Mechanism of DNA repair gene ERCC1 affecting breast cancer metastasis through NF- κ B signaling pathways

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Abstract

To explore the role of DNA repair gene ERCC1 in breast cancer metastasis and its signaling pathway. Through prospective cohort studies and cell tests, Western Blot and IHC were used to detect the expression of DNA repair gene ERCC1 and NF-κB signaling pathways related proteins, scratch test and Transwell to detect the ability of migration and invasion. To explore the mechanism of ERCC1 affecting breast cancer metastasis. Population results: There was no significant difference in clinicopathological features between the two groups ($P>0.05$). ERCC1 has a positive correlation with breast cancer metastasis ($r = 0.543$, $P<0.05$). And the metastasis-free survival rate of ERCC1 low expression group was higher than that of high expression group ($\chi^2=17.571$, $P<0.05$). The expression level of p65, p50, TNF α , IKK β in ERCC1 low expression group was significantly lower than that in ERCC1 high expression group ($P<0.05$), I κ B α was significantly higher than ERCC1 high expression group ($P<0.05$). The analysis of Spearman correlation method shows that ERCC1 expression is positively correlated with the

IKK β ($r=0.481$, $P<0.05$), IKK β and I κ B α were negatively correlated($r = -0.203$, $P<0.05$), I κ B α and p50 were negatively correlated($r = -0.275$, $P<0.05$), p50 and p65 were positively correlated($r=0.386$, $P<0.05$), positive correlation between p65 and TNF α ($r = 0.476$, $P<0.05$), TNF α is positively associated with breast cancer metastasis($r = 0.281$, $P<0.05$). Cell tests:At 24 hours, the migration distance of the MCF-7-ERCC1 knockdown group was less than that of the negative control group($P<0.05$). At 48 hours, the migration distance of MCF-7-ERCC1 knockdown group was less than that of blank control group and negative control group($P<0.05$). Compared with the blank control group and the negative control group, the number of cell invasion in the MCF-7-ERCC1 knockdown group decreased significantly ($P<0.001$). Compared with the blank control group, the number of cell invasion decreased in the negative control group($P<0.05$). The protein level of p65, p50, TNF α , IKK β in ERCC1 knockdown group was significantly lower than that in blank control group and negative control group($P<0.05$), I κ B α protein levels were significantly higher than those of the blank and negative control groups($P<0.05$). DNA repair gene ERCC1 may affect breast cancer metastasis by regulating NF- κ B signaling pathways.

Key words: ERCC1; Breast Cancer; metastasis; NF- κ B signaling pathways

Breast cancer is a very high incidence of malignant tumors, among American women, it ranks first in the mortality of malignant tumors¹.The study found that breast cancer metastasis is closely related to the regulatory system of DNA repair. In clinical treatment of tumors, related drugs are usually used to destroy their cell DNA, thus inducing apoptosis to eliminate cancer cells²⁻³.However, some tumor cells can induce metastasis by activating DNA repair⁴.

ERCC1 is a DNA repair gene, located on autologous chromosome 19 and containing 10 exons, which can encode ERCC1 proteins and act as structure-specific endonuclease in NER. NER pathways identify DNA damage by GG-NER or TC-NER and recruit multiple related factors, ERCC1/XPF bind to the 5' end of the damaged single strand and cut the DNA chain, remove the 25-30 nucleotide long oligonucleotides containing the damaged DNA, repair the nucleotide defects with DNA polymerase, and finally repair the nucleotide defects with DNA ligase⁵.Furthermore, ERCC1 also plays an important role in some aspects as inter-chain cross-linking repair, double-strand break repair and telomere maintenance. DSB is a severe DNA injury that can lead to cell death, genomic alterations, and tumor formation.

