High excision repair cross-complementation group 1 expression is associated with favorable prognostic factors in breast cancer

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Abstract. Distortion of DNA can inhibit transcription and replication, resulting in cell death. The nucleotide excision repair (NER) pathway recognizes and repairs DNA adducts. Excision repair cross-complementation group 1 (ERCC1) is a nuclease that serves a vital role in the NER pathway. Few studies have investigated ERCC1 expression in breast cancer. The aim of the present study was to analyze the association between clinicopathological features and ERCC1 expression in breast cancer. ERCC1 expression was studied in 224 invasive ductal carcinomas by immunohistochemical staining. ERCC1 expression was analyzed as an immunoreactive score, and classified into low and high expression groups. The association between immunohistochemical parameters and clinicopathological features was evaluated. High expression of ERCC1 was observed in 33 cases (14.7%) and was statistically associated with lower T stage (P=0.005), lower tumor size (P=0.001), no lymph node metastasis (P=0.044) and no lymphovascular invasion (LVI; P=0.004). Additionally, high ERCC1 expression was associated with a positive estrogen receptor (ER) (P=0.006) and progesterone receptor (PR) (P=0.001) expression status. Non-triple-negative breast carcinoma occurred more frequently in the high expression group (97%) than the low expression group; however, the difference was not statistically significant (P=0.056). Overall and disease-free survival were also not significantly different between the two groups (P=0.989 and P=0.215, respectively). In conclusion, high ERCC1 expression is statistically associated with lower T stage, smaller tumor size, no lymph node metastasis, no LVI, and positive ER and PR expression. This suggests that ERCC1 is associated with favorable prognostic parameters in breast cancer.

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Introduction

Breast cancer is the most common malignancy in women worldwide. Various histological types of breast cancer have been reported, with invasive ductal carcinoma (invasive carcinoma of no special type) being the most frequently occurring type (1,2). Therefore, considerable effort has been devoted to identifying factors of prognostic and therapeutic significance in invasive ductal carcinoma. The immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR) and human epithelial growth factor receptor 2 (HER2) has been widely used for predicting the prognosis of breast cancer and for providing therapeutic strategies (3). Since Perou *et al* (4) reported the molecular features of breast cancer cells in 2000, the improvements in molecular techniques have provided a framework to establish molecular subtypes, namely luminal A, luminal B HER2$^-$, luminal B HER2$^+$, triple-negative, HER2 type, 5 negative phenotype and basal phenotype breast cancer (5,6). Breast cancer-expressed hormonal receptors, including ER and PR, or amplification of HER2, have been used in various targeted treatment approaches (7,8).

A targeted therapy has not yet been established for TNBC. Therefore, chemotherapy with a platinum-based agent remains in use as a common treatment of choice for TNBC (9). Excision repair cross-complementation group 1 (ERCC1)-xeroderma pigmentosum complementation group F (XPF) complex repairs DNA damaged by anticancer agents; studies have reported that ERCC1 expression is an important factor in determining the poor response of chemotherapy (10,11). Certain studies have also reported that the expression of ERCC1 in TNBC may be a predictive factor of a poor response to platinum-based chemotherapy (12,13). By contrast, others studies have reported that there is no association between ERCC1 expression and TNBC (14,15). Another study reported that TNBC showed the lowest ERCC1 expression among other breast cancer subtype based on the expression of hormonal receptor (16). Furthermore, these studies showed that the high expression of ERCC1 was correlated with the clinicopathological factors associated with a good prognosis (14,15).

Thus, the expression of ERCC1 in breast cancer has provided ambivalent results. Therefore, the present study evaluated the association between various clinicopathological parameters and ERCC1 expression in invasive ductal breast carcinoma. Furthermore, the study also analyzed the prognosis, depending on the level of ERCC1 expression, in this carcinoma.

