The Diagnostic Value of DNA Repair Gene in Breast Cancer Recurrence and Metastasis

Yongxin Yang

Southwest Medical University

Xiabin Li

Southwest Medical University

Liyue Hao

Southwest Medical University

Deyong Jiang

Centers for Disease Control and Prevention

Bin Wu

Southwest Medical University

Tao He

Southwest Medical University

Yan Tang (✉ tangyan200310@163.com)

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Abstract

Background: DNA repair genes play a vital role in the treatment of many cancers, and DNA repair genes can be used in breast cancer recurrence and metastasis research. We found that the expression of DNA repair genes in breast cancer patients after recurrence and metastasis is abnormal, however, the clinical predictive significance of DNA repair genes is still elusive.

Methods: The nested case-control method was used in patients with breast cancer recurrence and metastasis after surgery (n=109) and patients without recurrence and metastasis after surgery (n=109). The proteins and mRNA of DNA repair genes were detected by immunohistochemistry and Real-time PCR respectively.

Results: PARP1($OR=1.485$, $95\%CI:1.279\sim1.725$, $P<0.05$), XRCC4($OR=1.419$, $95\%CI:1.217\sim1.656$, $P<0.05$) and ERCC1 ($OR=1.181$, $95\%CI:1.032\sim1.353$, $P<0.05$) were risk factors for postoperative recurrence and metastasis of breast cancer. Therefore, we used the ROC curve to study the optimal critical values of PARP1, XRCC4, and ERCC1, combined with the comprehensive judgment of clinical experience, we can conclude that PARP1 (cutoff value = 6, Se = 75.23%, Sp = 79.82%), XRCC4 (cutoff value = 6, Se = 78.9%, Sp = 79.82%), ERCC1 (cutoff value = 3, Se = 89.91%, Sp = 47.71%), suggesting that when the PARP1 score is higher than 6 or the XRCC4 score is higher than 6 or the ERCC1 score is higher than 3, the risk of recurrence and metastasis will increase ($OR_{PARP1} = 14.941$, $95\% CI_{PARP1}: 4.004\sim55.758$, $P<0.05$; $OR_{XRCC4} = 16.740$, $95\% CI_{XRCC4}: 6.433\sim43.560$, $P<0.05$; $OR_{ERCC1} = 5.285$, $95\% CI_{ERCC1}: 1.843\sim15.156$, $P<0.05$). Due to PARP1, ERCC1 and XRCC4 belong to a part of DNA repair gene system, and the three proteins are positively correlated by correlation analysis ($r_{PARP1-ERCC1}=0.317$; $r_{PARP1-XRCC4}=0.329$; $r_{ERCC1-XRCC4}=0.377$). The combined diagnosis of the PARP1, ERCC1 and XRCC4 have greater predictive value for the risk of recurrence and metastasis of breast cancer (Se = 88.99%, Sp = 82.57%, $OR = 50.914$, $95\% CI: 10.918, 237.417$, $P<0.05$).

Conclusions: The recurrence and metastasis of breast cancer are predictive after PARP1>6, XRCC4>6, and ERCC1>3. The combined diagnosis of the three indicators have greater predictive value for the risk of recurrence and metastasis of breast cancer.

1. Background

Breast cancer is the most general malignancy in China and either world, and its mortality rate firstly in female malignancy[1]. In recent years, the survival rate of breast cancer has been prominently improved by comprehensive treatment such as surgery and chemotherapy. Nevertheless, approximately one-third of breast cancer patients will present metastases. Recurrence and metastasis are bound up with the prognosis of breast cancer patients and it is also the soprattutto cause of death in breast cancer patients[2]. Studies have found that breast cancer patients' postoperative recurrence and metastasis are related to age, tumor pathological tissue type, clinical analysis, postoperative chemotherapy, and endocrine therapy[3]. At the same time, some people have also studied tolerance to treatment as one of

the influencing factors. However, some tumor cells can pass activating self DNA repair mechanisms to resistance to DNA damage drugs[4–6]. So some studies have proposed that DNA repair genes have a relationship with the recurrence and metastasis of breast cancer[7].

