnologies, Beijing, China). Tissue lysates were centrifuged at 12,000 rpm for 30 min at 4°C. Supernatant was collected and protein was quantified with a BCA reagent kit. Lysates were boiled at 100°C for 5 min and 40 g of total protein was separated by 4-12% SDS-PAGE and then transferring to polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were blocked with no-fat milk for 1 h and then incubated with primary antibodies at 4°C overnight. The membranes were then incubated with secondary antibodies for 1–2 h at room temperature(22-25^{o}C). Chemiluminescence reagent ECL Plus was used to visualize the bands and the results were analyzed by Image J software.

Statistical analysis

All statistical analyses were performed using SPSS18.0 software (Chicago, IL, USA). Comparison of gene differential expression between SKOV3NC, Caov4NC and SKOV3KD, Caov4OE was performed by student's t test. Two-tailed P values <0.05 was considered statistically significant.

Results

Expression of ERCC5 in SKOV3 cells

We found that the expression of ERCC5 in SKOV3 cells was significantly higher than that in Caov4 cells by real-time PCR and Western blot at both mRNA and protein level (P <0.05). SKOV3 cells with ERCC5 low expression (SKOV3 KD), Caov4 cells with ERCC5 overexpression (Caov4 OE) and negative control (SKOV3NC, Caov4NC) were constructed by transient transfection reagent lipo2000. Real-time PCR and western blot were performed to confirm the results at the mRNA and protein levels, respectively (Fig. 1).

Low expression of ERCC5 promotes SKOV3 cell apoptosis but inhibit its proliferation and invasion

We found that low ERCC5 expression can significantly inhibit the proliferation and invasion of SKOV3 cells by detection the expression of Ki-67, PNCA, NM23 and MMP9, while significantly promote SKOV3 cell apoptosis by detecting the expression of caspase-3 and TFAR-19 (Fig. 2).

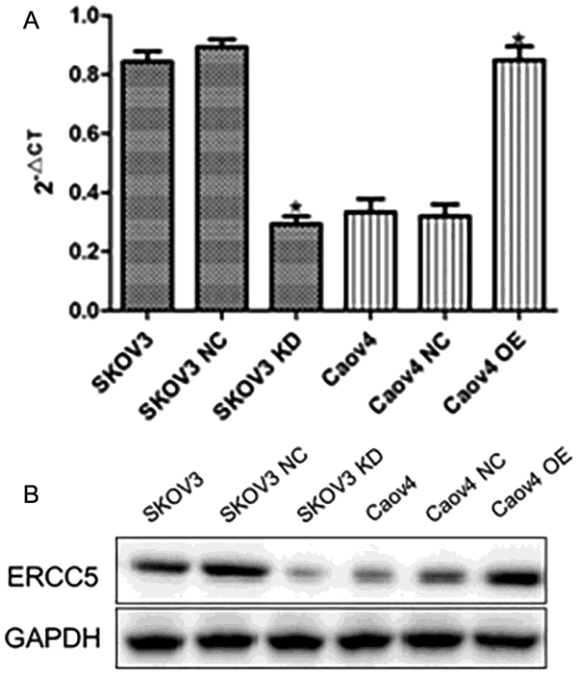

Fig. 1 — Expression of ERCC5 in SKOV3 and Caov4 cells. (A) RT-PCR; and (B) Western blot

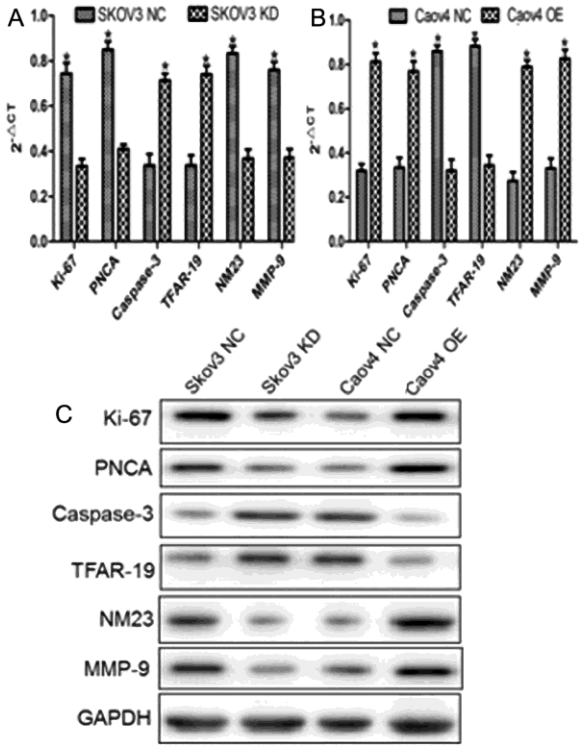

Fig. 2 — Abnormal expression of ERCC5 in SKOV3NC, SKOV3KD and Caov4NC Caov4OE cells. (A and B) RT-PCR; and (C) Western blot

Overexpression of ERCC5 can significantly promote proliferation and invasion of Caov4 cells but inhibit its apoptotic ability

We found that overexpression of ERCC5 can significantly promote the proliferation and invasion of Caov4 cells by detection the expression of Ki-67, PNCA, NM23 and MMP9, while significantly inhibit Caov4 cell apoptosis by detecting the expression of caspase-3 and TFAR-19 (Fig. 2).