Materials and methods

Patient selection. A total of 224 patients with invasive ductal breast cancer, who were diagnosed and treated at the Kangbuk Samsung Hospital (Sungkyunkwan University School of Medicine, Seoul, South Korea) between January 2006 and April 2010 were enrolled. Patients who received preoperative treatment and had other diseases were excluded. Patients who performed biopsy for pathologic diagnosis were also excluded. All studies were conducted with the prior approval of the Institutional Review Board of Kangbuk Samsung Hospital. The requirement for patient consent for publication of this study was waived. The following clinicopathological parameters were included: Patient age, presence of an extensive intraductal component (EIC), skin or chest wall invasion, Paget's disease, lymphovascular invasion (LVI), tumor borders, ER positivity, PR positivity, HER-2 positivity, triple negativity, Tumor-Node-Metastasis (TNM) stage (17), presence of lymph node metastasis, distant metastasis and mortality due to breast cancer. Histological grades were assigned using tubule formation, nuclear pleomorphism, and mitotic counts based on the modified Bloom-Richardson grading system (18). The tissue samples were formalin fixed at room temperature for more than 8 h and they were paraffin embedded representatively. Tissue section (3-μm-thick) were stained with hematoxylin (at room temperature for 90 sec) and eosin (at room temperature for 40 sec) using Dako Coverstainer fully automated system (Dako, Agilent Technologies, Inc., Santa Clara, CA, USA) and slides from all patients were reviewed by two pathologists in a blind manner with an Olympus BX51 microscope, and the histological data such as T and N stage, and lymphatic invasion, were confirmed again. The discrepant cases were reviewed by the two pathologists together to achieve a consensus result.

Tissue microarray (TMA) construction. The surgically resected specimens were fixed in 10% buffered formalin at room temperature for 24 h, processed and embedded in paraffin using a standard protocol. All H&E-stained slides were reviewed and the most representative tumor area was carefully selected and marked on individual paraffin blocks. The most representative tissue core was obtained from each tumor specimen. The TMA specimens were assembled using a tissue-array instrument (TMA Master; 3D HISTECH Kft., Budapest, Hungary) consisting of thin-walled stainless steel punches and stylets for emptying and transferring the needle contents. The assembly was held in an X-Y position guide with a 1-mm increment between the individual samples, a 4-mm punch depth stop device and semiautomatic micrometers. The instrument was used to create holes in the recipient block with defined array cores. The fit needle was used to transfer the tissue cores into the recipient block. Taking into consideration the limitations of the representative areas of the tumor, duplicate 2-mm-diameter tissue cores were used from each donor block. The percentage of tissue cores with tumor was ≥70%.

Immunohistochemistry and immunohistochemical evaluation. Immunohistochemistry analysis was performed using Leica BOND-MAX™ fully automated immunohistochemistry system, according to the manufacture's protocol (Leica Microsystems GmbH, Wetzlar, Germany). Briefly, 4-μm-thick sections were deparaffinized and pre-treated with the Epitope Retrieval Solution 2 (EDTA-buffer pH 8.8) at 98˚C for 20 min. After the tissue washed three times with Bond TM Wash Solution 10X concentrate (cat. no. AR9590), peroxidase blocking was performed for 10 min using the Bond Polymer Refine Detection kit DS9800 (Leica Microsystems GmbH). Tissues were again washed three times with Bond TM Wash Solution 10X concentrate (cat. no. AR9590) and then incubated with the primary antibodies at room temperature for 60 min. Subsequently, tissues were incubated with polymer for 10 min and developed using 3,3-diaminobenzidine at room temperature for 10 min. ER (cat. no. RM-9101-F; 1:200 dilution; SP1 clone; Labvision Corporation, Fremont, CA, USA), PR (cat. no. M3569; 1:200 dilution; PgR636 clone; Dako; Agilent Technologies, Inc.) and HER2 (cat. no. RM-9103-R7-A; 1:200 dilution; SP3 clone; Labvision Corporation) antibodies were used. ER and PR expression was evaluated by Allred scoring (19) and HER2 expression was evaluated by American Society of Clinical Oncology/College of American Pathologists guideline recommendations (20). In case with equivocal scores (HER2 score 2) (20), silver *in situ hybridization* was performed for the determination of HER2 gene status (Fig. 1).