More and more studies have found that tumor response to chemotherapy drugs is closely related to the regulation of the DNA repair system [8]. Four major DNA repair pathways are currently known: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), and double strand break repair (DSBR). In cancer, we found that ERCC1, XPA, XRCC1, PARP1, MSH2, MLH1, 53BP1, XRCC4 are closely related to cancer recurrence and metastasis. The ERCC1 and XPA genes in the NER pathway have confirmed that ERCC1 is associated with recurrence and metastasis in ovarian cancer, testicular germ cell tumors and gastric cancer, and high expression of ERCC1 will lead to an increased risk of recurrence and metastasis [9]. BER as one of the DNA repair mechanisms, PARP1 may be one of the major genes involved in tumor cell recurrence and metastasis [10]. In vitro and in vivo studies have suggested that inhibition of PARP1 can reduce tumor cell repair function, thereby enhancing the therapeutic effect of radiotherapy and chemotherapy on tumors [11,12]. DSBR is the most common but most severe type of DNA damage in eukaryotic cells, and is mainly repaired in mammals through non-homologous end joining (NHEJ). Xiaoyan Li et al. found that 53BP1 affects breast cancer patients' sensitivity to 5-Fu, it will results poor prognosis [13]. Some scholars have found that the research on DNA repair genes such as MLH1, XRCC4, 53BP1, ERCC1 and XPA in breast cancer is mainly expressed in breast cancer tissues and its clinical significance[14–16]. XRCC1 overexpression can inhibit breast cancer cell proliferation and metastasis[17]. MSH2 mutation may be involved in the occurrence and development of early-onset breast cancer in the family of Lynch syndrome[18]. Among them, PARP1 inhibitors have entered the trial stage of clinical treatment of breast cancer[19]. But no further study of their recurrence and metastasis with breast cancer.

DNA repair requires the role of multiple enzymes and genes. A single gene has a limited role in damage repair. Analyzing only an enzyme or gene is not enough to reflect the complexity of DNA repair. Due to ERCC1, XPA, XRCC1, PARP1, MSH2, MLH1, 53BP1, and XRCC4 are more studied in other cancer. But there are few studies in breast cancer recurrence and metastasis. So in this study, nested case-control study was used to explore the expression levels of major molecules of the DNA repair system ERCC1, XPA, XRCC1, PARP1, MSH2, MLH1, 53BP1, and XRCC4 in patients with recurrent and metastatic breast cancer, in order to provide theoretical support for clinical treatment and prognosis.

2. Methods

2.1 Sample

The data come from the follow-up cohort of the Cancer Institute of Southwest Medical University. The cohort was collected and followed up in January 2013 at the Department of Breast Medicine, Southwest Medical University Hospital. Cancer patients have collected approximately 1360 cases. Recurrence and metastasis cases and controls selected in this study were collected from this cohort. Patients with

recurrence and metastasis during the follow-up period were included in the recurrence and metastasis case group. At the same time, the control group(non-recurrence) was selected according to the 1: 1 pairing principle in this cohort (n = 109, the matching condition was age $\pm$ 3 years, the operation time was consistent, and the treatment plan was consistent). The control group(non-recurrence) was surviving patients in the cohort, and no recurrence and metastasis occurred. Finally, 109 cases and 109 controls were included in January 2018. The pathological data used in this study were from the Department of Pathology, Affiliated Hospital of Southwest Medical University. The data collected included clinical data, pathological data, and treatment options, as well as paraffin specimens from patients with breast cancer. After preliminary diagnosis of breast cancer patients in the affiliated hospital of Southwest Medical University, materials were obtained from the Department of Pathology.

Ethical issues: (1) Patients with informed consent to participate. (2) This research program has been reviewed by the ethics committee of the unit, and it is considered that it meets the relevant ethical requirements of clinical research and agrees to the research plan. Application acceptance number: XNYD2018001.