A number of clinical studies have confirmed that the expression level of ERCC1 is closely related to the efficacy and prognosis of platinum-containing chemotherapy in tumor patients.Metro G⁶ pointed out that in breast cancer, the efficacy of ERCC1 expressed in platinum-containing chemotherapy regimen was negatively correlated. Kasimir⁷ study found that patients with low ERCC1 expression were more likely to benefit from chemotherapy regimens containing platinum-based therapy drugs, while low ERCC1 expression in invasive breast cancer has been found to be associated with negative lymph node status. Abdel⁸ and other examined the relationship between ERCC1 expression and chemotherapy in breast cancer, showed that patients with negative ERCC1 expression had higher chemotherapy efficiency. Xiaosong Chen's⁹ study found that ERCC1 was an independent predictor of complete pathological remission in breast cancer patients receiving paclitaxel combined with carboplatin weekly regimen neoadjuvant chemotherapy, and the rate of complete pathological remission was higher in ERCC1 negative patients.EL Baiomy Mohamed Ali et al¹⁰ studies showed that the expression and survival time ERCC1 advanced triple negative breast cancer were negatively correlated with platinum effect. Above studies show that ERCC1 has a certain role in breast cancer resistance, its high expression suggests that chemotherapy effect is poor.

Current studies have found that ERCC1 expression is associated with cancer metastasis, Jianmin Fu¹¹ found that the expression level of ERCC1 in breast cancer was associated with lymph node metastasis in unchemotherapy group. Ma Wang¹² study found that the overall survival rate of ERCC1 negative expression was significantly higher than that of patients with positive expression. The higher the TNM stage and lymph node metastasis, the higher the positive predictive value of ERCC1 expression, which suggests a positive correlation between ERCC1 expression and tumor invasiveness and poor prognosis, consistent with studies such as Ota¹³. He Lang¹⁴ study found that ERCC1 highly expressed tumor tissue enhanced DNA injury repair and tumor more invasive. Xia Anzhou¹⁵ study found that adjuvant chemotherapy can reduce ERCC1 and inhibit tumor invasion and metastasis, suggesting that ERCC1 may be associated with tumor metastasis. Wang Yan¹⁶ study found that ERCC1 expression rate in esophageal squamous cell carcinoma was associated with tumor differentiation, lymph node metastasis, ERCC1 tumor recurrence and distant metastasis. Steffensen¹⁷ study found that the PFS and OS of patients with ERCC1 positive expression in ovarian cancer were lower than those in the negative expression group, and Hui EP¹⁸ found that ERCC1 can repair the damage of tumor cells DNA and reduce the body's autoimmune ability to remove cancer cells, so that ovarian cancer cells from macrophages, T lymphocytes and natural killer cells, which will lead to the continuous proliferation and metastasis of cancer cells in the cancer tissue of ovarian cancer patients, and then make the patient's condition deteriorate, suggesting that high level of ERCC1 can promote the proliferation, expansion and metastasis of tumor cells, and make the patients more prone to recurrence and after operation¹⁹. But the relationship between ERCC1 and metastasis in breast cancer is rarely studied.

Some studies have found that cancer cell DNA repair is closely related to IKK β , when drugs in cancer cells cause DNA damage, ATM is activated as the main kinase in many signal transduction pathways. It is involved in cell cycle checkpoint and repair signal network after DNA injury. IKK β plays an important role in NF- κ B pathway. Although there are several signal sources upstream, they all converge on the IKK. generally, the NF- κ B in the cytoplasm is in a state of inactivation due to its dimerization binding NF- κ B inhibiting protein formation of a trimer complex. NF- κ B has no activity in trimeric form. IKK β as kinases can phosphorylate I κ B α proteins, causing it to dissociate from trimers, NF- κ B dimers can be exposed and rapidly enter the nucleus from the cytoplasm, binding to specific sequences on nuclear DNA, and promoting transcription of related genes²⁰. The activated I κ B α is combined with p65/p50 to repair DNA injury. In ovarian and lung adenocarcinoma, overexpression of p65/p50 leads to rapid repair of cell injury at the G2/M stage, resulting in tumor necrosis factor TNF- α , which stimulates epithelial mesenchymal transformation to produce cancer cell metastasis, and repair the TNF- α caused by DNA injury can produce positive feedback on the NF- κ B pathway and activate the pathway continuously, studies have shown that blocking NF- κ B pathways attenuates MCF-7 cell invasion and migration²¹.

Therefore, in this study, patients with breast cancer in affiliated hospitals were studied, through in vitro cell tests and population cohort studies, Western Blot and IHC methods were used to detect the expression of DNA injury repair gene ERCC1 and NF- κ B pathway related proteins. To explore the mechanism of postoperative metastasis and provide important scientific basis for the prevention of postoperative metastasis and survival rate.