ERCC1 immunohistochemistry staining and immunohistochemical evaluation. Human tissues obtained were fixed in 10% formalin solution at room temperature for 24 h, dehydrated through a graded ethanol series, washed in xylene and processed for embedding in paraffin wax, according to routine protocols. Sections were incubated in a solution of 0.3% H_2O_2 at room temperature for 15 min to inhibit endogenous peroxidase activity. Antigen retrieval procedure was performed using 10 mM Tris + 1 mM EDTA + 0.03% Tween-20 Solution for 30 min in a presser cooker chamber. Non-specific blocking was quenched by incubation with 4% bovine serum albumin for 30 min. Sections were then incubated for 1 h at room temperature with primary antibodies against ERCC1 (cat. no. ab2356; Abcam, Cambridge, MA, USA) diluted to 1:100. The detection system EnVision+ for secondary horseradish peroxidase-conjugated mouse antibodies (cat. no. K4001; 1:2,000; Dako; Agilent Technologies, Inc.) was applied according to the manufacturer's instructions. The secondary antibodies were incubated at room temperature for 8 min. Slides were stained with liquid diaminobenzidine tetrahydrochloride, a high-sensitivity substrate-chromogen system (cat. no. K5007; Dako; Agilent Technologies, Inc.). Counterstaining was performed with Meyer's hematoxylin at room temperature for 1 min.

The images on the slides were visualized with an Olympus BX51 light microscope (Olympus, Tokyo, Japan). Staining intensity was scored on a scale of 0 to 3 (0, negative; 1, weak;

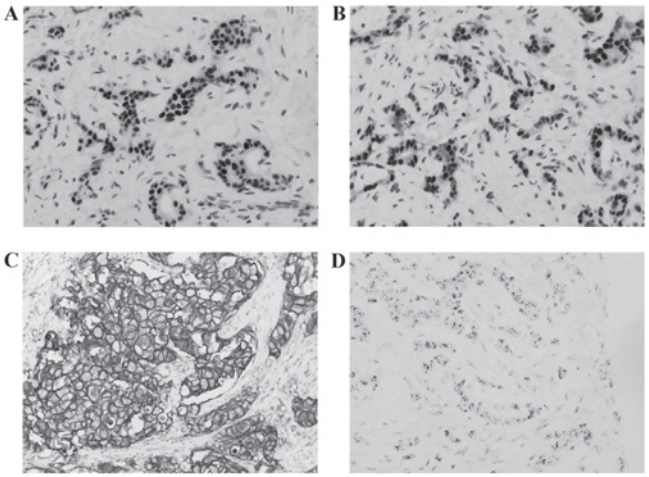

Figure 1. Immunohistochemical staining for receptors in invasive breast carcinoma. (A) Estrogen receptor (magnification, x200), (B) progesterone receptor (magnification, x200), (C) HER2 (magnification, x200) and (D) silver *in situ* hybridization for HER2 gene expression in breast invasive carcinoma (magnification, x1,000). HER2, human epidermal growth factor receptor-2.

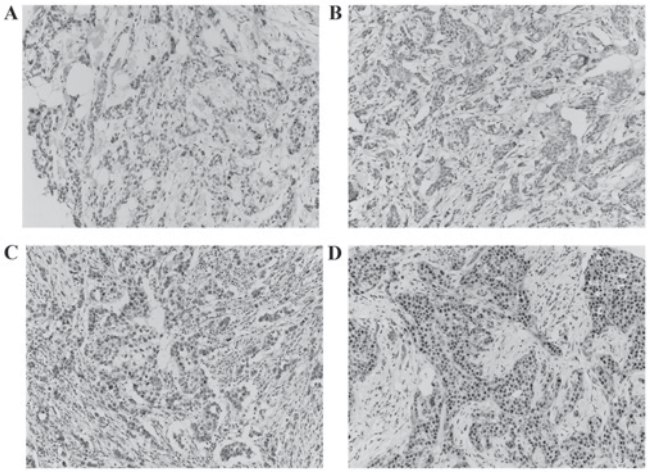

Figure 2. Immunohistochemical staining for ERCC1 in invasive breast carcinoma. (A) Negative nuclear expression for ERCC1 (magnification, x200). (B) Weak nuclear expression for ERCC1 (magnification, x200). (C) Moderate nuclear expression for ERCC1 (magnification, x200) and (D) strong nuclear expression for ERCC1 (magnification, x200). ERCC1, excision repair cross-complementation group 1.