2.2 Detection of DNA repair genes ERCC1, XPA, XRCC1, PARP1, MSH2, MLH1, 53BP1 and XRCC4 in paraffin-embedded tissues of breast cancer patients by Real-time PCR

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 – $\Delta\Delta$ 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

Gene	Primer sequences
53BP1	sense primer 5'-CCAGACTCCACCAGACGAACA-3' Anti-sense 5'-ACCACTTGGCTACAACACGGA-3'
ERCC1	sense primer 5'-TATGAGCAGAAACCAGCGGAC-3' Anti-sense 5'-GCTCGTGCAGGACATCAAACA - 3'
MLH1	sense primer 5'-TGAGGAAGGGAACCTGATTGG - 3' Anti-sense 5'-CCGGATGGAATAGAACATAGCG - 3'
XRCC4	sense primer 5'-TCTGTTCTGAAATGACTGCTGACC-3' Anti-sense 5'-GGTGCAATATCAGTGACATCAAGAC-3'
MSH2	sense primer 5'-GGAAGTTCTACCTACGATGGATTTG-3' Anti-sense 5'-TCAGTGGTGAGTGCTGTGACATG-3'
XRCC1	sense primer 5'-TCGAGGACTATATGAGTGACCGG-3' Anti-sense 5'-ACGAACGAATGCCAGGGAG-3'
PARP1	sense primer 5'-CAGAAGCCGAAACTCTT-3' Anti-sense 5'-GATGCCAAATCACCAGGT-3'
XPA	sense primer 5'-TGTTTTGCCTCTGTTTTGGTT-3' Anti-sense 5'-GTAATATGCGAAGAATGTGGG-3'
β -actin	sense primer 5'-CCACGAACTACCTTCAACTCC-3' Anti-sense 5'-GTGATCTCCTTCTGCATCCTGT-3'

2.3 Immunohistochemical detection of DNA repair gene protein expression in paraffin-embedded tissues of breast cancer patients

Paraffin sections (3 μ m) were dried, deparaffined, and rehydrated in graded alcohol to water. Heat-mediated antigen retrieval was performed using pressure cooker treatment for 10 min in EDTA buffer (pH 9.0). The slides were incubated for 120 min at 25°C with primary mouse anti-human monoclonal antibodies to ERCC1, XPA, XRCC1, PARP1, MSH2, MLH1, 53BP1 and XRCC4 (Dako, DK). After washing, the sections were incubated with the second antibody (Envision, HRP rabbit/mouse, Dako, DK) for 30 min at 25°C. Negative controls were obtained by omitting the primary antibody. The slides were visualized by DAB.

Expression of 8 DNA repair protein was determined in the nucleus of tumor cells. Five high-power fields (200 $\times$) 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[20].

2.4 Statistical analysis

All data were analyzed using SPSS 22.0 statistical software and MedCalc software, and bilateral *P* values below 0.05 were considered statistically significant. The correlation between DNA repair genes and breast cancer recurrence and metastasis was analyzed by χ^2 test, rank sum test, cox risk model and other statistical methods.

3. Results

3.1 The protein expression of DNA repair genes

Immunohistochemical staining results (Fig. 1) shows that: DNA repair gene protein positive expression mainly in the cytoplasm, repair gene is highly expressed in the transfer of recurrence group in the breast tissue. The MSH2, MLH1, PARP1, XRCC1, XRCC4, 53BP1, ERCC1, XPA of the recurrence and metastasis group were higher than those of the control group(non recurrence and metastasis group)(*P*< 0.05), we can concluded that all of them are related to the prognosis of recurrence and metastasis of breast cancer.

3.2 The mRNA expression of DNA repair genes

Fig.2 shows the comparison of the expression of DNA repair gene mRNA in breast cancer patients in the recurrence and metastasis group and the control group(non recurrence and metastasis group). The mRNA expressions of MSH2, MLH1, PARP1, XRCC1, XRCC4, 53BP1, ERCC1 and XPA in breast cancer recurrence and metastasis group were higher than those in control group(non recurrence and metastasis group)(*P*<0.05).

3.3 Clinicopathologic feature of breast cancer patients

The PR index of the recurrence and metastasis group was lower than that of the control group(non recurrence and metastasis group)(*P*< 0.05). P53, WHO Grade and Lymph node metastasis of the recurrence and metastasis group was higher than that of the control group(non recurrence and metastasis group) (*P*< 0.05). There was no significant difference in Age, ER, HER2, E-cad, Ki67, TNBC, Infiltration and Tumor diameter between the two groups (*P*> 0.05), as shown in Table 2.