Methods

1 Population aspects

1.1 Follow-up cohort

A follow-up cohort of breast cancer patients was established after the first confirmed operation in the affiliated Hospital of Southwest Medical University. The cohort was established in January 2013 and included in the study year by year. At present, there are 1360 patients in the cohort, who visit each year. Inclusion criteria: primary breast cancer patients diagnosed by pathological diagnosis after surgery, followed up for 5 years, the main treatment methods and chemotherapy methods are the same, pathological data are complete, and breast cancer metastasis as the outcome. Exclusion criteria: follow-up less than 5 years, pathological data is not complete, breast cancer recurrence or metastasis with recurrence or death as the outcome. Ethical question : patient informed consent to participate. The research plan was approved by the Biomedical Ethics Committee of Southwest Medical University and met the ethical requirements of clinical research. Application No.: XNYD2018001.

1.2 Determination of grouping and sample size

Based on the previous research results²², the ERCC1 IHC score was 3 as critical value, more than 3 as high expression group, less than 3 as low expression group. On the basis of metastasis rate of ERCC1 patients with high expression was $p_1=0.643$, that of ERCC1 patients with low expression was $p_0=0.175$; $\alpha=0.05$, $\beta=0.10$. Sample content formula based on cohort study(1) and calculated samples $n=63$.

$$n = \left[\frac{(Z_{\alpha/2} \sqrt{2pq} + Z_{\beta} \sqrt{p_0 p_1 + q_0 q_1})}{p_1 - p_0} \right]^2 \quad (1)$$

1.3 Data collection

The clinicopathological data (clinical data, pathological data and treatment plan) used in the study were from the Department of Pathology and Breast surgery of the affiliated Hospital of Southwest Medical University; The paraffin samples of breast cancer patients were from the pathology department, and the paraffin block samples were 100% tumor cells (determined by the pathologist).

1.4 Measurement of ERCC1 and NF-κB signaling pathway-related proteins in paraffin blocks of breast cancer patients

Paraffin sections (3μm) were dried, deparaffined, and rehydrated in graded alcohol to water. Heat-mediated antigen retrieval was performed using pressure cooker treatment for 10min in EDTA buffer (PH 9.0). The slides were incubated for 120min at 25°C with primary mouse anti-human polyclone antibodies to p65, p50, IKKβ, TNFα, IκBα. After washing, the sections were incubated with the second antibody (Envision, HRP rabbit/mouse, Dako, DK) for 30min at 25°C. Negative controls were obtained by omitting the primary antibody. The slides were visualized by DAB.

Expression of NF-κB signaling pathways was determined in the nucleus of tumor cells. Five high-power fields (200×) were randomly selected. The extent of the staining was categorized into five semi-quantitative classes based on the percentages of positive tumor cells: 0, <5% positive cells; 1, 6–25% positive cells; 2, 26–50% positive cells; 3, 51–75% positive cells; and 4, >75% positive cells. Staining intensity was scored as 0, negative; 1, weak; 2, moderate; and 3, intensive. Multiplication of the intensity and the percentage scores gave rise to the final staining score. The review process is done independently by two senior pathologists in a double-blind manner, and the superior physician will again judge any dissenting cases to ensure that the results are accurate.

2 Cell testing

2.1 Cell lines

The breast cancer cell lines with ERCC1 gene expression were queried in the <https://www.proteinatlas.org/>, and the MCF-7 cell lines with high expression and more research in breast cancer metastasis and invasion were selected. Human breast cancer cell line MCF-7 borrowed from the Institute of Oncology, Southwest Medical University. The human breast cancer cell line MCF-7-ERCC1 knockdown lentivirus stable strain and MCF-7-NC negative control were purchased from Shanghai Hanheng Biotechnology Co., Ltd.

2.2 ERCC1 knockdown expression plasmid

The ERCC1 knockdown plasmid was designed and synthesized by Shanghai Hanheng Biotechnology Co., Ltd. The human breast cancer cell lines MCF-7 cells transfected with ERCC1 knockdown plasmid were stored in -80°C refrigerator.