2, moderate; and 3, strong) (Fig. 2). The percentage of positive cells was also classified into one of four categories: Score of 1, 0-25%; score of 2, 26-50%; score of 3, 51-75%; and score of 4, 76-100%. When a discrepancy occurred between duplicate cores, the higher score of the two tissue cores was used as the final score. The level of staining was analyzed as an immunoreactive score (IRS), which was calculated by multiplying together the score of the staining intensity and the percentage of positive cells (21). The expression was classified into low expression (IRS≤7) and high expression (IRS>7) groups, according to a previous study (21).

Statistical analysis. Statistical analyses were performed with PASW Statistics for Windows, version 18.0 (SPSS, Inc., Chicago, IL, USA). The χ^2 test, Fisher's exact test and Student's t-test were used to evaluate the associations between ERCC1 expression and clinicopathological parameters. Multivariate logistic regression analysis was used to identify the clinicopathological predictors of ERCC1 high expression. Disease-free survival (DFS) was defined from the day of surgery to the day of recurrence. Overall survival (OS) was defined from the day of diagnosis to the day of the patient mortality from breast cancer or last known follow-up. Survival probability curves were calculated by life table method, and Gehan's generalized Wilcoxon method was applied for analyzing the univariate survival clinicopathological parameters. P≤0.05 was considered to indicate a statistically significant difference. Multivariate survival parameters were detected among parameters that were statistically significant in univariate analysis by applying the Cox proportional hazards model (95% confidence interval) with a backward stepwise elimination method.

Results

ERCC1 immunohistochemical staining was performed for all 224 invasive ductal carcinoma cases. ERCC1 showed a nuclear expression pattern in all cases. The cut-off value of IRS was 7, and IRS>7 referred to high expression. Among the 224 cases, high expression of ERCC1 was observed in 33 cases (14.7%). Clinicopathological and immunohistochemical parameters, including the expression of ERCC1, are shown in Table I.

With regard to the clinicopathological parameters, high expression of ERCC1 was statistically associated with smaller tumor size (<2.0 cm; P=0.001), no lymph node metastasis (P=0.044), lower pathological stage (stage I; P=0.001) and no LVI (P=0.004). However, age (P=0.253), N stage (P=0.131), histological grade (P=0.373), EIC (P=0.935), skin and chest wall invasion (P=0.442), Paget's disease (P=0.999), the presence of metastasis (P=0.750) and the recurrence rate (P=0.999) were not statistically associated with ERCC1 expression (Table II). To detect parameters that were independently associated with high expression of ERCC1, the four parameters found to be significant on univariate analysis were analyzed by multivariate logistic regression analysis. It was found that smaller tumor size (<2.0 cm; P=0.002 relative risk, 3.815; 95% confidence interval, 1.638-8.888), no lymph node metastasis (P=0.048; relative risk, 2.229; 95% confidence interval, 1.007-4.9340), lower pathological stage (stage I; P=0.001; relative risk, 3.617; 95% confidence interval, 1.685-7.764) and no LVI (P=0.007; relative risk, 3.608; 95% confidence interval, 1.424-9.141) were independent clinicopathological parameters in accordance with the high expression of ERCC1 (Table II).

With regard to immunohistochemical parameters, high expression of ERCC1 was associated with positive ER (P=0.006) and PR (P=0.001) expression. Non-triple-negative breast carcinoma (TNBC) occurred more frequently in the high expression group (97%) than the low expression group, however, the difference was not statistically significant (P=0.056). Additionally, HER2 expression was also not associated with ERCC1 expression. Multivariate logistic regression analysis was also applied for the detection of independent parameters that were associated with the high expression of ERCC1. It was found that positive ER (P=0.012; relative risk, 4.806; 95% confidence interval, 1.412-16.359)

Table I. Clinicopathological parameters and immunohistochemical results.

