Original Article

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BRCA1 Expression and Its Combined Low Expression with PARP1 and ERCC1 Predict Chemotherapeutic Response in Ovarian Cancer

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Abstract:

Objective: This study aimed to evaluate the associations of immunohistochemical expressions of various deoxyribonucleic acid repair proteins, either individually or combined, with the response to platinum-based chemotherapy and overall survival in epithelial ovarian cancer.

Material and Methods: This retrospective cohort study included patients with epithelial ovarian cancer who were treated by primary cytoreductive surgery with adjuvant platinum-based chemotherapy at Songklanagarind Hospital between January 2008 and December 2019. Immunohistochemistry analysis of breast cancer type 1 (BRCA1), poly (ADP-ribose) polymerase 1 (PARP1), X-ray repair cross-complementing 1 (XRCC1), and excision repair cross-complementation group 1 (ERCC1) expression was performed. Logistic regression was used to evaluate factors associated with chemotherapeutic response and Cox regression was applied for survival analysis.

Results: Chemotherapeutic response was achieved in 205 of 249 patients (82.3%). Low BRCA1 expression was associated with good response (odds ratio [OR] 5.01, 95% confidence interval [CI] 1.78–14.1) and favorable overall survival (hazard ratio 0.61, 95% CI 0.38–0.98). PARP1, XRCC1, and ERCC1 showed no significant predictive or prognostic roles; however, combined low expression of PARP1/BRCA1 (OR 7.62, 95% CI 1.69–34.31) and ERCC1/BRCA1 (OR 6.98, 95% CI 1.5–32.52) additively enhanced response compared to high/high expressions.

Conclusion: This study provides evidence that epithelial ovarian cancer (EOC) with low BRCA1 expression is more likely to be responsive to platinum-based therapy and is associated with favorable overall survival compared to tumors with high BRCA1 expression. The study supports a potential therapeutic strategy involving co-depletion of PARP1/BRCA and ERCC1/BRCA1 expression, although additional studies are needed.

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Introduction

Ovarian cancer ranks as the eight most frequent cancer in women worldwide and the most lethal gynecological cancer[1,2]. It is histologically classified according to cell origin and epithelial ovarian cancer (EOC) accounts for 90% of the cases. Although 80% of patients respond to the standard treatments of cytoreductive surgery and adjuvant platinum-based chemotherapy, a considerable proportion of them experience disease relapse within 2 years[3]. Currently, there is a lack of biomarkers in routine use to help predict chemotherapy (CMT) response, therefore, identifying such biomarkers would be helpful in treating these patients appropriately. Additionally, identifying therapeutic strategies which are less toxic is needed.

Platinum-based chemotherapy kills cancer cells by causing single-strand or double-strand deoxyribonucleic acid (DNA) DNA breaks which trigger DNA repair processes[4]. Therefore, the ability of cancer cells to repair DNA may indicate tumor sensitivity or resistance to CMT. Key proteins involved in these processes include poly (ADP-ribose) polymerase 1 (PARP1) and X-ray repair cross-complementing protein 1 (XRCC1), both of which function in base excision repair, while excision repair cross-complementation group 1 (ERCC1) functions in nucleotide excision repair, and breast cancer type 1 (BRCA1) is implicated in homologous recombination[4]. These pathways are functionally related, in that deficiency in one pathway leads to dependency on the remaining repair pathways. The generation of PARP inhibitors, a promising novel strategy in BRCA-deficient ovarian cancers, has been developed as a method to exploit this synthetic lethality concept[5,6]. However, a substantial portion of ovarian cancer patients harboring *BRCA1* or *BRCA2* germline mutation fail to respond to the PARP inhibitors[7], and searching for alternative targets has been a recent research focus.

Many studies have evaluated the expression of DNA-repair proteins in association with survival outcomes in patients with EOC, however, they have often reported conflicting results[8-14]. Only a few of these studies focused on CMT responsiveness while fewer evaluated the co-expression of proteins from different DNA-repair pathways to determine targets for a possible therapeutic strategy[12,14,15]. In this study, we investigated the associations of immunohistochemical expressions of PARP1, XRCC1, ERCC1, and BRCA1 with CMT response and overall survival (OS) in patients with EOC. Additionally, we evaluated the co-expression of these DNA repair proteins in order to consider the potential role on their therapeutic synthetic lethality.

Material and Methods

Patients and clinical data

This retrospective cohort study was approved with a waiver of informed consent by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC.63-274-5-4). The patients were consecutive cases of EOC treated by cytoreductive surgery and adjuvant platinum-based chemotherapy at Songklanagarind Hospital between January 2008 and December 2019. This included patients at any stage of clear cell carcinoma or stage 1C or above of other histologic types. Only cases with tissue samples available for immunohistochemical evaluation were included.