(1)Carrier: pHBLV-U6-MCS-CMV-ZsGreen-PGK-PURO;

(2)Knockdown target design and primer sequences:

Gene	Primer sequences
ERCC1 siRNA	CCTCAAGGAGCTGGCTAAGATGTGT
ERCC1-shRNA	GATCCGCTCAAGGAGCTGGCTAAGATGTGTTTCAAGAGAACACAT
(Top strand)	CTTAGCCAGCTCCTTGAGGTTTTTTG
ERCC1-shRNA	AATTCAAAAAACCTCAAGGAGCTGGCTAAGATGTGTTCTCTTGAAA
(Bottom strand)	CACATCTTAGCCAGCTCCTTGAGGCG

2.6 RT-qPCR

Total RNA were extracted using the RNeasy FFPE Kit (QIAGEN, shanghai, China), according to manufacturer's instructions. cDNA was reversely transcribed using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, Liaoning, China). Gene expression was quantified by SYBR Premix Ex Tap II (TaKaRa, Dalian, Liaoning, China) and performed in a real-time thermal cycler qTOWER 2.0/2.2 (Analytik Jena, Germany) Relative gene expression was calculated using the $2^{-\Delta CT}$ method and the results were normalized with β -actin as an internal control. The primer sequences are shown in Table 1.

Table.1 The primers used for PCR

Primer Name	Primer Sequence
ERCC1-F	GCGACGTAATTCCCGACTA
ERCC1-R	CCGCTGGTTTCTGCTCAT
GAPDH-F	TCAAGGCTGAGAACGGGAAG
GAPDH-R	TCGCCCCACTTGATTTTGGA

2.7 Western Blot

Stable transfection ERCC1 knockdown, using RIPA lysis buffer (Biotেকে,Wuxi) to cleave three groups of MCF-7 cell proteins. Protein concentration was determined by BCA method. Proteins were separated and transferred to the PVDF membrane (Millipore) by 10% SDS-PAGE and then incubated overnight with the following antibody at 4°C. The membrane was incubated with horseradish peroxidase labeled goat anti-rabbit IgG (Bioss, Beijing) for 2h at room temperature. GAPDH as internal control. A ECL method is used to develop and capture the generated image using a digital imaging system. The diluted antibodies ratio in this part of the experiment of proportion is as follows:

Antibody, manufacturer	Optimal dilution ratio
β -actin, Cell Signaling Technology	1:2000

ERCC1,Proteintech	1:2000
TNF- α ,AtaGenix	1:1000
I κ B α ,Abcam	1:10000
IKK β ,AtaGenix	1:1000
p65,Abcam	1:2000
p50,Abcam	1:1000
Goat Anti-Rabbit IgG/HRP	1:7000

2.8 Cellular scratches

The samples that have been successfully established and identified by the RT-qPCR and Western blot are added to the plate hole, and the stage of the cells is paid attention to, the quantity is controlled to ensure the sufficient reaction source; the changes in the template are observed, once the cells are fused, the tool is the gun head and the bottom area; and use PBS wash twice to remove suspended cells; add medium containing 1% FBS to culture; observe, photograph and detect scratch healing at different time points to judge cell motility. Each time three scratch fields were taken, the average distance was calculated using ImageJ, and repeated 3 times to evaluate cell migration ability.

2.9 Transwell

Preparation Corning Costar Transwell (24 holes ,8 μ m diameter); Spread 1:8 Matrigel diluted without serum medium, logarithmic cells were starved in serum-free medium for 12 hours, then digest and re-suspend cells in serum-free medium, and then inoculated in the upper chamber (200 μ l, 4 $\times$ 10⁴ per hole). Next put 600 μ l complete medium containing FBS in the lower chamber and place it in incubator 24h.

3 Statistical analysis

Data was double entered and checked by Epidata3.1 software. Apply SPSS25.0 statistical software for statistical analysis. The measured data in accordance with the normal distribution are expressed as mean $\pm$ standard deviation ($\bar{X} \pm S$), and the median and quartile range are expressed as not in accordance with the normal (*Median(IQR)*). When comparing the two independent samples, if the normal distribution and variance are uniform, the *t* test is adopted, and the *Mann-Whitney U* test is used if the normal distribution is not consistent. When comparing paired samples, the paired *t* test is used for normal distribution, and the Wilcoxon paired symbol rank test is used for non-normal distribution. To analyze the correlation between the two groups of quantitative data, the use Pearson correlation analysis with normal distribution, and the Spearman grade correlation for those without normal distribution. The analysis was used for survival analysis, Cox risk regression model was used for multivariate analysis, $P < 0.05$ was statistically significant.