Parameter	No.	%
Age, years		
≤52	129	57.6
>52	95	42.4
T stage		
1	111	49.6
2	102	45.5
3	11	4.9
4	0	0
Tumor size, cm		
≤2.0	111	49.6
>2.0	113	50.4
N stage		
0	120	53.6
1	66	29.5
2	21	9.4
3	17	7.6
TMN stage		
1	77	33.4
2	107	47.8
3	40	17.9
Lymph node metastasis		
Absence	120	53.6
Presence	104	46.4
Histological grade		
1	54	24.1
2	99	44.2
3	71	31.7
EIC		
Absence	189	84.4
Presence	35	15.6
Skin and chest wall invasion		
Absence	197	87.9
Presence	5	2.2
Not examined	22	9.9
Paget's disease		
Absence	205	91.5
Presence	4	1.8
Not examined	15	6.7
Lympho-vascular invasion		
Absence	133	59.4
Presence	91	40.6
ER		
Negative	65	29.0
Positive	159	71.0
PR		
Negative	77	34.4
Positive	147	65.6
HER2		
Negative	167	74.6
Positive	57	25.4
Triple-negative		
Yes	32	14.3
No	192	85.7
Distant metastasis		
Absence	202	90.2
Presence	22	9.8
ERCC1		
Low	191	85.3
High	33	14.7

EIC, extensive intraductal component; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; ERCC1, excision repair cross complementation 1; TNM, Tumor-Node-Metastasis.

and positive PR (P=0.003; relative risk, 6.325; 95% confidence interval, 1.864-21.466) expression are independent immunohistochemical parameters that correspond to the high expression of ERCC1 (Table III).

The 5-year OS rate for all patients in this study was 95.1% (213/224 patients). In the high expression group, the 5-year OS rate was 100% (33/33 patients). In the low expression group, the 5-year OS rate was 94.2% (180/191 patients). ERCC1 expression was not statistically associated with the OS rate (P=0.375). The 5-year DFS rate for all patients in this study was 85.7% (192/224 patients). In the high expression group, the 5-year DFS rate was 87.9% (29/33 patients). In the low expression group, the 5-year DFS rate was 85.3% (163/191 patients). ERCC1 expression was also not statistically associated with the DFS rate (P=0.999).

To evaluate OS, univariate analysis was performed. Advanced T stage (T stage 2-3; P=0.006), presence of lymph node metastasis (P=0.038), advanced pathological stage (stage 2-3; P=0.023), presence of skin and chest wall invasion (P=0.015), presence of LVI (P=0.011) and presence of distant metastasis (P=0.001) were statistically associated with poor OS. Status of ERCC1 expression and immunohistochemical parameters were not associated with OS. Multivariate analysis for OS was performed using these statistically significant parameters. It was found that advanced T stage (P=0.034; hazard ratio, 9.283; 95% confidence interval, 1.188-72.538), presence of lymph node metastasis (P=0.04; hazard ratio, 4.989; 95% confidence interval, 1.078-23.097), presence of skin and chest wall invasion (P=0.001; hazard ratio, 12.647; 95% confidence interval, 2.718-58.839), presence of LVI (P=0.017; hazard ratio, 6.448; 95% confidence interval, 1.393-29.854) and presence of distance metastasis (P=0.000; hazard ratio, 22.361; 95% confidence interval, 6.486-77.095) independently predicted poor OS (Table IV).

Table II. Association between ERCC1 expression and clinicopathological parameters.