Table.2 Clinicopathologic feature of breast cancer patients [n(%)]

Variable		Cases (recurrence and metastasis group) (N = 109)	Control (non recurrence and metastasis group) (N = 109)	χ^2	P
Age	< 50	51(48.1)	55(51.9)	0.294	0.588
	≥ 50	58(51.8)	54(48.2)		
ER	Negative	48(58.5)	34(41.5)	3.638	0.056
	Positive	61(45.2)	74(54.8)		
PR	Negative	65(58.0)	47(42.0)	6.008	0.014
	Positive	43(41.3)	61(58.7)		
HER2	-/+ / ++	82(48.8)	86(51.2)	0.415	0.519
	+++	27(54.0)	23(46.0)		
E-Cad	Negative	11(47.8)	12(52.2)	0.020	0.886
	Positive	84(49.4)	86(50.6)		
P53	Negative	50(40.0)	75(60.0)	5.341	0.021
	Positive	27(60.0)	18(40.0)		
Ki67	< 20	23(42.6)	31(57.4)	1.305	0.253
	≥ 20	79(51.6)	74(48.4)		
TNBC	No	91(48.1)	98(51.9)	2.540	0.111
	Yes	18(64.3)	10(35.7)		
Infiltration	No	5(38.5)	8(61.5)	0.679	0.410
	Yes	102(50.2)	101(49.8)		
WHO Grade	Ⅰ	1(5.9)	16(94.1)	2.294	0.022
	Ⅱ	71(53.0)	63(47.0)		
	Ⅲ	37(55.2)	30(44.8)		
Lymph node metastasis	0	28(34.1)	54(65.9)	30.345	0.000
	1 ~ 3	18(38.3)	29(61.7)		
	4 ~ 9	27(69.2)	12(30.8)		
	≥ 10	41(71.9)	16(28.1)		

Variable		Cases (recurrence and metastasis group) (N = 109)	Control (non recurrence and metastasis group) (N = 109)	χ^2	P
Tumor diameter	< 2cm	28(43.8)	36(56.2)	1.416	0.234
	≥ 2cm	81(52.6)	73(47.4)		

3.4 Binary Logistic Regression Analysis

To reduce confounding bias, at the protein expression level and the mRNA expression level, respectively, binary logistic regression analysis was performed on variables related to prognosis in univariate analysis. The results showed that at the protein level, PARP1(*OR* = 1.485, 95%*CI*: 1.279 ~ 1.725, *P* < 0.05), XRCC1(*OR* = 1.293, 95%*CI*: 1.083 ~ 1.543, *P* < 0.05), XRCC4(*OR* = 1.419, 95%*CI*: 1.217 ~ 1.656, *P* < 0.05) and ERCC1(*OR* = 1.181, 95%*CI*: 1.032 ~ 1.353, *P* < 0.05) were risk factors for postoperative recurrence and metastasis of breast cancer. The results of mRNA levels showed that the PARP1(*OR* = 1.770, 95%*CI*: 1.376 ~ 2.276, *P* < 0.05), XRCC4(*OR* = 1.264, 95%*CI*: 1.055 ~ 1.515, *P* < 0.05) and ERCC1(*OR* = 1.489, 95%*CI*: 1.182 ~ 1.876, *P* < 0.05) were risk factors for postoperative recurrence and metastasis of breast cancer. For details, see Tables 3 and 4.

Table 3
Binary logistic regression of protein expression

Variable	<i>B</i>	<i>S.E.</i>	<i>Wald</i>	<i>P</i>	<i>OR</i> (95% <i>CI</i>)
PR	-0.845	0.492	2.950	0.086	0.430(0.164,1.127)
PARP1	0.396	0.076	26.891	0.000	1.485(1.279,1.725)
XRCC1	0.257	0.090	8.082	0.004	1.293(1.083,1.543)
XRCC4	0.350	0.079	19.887	0.000	1.419(1.217,1.656)
ERCC1	0.167	0.069	5.822	0.016	1.181(1.032,1.353)
Lymph node metastasis					
0	-	-	-	-	Reference
1 ~ 3	0.193	0.649	0.089	0.766	1.213(0.340,4.327)
4 ~ 9	2.098	0.746	7.918	0.005	8.150(1.890,35.142)
≥ 10	2.704	0.672	16.197	0.000	14.941(4.004,55.758)