Results

1 Population aspects results

1.1 Comparison of pathological characteristics of two group patients

The pathological characteristics analysis showed that there was no significant difference between the pathological characteristics of all breast cancer patients in ERCC1 low expression group and the expression of breast cancer patients in ERCC1 high expression group ($P > 0.05$), see Table 2.

Table 2 Analysis of pathological characteristics of two groups (n(%))

Pathological characteristics	<i>n</i>	Low ERCC1	High ERCC1	χ^2	<i>P</i>
Age (years)					
0~	59	30(47.6)	29(46.0)	0.032	0.858
50~	67	33(52.4)	34(54.0)		
ER					
Negative	47	23(36.5)	24(38.1)	0.034	0.854
Positive	79	40(63.5)	39(61.9)		
PR					
Negative	65	30(47.6)	35(55.5)	0.749	0.373
Positive	61	33(52.4)	28(44.5)		
HER2					
-/+	89	41(65.1)	48(76.2)	1.875	0.171
+++	37	22(34.9)	15(23.8)		
E-Cad					
Negative	15	7(11.1)	8(12.7)	0.076	0.783
Positive	111	56(88.9)	55(87.3)		
P53					
Negative	76	39(61.9)	37(58.7)	0.133	0.716
Positive	50	24(38.1)	26(41.3)		
Ki67					
0~	36	19(30.1)	17(27.0)	0.156	0.693
20~	90	44(69.9)	46(73.0)		
Molecular subtypes					
Luminal A	24	14(22.2)	10(17.5)	2.639	0.620
Luminal B	37	21(33.3)	16(28.1)		
Luminal HER2	21	8(12.7)	13(22.8)		
HER2-enriched	10	6(9.5)	4(7.0)		
Basal-like	28	14(22.2)	14(24.6)		
Lymph node metastasis					
0	48	25(39.7)	23(36.5)	0.932	0.818
1~	23	13(20.6)	10(15.9)		
4~	24	11(17.5)	13(20.6)		
10~	31	14(22.2)	17(27.0)		
Pathological type					
Carcinoma in situ	7	3(4.8)	4(6.3)	1.143	0.565
Non-specific invasive carcinoma	118	59(93.7)	59(93.7)		
Invasive special type carcinoma	1	1(1.6)	0(0.0)		
Tumor size(cm)					
0~	37	19(30.2)	18(28.6)	0.041	0.980
2~	71	35(55.6)	36(57.1)		
5~	18	9(14.3)	9(14.3)		

WHO Grade					
I	6	2(3.2)	4(6.3)		
II	80	41(65.1)	39(61.9)	0.717	0.699
III	40	20(31.7)	20(31.7)		

1.2 Effects of ERCC1 on breast cancer metastasis

ERCC1 expression was positively correlated with breast cancer metastasis($r=0.543$, $P<0.001$), see table 3. The survival rate of breast cancer patients with ERCC1 low expression group was higher than that of ERCC1 high expression group in metastasis-free survival analysis ($\chi^2=17.571$, $P<0.001$), see figure 1.

Table 3 ERCC1 correlation with breast cancer metastasis

Variable	ERCC1	Breast cancer metastasis
ERCC1	1	0.543*
Breast cancer metastasis	-	1

Note : * Represents $P<0.05$.

Figure 1 Analysis of metastasis-free survival of breast cancer patients after ERCC1 grouping

1.3 Comparison of the Expression of NF- κ B Signal Pathway-related Protein in two Groups

p65、p50、TNF α 、IKK β IHC positive expression was yellow. The protein level of p65、p50、TNF α 、IKK β in paraffin block of breast cancer patients with ERCC1 high expression group was significantly higher than that in low expression group($P<0.05$), I κ B α protein levels were significantly lower than those of low expression groups($P<0.05$), see figures 2,3.

Figure 2 IHC of NF- κ B signaling pathway related proteins in breast cancer patients

Figure 3 Comparison of NF-κB signaling pathway-related protein expression in two groups of breast cancer patients(Median(IQR), * Represents $P<0.05$.)