	Univariate analysis			Multivariate analysis	
Clinicopathological parameters	ERCC low expression (IRS≤7), n (%)	ERCC high expression (IRS>7), n (%)	P-value	RR (95% CI)	P-value
Age, years			0.253		
≤52	107 (56.0)	22 (66.7)		Not applicable	
>52	84 (44.0)	11 (33.3)			
Tumor size, cm			0.001[a]		0.002[a]
≤2.0	86 (45.0)	25 (75.8)		3.815 (1.638-8.888)	
>2.0	105 (55.0)	8 (24.2)			
N stage			0.131		
0	97 (50.8)	23 (69.7)		Not applicable	
1	58 (30.4)	8 (24.2)			
2	19 (9.9)	2 (6.1)			
3	17 (8.9)	0 (0.0)			
Lymph node metastasis			0.044[a]		0.048[a]
Absence	97 (50.8)	23 (69.7)		2.229 (1.007-4.934)	
Presence	94 (49.2)	10 (30.3)			
TNM stage					
1	57 (29.8)	20 (60.6)	0.001[a]	3.617 (1.685-7.764)	0.001[a]
2 and 3	134 (70.2)	13 (39.4)			
Histological grade			0.373		
1	45 (23.6)	9 (27.3)		Not applicable	
2	82 (42.9)	17 (51.5)			
3	64 (33.5)	7 (21.2)			
EIC			0.935		
Absence	161 (84.3)	28 (84.8)		Not applicable	
Presence	30 (15.7)	5 (15.2)			
Skin and chest wall invasion[a]			0.442		
Absence	176 (97.8)	21 (95.5)		Not applicable	
Presence	4 (2.2)	1 (4.5)			
Paget's disease[b]			0.999		
Absence	176 (97.8)	29 (100)		Not applicable	
Presence	4 (2.2)	0 (0)			
Lymphovascular invasion			0.004[a]		0.007[c]
Absence	106 (55.5)	27 (81.8)		3.608 (1.424-9.141)	
Presence	85 (44.5)	6 (18.2)			
Distant metastasis			0.750		
Absence	171 (89.5)	31 (93.9)		Not applicable	
Presence	20 (10.5)	2 (6.1)			
Recurrence			0.999		
Negative	163 (85.3)	29 (87.9)		Not applicable	
Positive	28 (14.7)	4 (12.1)			

[a]Medical records regarding skin and chest wall invasion were missing for 22 patients; [b]medical records regarding Paget's disease were missing for 15 patients. [c]Statistically significant. EIC, extensive intraductal component; ERCC1, excision repair cross complementation 1; RR, relative risk; IRS, immunoreactive score; TNM, Tumor-Node-Metastasis.

Table III. Association between ERCC1 expression and immunohistochemical results.

Immunohistochemical results	Univariate analysis			Multivariate analysis	
	ERCC low expression (IRS≤7)	ERCC high expression (IRS>7)	P-value	RR (95% CI)	P-value
ER			0.006[a]		0.012[a]
Negative	62 (32.5)	3 (9.1)		4.806 (1.412-16.359)	
Positive	129 (67.5)	30 (90.9)			
PR			0.001[a]		0.003[a]
Negative	74 (38.7)	3 (9.1)		6.325 (1.864-21.466)	
Positive	117 (61.3)	30 (90.9)			
HER2			0.299		
Negative	140 (73.3)	27 (81.8)		Not applicable	
Positive	51 (26.7)	6 (18.2)			
Triple-negative			0.056		
No	160 (83.8)	32 (97.0)		Not applicable	
Yes	31 (16.2)	1 (3.0)			

[a]Statistically significant. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; ERCC1, excision repair cross complementation 1; IRS, immunoreactive score; RR, relative risk.

To evaluate DFS, univariate analysis was performed. Advanced T stage (P=0.007), high histological grade (P=0.035), presence of skin and chest wall invasion (P=0.001), presence of LVI (P=0.022), presence of distance metastasis (P=0.001), loss of PR expression (P=0.020) and triple-negative subtype (P=0.002) were statistically associated with a shorter DFS time. Multivariate analysis for DFS was performed using these statistically significant parameters. It was found that advanced T stage (P=0.008; hazard ratio, 2.968; 95% confidence interval, 1.333-6.607), presence of skin and chest wall invasion (P=0.001; hazard ratio, 8.991; 95% confidence interval, 2.713-27.796), presence of LVI (P=0.027; hazard ratio, 2.22; 95% confidence interval, 1.096-4.497), presence of distance metastasis (P=0.001; hazard ratio, 16.016; 95% confidence interval, 7.790-32.929), loss of PR expression (P=0.037; hazard ratio, 2.091; 95% confidence interval, 1.045-4.184) and triple-negative subtype (P=0.005; hazard ratio, 3.020; 95% confidence interval, 1.395-6.537) independently predicted a shorter DFS time (Table IV).