Table 4
Binary logistic regression of mRNA expression

Variable	<i>B</i>	<i>S.E.</i>	<i>Wald</i>	<i>P</i>	<i>OR(95%CI)</i>
PR	-1.107	0.497	4.956	0.026	0.331(0.125,0.876)
PARP1	0.571	0.128	19.744	0.000	1.770(1.376,2.276)
XRCC4	0.234	0.092	6.456	0.011	1.264(1.055,1.515)
ERCC1	0.398	0.118	11.430	0.001	1.489(1.182,1.876)
XPA	-0.744	0.265	7.866	0.005	0.475(0.282,0.799)
Lymph node metastasis					
0	-	-	-	-	Reference
1 ~ 3	0.564	0.643	0.771	0.380	1.758(0.499,6.195)
4 ~ 9	1.442	0.720	4.010	0.045	4.228(1.031,17.337)
≥ 10	2.352	0.622	14.280	0.000	10.509(3.102,35.598)

3.5 Diagnostic value of DNA repair genes

In the previous study, we found that the protein expression of PARP1, XRCC4 and ERCC1 is related to the recurrence and metastasis of breast cancer($P < 0.05$). However, the IHC score is a continuous variable and there is no accurate cut-off value for diagnosis. In order to further understand the role of PARP1, ERCC1 and XRCC4 in the prognosis of breast cancer recurrence and metastasis. Therefore, we used the ROC curve to study the optimal critical values of PARP1, XRCC4, and ERCC1, combined with the comprehensive judgment of clinical experience, we can conclude that PARP1 (cutoff value = 6, Se = 75.23%, Sp = 79.82%), XRCC4 (cutoff value = 6, Se = 78.9%, Sp = 79.82%), ERCC1 (cutoff value = 3, Se = 89.91%, Sp = 47.71%), suggesting that when the PARP1 score is higher than 6 or the XRCC4 score is higher than 6 or the ERCC1 score is higher than 3, the risk of recurrence and metastasis will increase. As shown in Fig. 3 and Table 5. Based on the cutoff value predicted by ROC curve and clinical experience, PARP1, XRCC4 and ERCC1 were divided into the high expression group and the low expression group according to the cutoff value, and the effects of PARP1, XRCC4 and ERCC1 on breast cancer recurrence and metastasis were verified again. Among them, the risk of recurrence and metastasis in the PARP1 high expression group was 14.235 times that of the low expression group ($OR = 14.235$, $95\%CI: 5.59 \sim 36.245$, $P < 0.05$). The risk of recurrence and metastasis in the XRCC4 high expression group was 16.740 times that of the low expression group ($OR = 16.740$, $95\%CI: 6.433 \sim 43.560$, $P < 0.05$). The risk of recurrence and metastasis in patients with ERCC1 high expression group was 5.285 times that of the low expression group ($OR = 5.285$, $95\%CI: 1.843 \sim 15.156$, $P < 0.05$). The risk of recurrence and metastasis in patients

with lymph node metastasis ≥ 10 was 9.175 times that without lymph node metastasis ($OR = 9.175$, $95\%CI: 2.842 \sim 29.625$, $P < 0.05$). As shown in Table 6.

Table 5
The best diagnostic value of PARP1, XRCC4 and ERCC1

Indicator	Cut-off Value	Sensitivity(%)	Specificity(%)	Youden index
PARP1	4	91.74	52.29	0.4403
	6	75.23	79.82	0.5505
	8	61.47	89.91	0.5138
XRCC4	4	96.33	50.46	0.4679
	6	78.90	79.82	0.5872
	8	56.88	86.24	0.4312
ERCC1	2	91.74	44.04	0.3578
	3	89.91	47.71	0.3761
	4	74.31	61.47	0.3578

Table 6
Binary logistic regression of protein high expression and low expression in postoperative recurrence and metastasis of breast cancer