Abnormal expression of ERCC5 can activate Wnt pathway

We further chose the node proteins including P38 (phosphorylation P38), JNK (phosphorylation JNK) and ERK (phosphorylation ERK) of MAPK pathway, the node protein Ik-Bα of NF-kB pathway and the node protein β-catenin of Wnt pathway for further study. Western blot was used to detect the expression of the above node proteins in SKOV3NC, SKOV3KD and Caov4NC, Caov4OE. The results showed that low or high expression of ERCC5 did not affect the expression of P38 (phosphorylated P38), JNK (phosphorylated JNK), IkB-α and ERK (phosphorylated ERK), but can significantly affect β-catenin expression (Fig. 3).

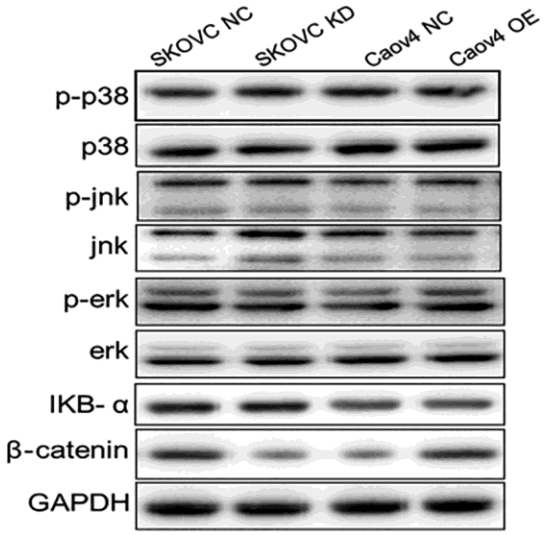

Fig. 3 — Abnormal expression of ERCC5 node proteins in SKOV3NC, SKOV3KD and Caov4NC Caov4OE cells

Discussion

DNA, due to its structural specificity, is vulnerable to attack by various intrinsic and exogenous physicochemical factors, causing genomic instability, while genomic instability is a tumor susceptibility root cause. DNA repair process is the most important part of the identification of DNA damage. Although there are many repair DNA damage mechanisms in human body, nucleotide excision repair (NER) is the most important DNA damage repair system. ERCC5 (Excision repair cross complementation group 5),

also known as XPG (Xeroderma pigmentosum pomplementaury group G)[15], belongs to the RAD2/ XPG family and is one of the key factors of NER repair pathway. It is located on chromosome 13q33, and is a kind of specific nuclease which is responsible for the 3' endonuclease in mammalian nucleic acid excision repair and is also involved in the xPF/ERCCl complex-mediated 5' endogenesis[16-18]. Studies have shown that ERCC5 gene is expressed in a variety of tumor tissues and cell lines and its expression level is related to the sensitivity of platinum drugs[10,19-23].

Many studies have found that ERCC5 gene plays an important role in the development and prognosis of many kinds of tumors[23,24]. However, the relationship between ERCC5 gene and the development of ovarian cancer is rarely reported at present. In this study, two ovarian cancer cell lines were selected. Cell lines with ERCC5 low expression and high expression were constructed by RNAi and plasmid transfection. Cell proliferation, apoptosis and invasion were assessed by real-time PCR and western blot to detect the expression of relevant indicators. The results showed that overexpression of ERCC5 could significantly promote the proliferation and invasion of ovarian cancer cells and inhibit the apoptosis of ovarian cancer cells. However, the low expression of ERCC5 could significantly inhibit the proliferation and invasion of ovarian cancer cells and promote their apoptosis. We further selected the node proteins of three classical signaling pathways including MAPK, Wnt and NF-kB pathway for western blot detection and found that abnormal expression of ERCC5 did not affect the activity of MAPK and NF-kB pathway, but can significantly activate the Wnt pathway, thereby affecting ovarian cancer biological behaviour of cells.

Conclusion

In this study, the role of ERCC5 gene in the development and progression of ovarian cancer and its mechanism was primarily explored by *in vitro* cytology experiments, in order to provide a new theoretical basis for the prevention and treatment of ovarian cancer.

Conflict of Interest

Authors declare no competing interests

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