Discussion

There are four major pathways to repair damaged DNA: NER, base excision repair, mismatch repair and double strand break repair (22). Among these pathways, NER plays an important role in recognizing and repairing the DNA adducts, particularly those induced by chemotherapeutic agents such as cisplatin (10). The NER pathway requires a number of factors. Among these factors, ERCC1 serves an essential role for the incision step and completion of the NER pathway (11). ERCC1 bind to XPF and forms the ERCC1-XPF complex (10,11). The ERCC1-XPF complex is important as a structure-specific endonuclease in the NER pathway (11). Therefore, certain studies have reported that the ERCC1-XPF complex can be an important factor for repairing the DNA damage induced by chemotherapeutic agents, including cisplatin; thus, the expression of ERCC1 has been considered as a predictive factor for resistance to platinum-based chemotherapy (10,11).

Certain studies have reported the association between ERCC1 expression and TNBC. Sidoni *et al* (12) reported that one-third of the triple-negative patients exhibited relevant ERCC1 expression. Additionally, Ozkan *et al* (13) reported that two-thirds of the triple-negative patients exhibited ERCC1 expression.

However, recently, good prognostic effects of ERCC1 expression have been reported by certain researchers. Goyal *et al* (14) reported that the overexpression of ERCC1 was associated with lower T stage, nodal negativity, an age >50 years and ER positivity, but was not associated with OS and DFS. Gerhard *et al* (15) reported that ERCC1 expression was significantly associated with smaller tumor size and ER positivity, but was not associated with OS and DFS. These two studies also reported that the triple-negative immunohistochemical phenotype was not statistically associated with ERCC1 expression. Furthermore, one report demonstrated that the level of ERCC1 expression was the lowest in triple-negative phenotypes compared with other phenotypes, and that negativity for ERCC1 expression occurred more frequently in TNBC and luminal B group breast cancer (16).

By contrast, other studies did not find any association between ERCC1 expression and clinical and immunohistochemical parameters. Fu *et al* (23) found that ERCC1 gene expression detected by reverse transcription-polymerase chain reaction was not significantly associated with age, tumor size, axillary lymph node metastasis, pathological type, histological grade, ER, PR or HER-2. Metro *et al* (24) also reported that

Table IV. Factors associating with poor overall and shorter disease-free survival time.

	Overall survival			Disease free survival		
	Univariate	Multivariate		Univariate	Multivariate	
Factors	P-value	HR (95% CI)	P-value	P-value	HR (95% CI)	P-value
Age, years (>52 vs. ≤52)	0.334	Not applicable		0.753	Not applicable	
T stage (2-3 vs. 1)	0.006[a]	9.283 (1.188-72.538)	0.034[a]	0.007[a]	2.968 (1.333-6.607)	0.008[a]
Lymph node metastasis (presence vs. absence)	0.038[a]	4.989 (1.078-23.097)	0.040[a]	0.169	Not applicable	
TNM stage (2-3 vs. 1)	0.023[a]	38.061 (0.196-7394.970)	0.176	0.081	Not applicable	
Histologic grade (2-3 vs. 1)	0.214	Not applicable		0.035[a]	2.404 (0.843-6.854)	0.101
EIC (presence vs. absence)	0.985	Not applicable		0.927	Not applicable	
Skin and chest wall invasion (presence vs. absence)	0.015[a]	12.647 (2.718-58.839)	0.001[a]	0.001[a]	8.991 (2.713-29.796)	0.001[a]
Paget disease (presence vs. absence)	0.261	Not applicable		0.062	Not applicable	
Lymphovascular invasion (presence vs. absence)	0.011[a]	6.448 (1.393-29.854)	0.017[a]	0.022[a]	2.22 (1.096-4.497)	0.027[a]
Distant metastasis (presence vs. absence)	0.001[a]	22.361 (6.486-77.095)	0.001[a]	0.001[a]	16.016 (7.790-32.929)	0.001[a]
ERCC1 (IRS>7 vs. IRS≤7)	0.215	Not applicable		0.989	Not applicable	
ER (negative vs. positive)	0.680	Not applicable		0.100	Not applicable	
PR (negative vs. positive)	0.255	Not applicable		0.020[a]	2.091 (1.045-4.184)	0.037[a]
HER2 (negative vs. positive)	0.116	Not applicable		0.237	Not applicable	
Triple-negative (yes vs. no)	0.171	Not applicable		0.002[a]	3.020 (1.395-6.537)	0.005[a]