Variable	B	S.E.	Wald	P	OR(95%CI)
PARP1	2.656	0.477	31.015	0.000	14.235(5.590,36.245)
XRCC4	2.818	0.488	33.351	0.000	16.740(6.433,43.560)
ERCC1	1.665	0.538	9.593	0.002	5.285(1.843,15.156)
Lymph node metastasis					
0	-	-	-	-	Reference
1 ~ 3	0.133	0.571	0.054	0.816	1.142(0.373,3.496)
4 ~ 9	2.423	0.731	10.975	0.001	11.278(2.690,47.290)
≥ 10	2.216	0.598	13.737	0.000	9.175(2.842,29.625)

3.6 The correlate and joint diagnostic value on between PARP1, XRCC4, ERCC1

Due to PARP1, ERCC1 and XRCC4 belong to a part of DNA repair gene system, and the three proteins are positively correlated by correlation analysis ($r_{\text{PARP1-ERCC1}}=0.317$; $r_{\text{PARP1-XRCC4}}=0.329$; $r_{\text{ERCC1-XRCC4}}=0.377$). See Table 7. These results indicate that there is an internal connection between these three proteins, and there is a certain synergy between them. So we combined PARP1, ERCC1 and XRCC4 to detect the prognosis of breast cancer. Joint diagnostic criteria: the high expression of a single indicator is judged as high, while the three indicators are simultaneously low and judged to be low.(Se = 88.99%, Sp = 82.57%;OR = 50.914, 95% CI: 10.918, 237.417, $P < 0.05$).See Fig. 4 and Table 8.

Table 7
The correlation coefficient of PARP1, XRCC4 and ERCC1

Variables	N	<i>r</i>	<i>P</i>
PARP1-XRCC4	218	0.329	0.000
PARP1-ERCC1	218	0.317	0.000
XRCC4-ERCC1	218	0.377	0.000
*:Spearman.			

Table 8
The Youden index and OR of combine detection

Joint indicator	Sensitivity(%)	Specificity(%)	Youden index	OR(95%CI)	<i>P</i>
PARP1 + XRCC4 + ERCC1	88.99	82.57	0.7156	50.914(10.918,237.417)	0.000

4. Discussion

Chemotherapy is one of the most important treatments for breast cancer after operation. At present, the survival rate of patients has been effectively improved by referring to ER, PR, HER-2, Ki67, TNBC and other indicators. However, the study found that there are still about 30% recurrence and metastasis rates [21]. It shows that the formulation of treatment plans based on the above pathological indicators may be incomplete, and there are other indicators for guiding treatment that can be excavated. In addition, there is still rise the survival rate of patients for adjustment in the formulation of treatment plans. Nowadays, more and more studies have found that the recurrence and metastasis of tumors are closely related to the DNA repair regulatory system related to drug resistance[22–24].

At present, not a few DNA repair genes, such as PARP1, XRCC1, 53BP1, ERCC1, have been found to be associated with the prognosis of breast cancer[25,26]. PARP1 promotes the expression of HIF-1α by

activating nuclear factor- κ B (NF- κ B) and promotes the polarization of macrophages M2, leading to the up-regulation of tumor-related macrophages (TAMs), such as tumor necrosis factor- α (TNF α) and IL-6, thus promoting the proliferation, invasion and metastasis of tumor cells, promoting the formation of tumor microvessels and microlymphatics[27]. And ERCC1 proteins can form heterodimers with DNA repair enzyme deficiency complementary gene (XPF) and perform functions by splicing at the 5' end of the damaged DNA single strand. Overexpression of ERCC1 proteins can lead to rapid repair of damaged DNA stagnating in G2/M, leading to resistance to cisplatin chemotherapeutics[28]. In this study, nested case-control single factor analysis showed that protein levels of PARP1, XRCC1, 53BP1, ERCC1 recurrence and metastasis group was higher than control group. We further included that PARP1 ($OR = 1.485$, 95% $CI: 1.279-1.725$), ERCC1 ($OR = 1.181$, 95% $CI: 1.032-1.353$) as independent factors for recurrence and metastasis of breast cancer after operation. The results are similar to those of Sunada, S. et al[29]. It shows that XRCC1 and 53BP1 become ineffective factors after excluding other confounding factors, while the high expression of PARP1 and ERCC1 in the course of chemotherapy will destroy the susceptibility of breast cancer cells, it can be make breast cancer cells less susceptible to chemotherapy, leading to resistance of breast cancer cells and even recurrence and metastasis of chemotherapy failure, which is affecting breast cancer. The independent prognostic factors of metastatic patients are not different from the existing studies.