1.4 Correlation between ERCC1-NF-κB signaling pathway factors and breast cancer metastasis

The results showed that ERCC1 expression was positively correlated with IKKβ expression($r=0.481$, $P<0.001$); IKKβ and IκBα were negatively correlated($r = -0.203$, $P=0.023$); IκBα and p50 were negatively correlated($r=-0.275$, $P=0.002$); Positive correlation between p50 and p65($r=0.386$, $P<0.001$); Positive correlation between p65 and TNFα($r=0.476$, $P<0.001$); TNFα is positively associated with breast cancer metastasis($r=0.281$, $P=0.001$), see table 4, 5.

Table 4 ERCC1 correlation with IKKβ

Variable	ERCC1	IKKβ
ERCC1	1	0.481*
IKKβ	-	1

Note : * Represents $P<0.05$.

Table 5 Correlation between NF-κB signaling pathways and breast cancer metastasis

Variable	breast cancer metastasis	p50	p65	TNFα	IKKβ	IκBα
breast cancer metastasis	1	0.250*	0.239*	0.281*	0.221*	-0.363*
p50	-	1	0.386*	0.424*	0.319*	-0.275*
p65	-	-	1	0.476*	0.374*	-0.219*
TNFα	-	-	-	1	0.453*	-0.224*
IKKβ	-	-	-	-	1	-0.203*
IκBα	-	-	-	-	-	1

Note : * Represents $P<0.05$.

2 Cellular aspects

2.1 ERCC1mRNA and protein expression of stable transfection of ERCC1 knockdown MCF-7 cells

The results showed that the ERCC1mRNA in MCF-7-ERCC1 knockdown group was significantly lower than that in MCF-7-NC negative control group($P<0.05$); ERCC1 protein levels in MCF-7-ERCC1 cells decreased significantly compared with those in MCF-7-NC negative control group and MCF-7 blank control group($P<0.05$), see figure 4; These results suggest that the ERCC1 expression in the MCF-7 can be effectively disturbed and subsequent experiments can be carried out.

Figure 4 Transfection efficiency of MCF-7 cell lines with stable knockdown ERCC1 (* Represents $P<0.05$) (A)&(B): RT-qPCR ;(C)&(D): Western Blot

2.2 The detection of migration ability of MCF-7 cells with stable knockdown ERCC1 in vitro

At 24 hours, the migration distance of the MCF-7-ERCC1 knockdown group was less than that of the negative control group($P<0.05$), compared with the blank control group, the migration distance of the MCF-7-ERCC1 knockdown group was not different($P>0.05$). At 48 hours, the migration distance of MCF-7-ERCC1 knockdown group was less than that of blank control group and negative control group($P<0.05$), as show in figure 5,6.

Figure 5 Scratch test of low ERCC1 MCF-7 breast cancer cell line migration in vitro

Figure 6 The Comparison of Mean Scratch Width of MCF-7 Breast Cancer Cell Lines with

Stable ERCC1 Knockdown (* Represents $P<0.05$) (A):0 h; (B)24 h; (C):48h

2.3 The Detection of Invasion Ability of MCF-7 Cells with Stable and ERCC1 Knockdown

Compared with the blank control group and the negative control group, the number of cell invasion in the MCF-7-ERCC1 knockdown group decreased significantly($P<0.05$); compared with the blank control group, the number of cell invasion decreased in the negative control group($P<0.05$), see figure 7.

Figure 7 Detection of invasive ability in vitro of MCF-7 breast cancer cell lines with stable knockdown ERCC1; (Note : * means $P<0.05$)

2.4 The affect of ERCC1 on NF- κ B signaling pathways

The protein level of p65、 p50、 $\text{TNF}\alpha$ 、 $\text{IKK}\beta$ in ERCC1 knockdown group was significantly lower than that in blank control group and negative control group($P<0.05$), $\text{I}\kappa\text{B}\alpha$ protein levels were significantly higher than those of the blank and negative control groups($P<0.05$), see figure 8.

Figure 8 Stable knockdown ERCC1 expression of key factors in NF- κ B signaling pathway in MCF-7 cells(Note : * Represents $P < 0.05$) (A): Western Blot electrophoresis; (B): Western Blot electrophoresis protein grayscale analysis

Discussion

The survival analysis of 63 pair patients with ERCC1 high expression group and ERCC1 low expression group showed that the survival rate of ERCC1 high expression group was significantly lower than that of ERCC1 low expression group. The results were consistent with those reported Huang et al²³. It suggested that high expression had an effect on postoperative metastasis and poor prognosis.