[a]Statistically significant. EIC, extensive intraductal component; ERCC1, excision repair cross complementation 1; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; CI, confidence interval.

there was no significant association between *in situ* protein expression of ERCC1 and various clinico-pathological parameters, including age, tumor stage at diagnosis, histology, hormone receptor status, HER-2 status, presence of visceral disease and pretreatment of metastatic disease.

Besides cases of breast cancer, in cases of non-small cell lung cancer and gastric cancer, a association has been reported between ERCC1 expression and good prognosis. Lee *et al* (25) showed that in patients with resected non-small cell lung cancer, ERCC1 expression was an independent prognostic factor of longer survival, and that EGFR mutation was more frequent in ERCC1-negative patients. Another study also showed that in patients with resected non-small cell lung cancer, the 5-year survival rate of ERCC1-positive patients was longer than that of ERCC1-negative patients (76 vs. 49%, P=0.004) (26). Wang *et al* (27) reported that ERCC1 expression may be a good prognostic factor in patients with resected gastric cancer.

In addition, Han *et al* (28) reported that single nucleotide polymorphism (SNP)-SNP interaction of NER pathway genes increased the risk of breast cancer. Another study reported that ERCC polymorphism was associated with the increase in the breast cancer risk (29). Notably, Mo *et al* (30) reported that the mRNA level of ERCC1 expression was significantly associated with water arsenic concentration and nail arsenic concentration. Moreover, the study suggested that the DNA repair response was induced by arsenic exposure. Therefore, high ERCC1 expression may be a compensatory response against the DNA injury that is induced by various carcinogens.

In the present study, the immunohistochemical expression of ERCC1 was analyzed in patients with invasive ductal carcinoma. ERCC1 high expression (IRS>7) was statistically associated with the smaller tumor size (≤2 cm), no lymph node metastasis, low pathological stage (TNM stage 1) and no LVI. In addition, high ERCC1 expression was statistically associated with positive estrogen receptor (ER) and progesterone receptor (PR) expression. The triple-negative phenotype was frequently expressed in the ERCC1 low expression group, but this result was not statistically significant. ERCC1 expression was not statistically associated with OS and DFS. Higher T stage (stage 2-3), the presence of skin and chest wall invasion, and LVI were independent of predictive factors of poor OS and shorter DFS. The presence of lymph node metastasis was associated with poor OS only. No immunohistochemical parameters influenced the OS, but the negative expression of PR and triple-negative status were statistically associated with a shorter DFS time. Although ERCC1 expression was not a direct predictor of OS and DFS, low T stage (size ≤2 cm), no lymph node metastasis and no LVI were significantly associated with the high expression of ERCC1. Therefore, the high expression of ERCC1 may be a more favorable factor of good OS and longer DFS times than low level ERCC1 expression.

In conclusion, in the present study, high ERCC1 expression was associated with several clinical and immunohistochemical parameters, namely lower T stage, smaller tumor size, no lymph node metastasis, no LVI, and positivity for ER and PR in invasive ductal carcinoma of the breast. However, no association was shown between the expression of ERCC1 and OS and DFS rate. Based on the results of previously reviewed studies, the role of ERCC1 is not yet fully understood. In order to evaluate the complete role of ERCC1 and the association between ERCC1 expression and clinical outcomes, a greater number of large-scale studies may be required.

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