In addition to the above genes related to breast cancer resistance[30], the prognosis-related DNA repair genes MSH2, MLH1, XRCC4 and XPA[31–33], which are frequently studied in the fields of colorectal cancer, lung cancer, prostate cancer and esophageal cancer, were also included in the study and analysis. Univariate analysis showed that MSH2, MLH1, XRCC4 and XPA in patients with recurrence and metastasis were higher than those without recurrence and metastasis. In binary logistic regression, only XRCC4 ($OR = 1.419$, 95% $CI: 1.217 \sim 1.656$) was a risk factor for recurrence and metastasis of breast cancer, suggesting that XRCC4 was also associated with recurrence and metastasis of breast cancer after operation. The results of high expression of XRCC4 in esophageal and colorectal cancer suggest poor prognosis. It is possible that XRCC4 itself could form dimers and interact with BRCT domain (BRCA1-terminal, BRCT) at the C end of Ligase IV protein through its α spiral stem ring structure in the central region. Damage repair defects lead to accumulation of genetic damage, and then increase the susceptibility of the body to tumors, thereby increasing the risk of recurrence and metastasis of breast cancer patients.

However, the direct use of IHC score to analyze the recurrence and metastasis of breast cancer after surgery is of little significance. In order to further understand the role of PARP1, ERCC1 and XRCC4 in predicting the prognosis, metastasis and recurrence of breast cancer, we also studied the best cut-off value of PARP1, ERCC1 and XRCC4. The results showed that PARP1 (Cut-off Value = 6, Se = 75.23, Sp = 79.82), ERCC1 (Cut-off Value = 3, Se = 89.91, Sp = 47.71), XRCC4 (Cut-off Value = 6, Se = 78.90, Sp = 79.82), suggesting that the risk of recurrence and metastasis increased at the scores of PARP1, ERCC1 and XRCC4 were higher than 6, 3, 6, respectively (PARP1: $OR = 14.235$, 95% $CI: 5.590 \sim 36.245$; ERCC1: $OR = 16.740$, 95% $CI: 6.433 \sim 43.560$; XRCC4: $OR = 5.285$, 95% $CI: 1.843 \sim 15.156$), the risk of breast cancer recurrence and metastasis will increase. The sensitivity of PARP1, ERCC1 and XRCC4 single detection is