In addition, in the cell scratch experiment, it was found that only the ERCC1 knockdown

group was different from the negative control group after 24h, which may be related to the slower growth of the MCF-7 cell line; therefore, we added the 48h group, and after 48h, the knockdown group different from the blank control group and the negative control group, it shows that the cell line in the knockdown ERCC1 group has a significantly reduced in vitro migration ability; and in the Transwell experiment, it was also found that the knockdown ERCC1 expression group had a lower in vitro invasion ability compared with the blank control group after 24 hours. Compared with the negative control group, it was significantly reduced. The study is consistent with Zhou Yingqiong's²⁴ conclusion that downregulation of ERCC1 in ovarian cancer leads to weakened invasion ability. Regarding the difference between the MCF-7 blank control group and the MCF-7 negative control group, we think it may be that the MCF-7 breast cancer cell line grows in clusters and overlaps. Therefore, the staining is prone to overlap staining after crossing the cell, so it is possible cause counting errors.

After confirming the relationship between ERCC1 and breast cancer metastasis, and knocking down ERCC1 will affect breast cancer metastasis, how does ERCC1 affect breast cancer metastasis? A review of related literature²² found that ERCC1 may have a certain relationship with TNF α , and studies have also found that cancer cell DNA repair is closely related to IKK β . Other studies²⁵ have shown that blocking the NF- κ B pathway can reduce the invasion and migration ability of MCF-7 cells, and can regulate the IL-4 / mi R-324-5p / CUEDC2 signaling pathway under the action of sinomenine. NF- κ B activity has an inhibitory effect and effectively inhibits the migration and invasion ability of breast cancer cells; therefore, it is speculated that ERCC1 may also affect breast cancer metastasis through the NF- κ B signaling pathway.

When cancer cells suffer DNA damage, they activate ATM, as the main kinase for a large number of signal transduction, ATM can not only participate in the DNA damage repair network, but also participate in cell cycle checkpoints, and through the activation of NEMO, phosphorylated NEMO and IKK β . In combination, IKK β plays a very important role in the NF- κ B pathway. Although there are many upstream signal sources, they all eventually converge to IKK. NF- κ B signaling pathway can participate in the pathological process of a large number of diseases, and this signaling pathway is closely related to human immunity and inflammation. Under normal circumstances, the inhibitory protein expressed by non-activated NF- κ B in the cytoplasm can bind to the dimer to form a trimeric complex. In this form, NF- κ B has no activity. The kinase IKK β can interact with the I κ B α protein, dissociate it from the trimer, and participate in the binding of specific sequences on nuclear DNA to promote the transcription of related genes²⁶. Activated I κ B α combined with p65/p50 repairs DNA damage and produces tumor necrosis factor TNF- α , which in turn stimulates cancer cells to undergo epithelial-mesenchymal transition to produce cancer cell metastasis, and TNF- α produced by repairing DNA damage can be used as an upstream stimulation signal activation pathway. The upstream IKK affects the dissociation of I κ B α and NF- κ B p65/p50, and achieves positive feedback by acting on inflammatory factors, and continuously activates the NF- κ B pathway.

In this study, it was found in Western blot experiments that the MCF-7 breast cancer cell line knocked down ERCC1. The levels of p65, p50, TNF α and IKK β in the MCF-7 cell ERCC1 knockdown group were lower, while the knockdown group had lower levels of proteins such as p65, p50, TNF α , and IKK β . On the contrary, I κ B α increased, and the effective trend of the pathway was consistent with that of Al-Sadi²⁷, indicating that knocking down ERCC1 has an inhibitory effect on p65, p50, TNF α and IKK β , and promotes the expression of I κ B α . After low ERCC1, the activation of IKK is inhibited, the phosphorylation of I κ B α is reduced, and the transcription of NF- κ B is inhibited; and the results of IHC detection in the population are consistent with the results of Western blot detection, and the p65 of breast cancer patients in the low expression group of ERCC1 Compared with the expression levels of breast cancer patients in the ERCC1 high expression group, the expression levels of, p50, TNF α and IKK β were