between 75.23% ~ 89.91%, the specificity is between 47.71% ~ 79.82%, the Youden index is between 0.3761 ~ 0.5872, the sensitivity is qualified, but the specificity and Youden index are low. It indicates that the diagnostic value of individual tumor markers in the prognosis of breast cancer needs to be further improved. Due to PARP1, ERCC1 and XRCC4 belong to a part of DNA repair gene system, and the three proteins are positively correlated by correlation analysis ($r_{\text{PARP1-ERCC1}}=0.317$; $r_{\text{PARP1-XRCC4}}=0.329$; $r_{\text{ERCC1-XRCC4}}=0.377$). These results suggest that there is an internal link among the three proteins and there is a certain synergy among them. So we combined PARP1, ERCC1 and XRCC4 to detect the prognosis of breast cancer. Joint diagnostic criteria: the high expression of a single indicator is judged as high, while the three indicators are simultaneously low and judged to be low. The results showed that after using the joint test, the specificity of diagnosis increased from 47.71–82.57%. The Youden index increased from 0.3761 to 0.7156. Sensitivity only decreased from 89.91–88.99%, a slight decrease, but the magnitude is very small. And in the binary logistic regression of breast cancer prognosis, the odds ratio of the combined indicators is as high as 50.914 (OR = 50.914, 95%CI:10.918 ~ 237.417). It can be seen that the combined detection of three DNA repair proteins has higher clinical diagnostic value than the single determination. It has been pointed out that PARP1 promotes the expression of HIF-1 α by activating nuclear factor κ B (NF- κ B) and promotes the polarization of macrophages M2, leading to the up-regulation of tumor-related macrophages (TAMs), such as tumor necrosis factor alpha (TNF α) and IL-6, thus promoting the proliferation, invasion and metastasis of tumor cells, promoting the formation of tumor microvessels and microlymphatics[34]. Overexpression of ERCC1 also up-regulates TNF- α , while XRCC4 in NHEJ pathway activates DNA-dependent protein kinase complex (DNA-PK), which is essential for the adhesion of monocytes to TNF- α treated endothelial cells. Other studies have found that in the process of tumorigenesis and development, most patients are accompanied by chronic inflammation, and the chronic protraction of inflammation affects the condition of tumors, leading to recurrence and metastasis of tumors[35]. Tumor necrosis factor- α (TNF α) is closely related to the occurrence of cancer. The secretion of TNF- α cytokines in tumor microenvironment can accelerate the growth and spread of cancer cells. At the same time, it can make cancer cells bypass the impact of the immune system, promote the EMT process of cells, and cause recurrence and distant metastasis of cancer[36]. These studies suggest that PARP1, ERCC1 and XRCC4 may act together on TNF- α to produce recurrence and metastasis of breast cancer. It can be seen that the combined detection of the three DNA repair proteins has higher clinical diagnostic value than the single determination, which is helpful for the early diagnosis of breast cancer prognosis.

Conclusions

PARP1 > 6, XRCC4 > 6 and ERCC1 > 3 can predict the recurrence and metastasis of breast cancer. In addition, the combined diagnosis of PARP1,XRCC4 and ERCC1 has great predictive value for the risk of breast cancer recurrence and metastasis. However, the mechanism of the effect of PARP1,XRCC4 and ERCC1 on the recurrence and metastasis of breast cancer remains unclear, which needs further study.

Abbreviations

ERCC1: DNA excision repair cross complementing 1; XPA: Xeroderma pigmentosum group A; XRCC1: X-ray repair cross-complementing 1; PARP1: Poly ADP-ribose polymerase-1; MSH2: MutS homolog 2; MLH1: MutL homolog 1; 53BP1: p53 binding protein; XRCC4: X-ray repair cross-complementing 4; ER: Estrogen receptor; PR: Progesterone receptor; HER2: human epidermal growth factor receptor-2; TNBC: Triple negative breast cancer; TAMs: Tumor-related macrophages; TNF- α : Tumor necrosis factor- α ; DNA-PK: DNA-dependent protein kinase complex; EMT: Epithelial - mesenchymal transition; NF- κ B: Nuclear factor-kappa B

Declarations

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Authors' contributions

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

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Availability of data and materials

The data and materials of this study are available from the corresponding authors for reasonable requests.

Ethics approval and consent to participate

Patients with informed consent to participate. This research program has been reviewed by the ethics committee of the unit, and it is considered that it meets the relevant ethical requirements of clinical research and agrees to the research plan. 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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Figures

Figure 1

ROC curve of PARP1, XRCC4 and ERCC1 combined to diagnose breast cancer recurrence and metastasis

Figure 2

The diagnose ROC curve of PARP1, XRCC4 and ERCC1

Figure 3

Shows the effect of breast cancer recurrence and metastasis on the mRNA expression of DNA repair gene. Data are described as Mean (SD), n = 109. Statistical differences are expressed as: *P < 0.05.

Figure 4

Shows the effect of breast cancer recurrence and metastasis on the protein expression of DNA repair gene as shown in Figure 1A. Data are described as Median (IQR), N = 109. Statistical differences are expressed as: *P < 0.05. Strong expression of immunohistochemical positive controls compared to negative controls (B). Immunohistochemistry (IHC) detection of DNA repair genes MSH2 (C), MLH1 (D), PARP1 (E), XRCC1 (F), XRCC4 (G), 53BP1 (H), ERCC1 (I), XPA (J) in paraffin tissues of patients with recurrence and metastasis breast cancer (1 for the recurrence and metastasis group, 2 for the control group(non recurrence and metastasis group); original magnification × 400).