significantly reduced. The expression levels of breast cancer patients in the ERCC1 low expression group were significantly higher than those in the ERCC1 high expression group. Through research and analysis, it can be found that TNF α can promote the occurrence and development of breast cancer²⁸. Inflammatory factors can induce and activate the NF- κ B signaling pathway, which will participate in the inflammatory response after activation, leading to the occurrence and metastasis of tumor cells. Eradication of related research reports shows that NF- κ B can affect the activity of NF- κ B in cancer cells while promoting the metastasis of breast cancer cells. Interfering with the expression of p65 can effectively inhibit its metastasis²⁹. After that, we also analyzed the correlation between ERCC1 and the key molecules of the NF- κ B signaling pathway. The results showed that I κ B α , p65, p50, TNF α and IKK β are all related to the expression of ERCC1; and the key molecules of the NF- κ B signaling pathway are related to the breast. Cancer metastasis is also related. Studies³⁰ found that inhibiting the activation of p65 can effectively inhibit the proliferation and invasion of MDA-MB-231 cells, suggesting that in breast cancer, ERCC1 may regulate the NF- κ B signaling pathway and affect the breast. The role of cancer metastasis. Another study found that most breast cancer patients are often accompanied by chronic inflammation during the development of the disease, and these chronic inflammations will affect cancer cells, and the tumor will metastasize as the cancer cells spread. According to research, TNF α is closely related to the occurrence of cancer. The cells will be analyzed in a large amount in the tumor microenvironment and promote the growth and further spread of cancer cells. Not only that, the cells can also prevent the immune system from recognizing cancer cells and metastasize. Therefore, it can be known that in breast cancer, ERCC1 may have the effect of regulating NF- κ B signaling pathway and affecting metastasis. The NF- κ B signaling pathway is involved in the pathological changes of various diseases in the human body, and can regulate the levels of various cytokines. If ERCC1 has a regulatory effect on various cytokines downstream of this pathway, then ERCC1 inhibitors will affect NF- κ B signaling pathway has a regulatory effect, so as to play a therapeutic role, relieve inflammation, and restore normal human functions.

Considering this reason, clinically, it is possible to analyze the postoperative cell metastasis by detecting the expression level of ERCC1, and to study the development of the patient's condition after breast cancer surgery. In order to prevent breast cancer postoperative metastasis, use the corresponding Molecular targeted drug inhibitor therapy provides theoretical support and provides a scientific basis for improving survival rates.

Conclusions

DNA repair gene ERCC1 is positively associated with breast cancer metastasis and may affect breast cancer metastasis by regulating NF- κ B signaling pathways.

Limitation and advantage of the study

This study has a prospective paired case-control research basis with complete data, and on this basis, further uses prospective cohort studies to analyze the impact and correlation of DNA repair genes in breast cancer metastasis, confirming ERCC1 at the population and cell levels Signal pathways affecting breast cancer metastasis. However, this study only explored the impact on the NF- κ B signaling pathway, and did not involve other possible pathways; due to funding issues, all breast cancer cell line types inquired were not included, and there was a certain degree of one-sidedness; in the experimental design, we should coupled with animal experiments, we plan to increase animal experiments and verify other signaling pathways that may be related in

the next step.

Abbreviations

ERCC1: DNA excision repair cross complementing 1; ER: Estrogen receptor; PR: Progesterone receptor; HER2: human epidermal growth factor receptor-2; TNF- α : Tumor necrosis factor- α ; NF- κ B: Nuclear factor-kappa B; NER: Nucleotide excision repair; GG-NER: Global genome NER; TC-NER: Transcription-coupled NER; DSB: Double strand break repair; IHC: immunohistochemistry; Ki67: Nuclear-associated antigen ki67;

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Authors' contributions

Y-T designed, guided the study; XB-L, LY-H and TX-W performed the experiments; YX-Y analyzed the data and wrote the paper; B-W, HS-Z and ZH-T collected the samples. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due our study was at an early stage of the investigation and had entered into a privacy agreement with the patient but are available from the corresponding author on request.

Ethics approval and consent to participate

Patients with informed consent to participate. The study plan has been reviewed by the Biomedical Ethics Committee of Southwest Medical University, and it is considered to meet the ethical requirements of clinical research, and the study plan is approved. Application acceptance Number: XNYD2018001.

Consent for publication

All authors agree to submit the article for publication.

Competing interests

The authors declare that they have no conflicts of interest.

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Statement

We confirming all the experiment protocol for involving humans was in accordance to guidelines of national in the manuscript.