Cytoreductive surgery included hysterectomy, bilateral salpingo-oophorectomy, pelvic and paraaortic lymph node dissection, omentectomy, and/or removal of tumors to the greatest extent possible. Peritoneal washing was done during the surgery for surgical staging. The first-line adjuvant chemotherapy regimen included carboplatin and paclitaxel. Upon recurrence, carboplatin-paclitaxel

or carboplatin–liposomal doxorubicin was administered to the CMT response group, and a single regimen, such as liposomal–doxorubicin, topotecan, gemcitabine, or etoposide, was administered to the non-response group.

Platinum-based CMT responders were defined as patients who had no disease recurrence within 6 months after the final course of therapy, while non-responders were those with stable or progressive disease since treatment or who experienced disease recurrence within 6 months[16]. The surveillance protocol after CMT completion was a pelvic examination and CA125 test every 3 months for 2 years, followed by every 6 months for 3 years, and then annually after 5 years. Pelvic organ imaging was performed as clinically indicated.

Clinicopathological data, including age, serum CA125 level, clinical stage, the result of cytoreductive surgery (optimal or sub-optimal), histological type, and lymphovascular invasion status were retrieved from electronic medical records and pathological reports. CA125 was obtained at the time of laboratory investigation before (around 1 month) surgery in all patients clinically suspected of ovarian cancer, except those who were unexpectedly found to have cancer postoperatively. Optimal surgery was defined as residual tumor less than 1 cm. Clinical staging was based on the International Federation of Obstetrics and Gynecology (FIGO) system[17]. Mortality status at the end of the study (December 2020) was acquired from the national civil registration system.

Sample size determination

A maximum of 187 cases were obtained from a sample size calculation to test the association of protein expression with CMT response based on the results of previous studies on XRCC1[11] and BRCA1[18] at a 5% significance level and 80% power. With similar significance level and power, 292 cases were required to test survival differences based on a 7-year enrollment time, 12-year follow-up, and survival information from previous studies[8,12,14,18].

Tissue microarray construction

Histologic slides and formalin-fixed paraffin-embedded tissue blocks were retrieved from the archives of the Department of Pathology, Faculty of Medicine, Prince of Songkla University, Thailand. All histologic slides were reviewed. Two representative areas of each histologic type on the slides and corresponding areas on the tissue blocks (donor block) were marked for subsequent microarray construction using a Quick Ray manual tissue microarray (Unitma, Seoul, Korea) with a 2-mm needle.

Immunohistochemistry

The sections (3-μm thick) underwent deparaffinization with xylene, rehydration with a graded alcohol series, and were stained using an automated immunostainer (BOND-MAX; Leica, Melbourne, Australia). Antigens were retrieved using BOND peroxidase-blocking reagent, followed by incubation with anti-PARP1 (1:100; clone 7D3-6; BD Pharmingen, San Diego, USA), anti-XRCC1 (1:200; clone 33-2-5; Diagnostic Biosystems, Pleasanton, USA), anti-ERCC1 (ready to use; clone 4F9; Dako-Agilent Technologies, Santa Clara, USA), or anti-BRCA1 (1:2,500; clone MS110; Abcam, Cambridge, UK). A BOND Polymer Refine Detection Kit (Leica) was used to detect antigen–antibody reactions, followed by color development using chromogen (3,3'-diaminobenzidine) and counterstaining (Meyer's hematoxylin).

Immunohistochemistry evaluation

A third-year pathology resident and a senior pathologist independently evaluated protein expression under a light microscope. Both observers were blinded to the clinical information during the evaluations. In cases of discordance, consensus was reached by joint reassessment and discussion.

Nuclear immunoreactivity was assessed and the staining intensity was scored as 0, negative; 1, weak; 2, moderate; and 3, strong. The percentage of stained

tumor cells in each intensity score was estimated. The immunoreactivity score (0–300) was calculated by summation of each intensity multiplied by the percentage of positively stained cells with the average score from both tissue cores used as the final score. In accordance with previous studies[15,19], high BRCA1 expression was defined as a tumor with >10% of stained tumor cells showing moderate or strong intensity; otherwise, they were classified as low expression.

Statistical analysis

Descriptive data are presented by percentages, means, or medians. Associations between variables were analyzed using the chi-square or Fisher's exact test as appropriate. The associations between the protein expressions and CMT responses with the adjustment of other co-variates were analyzed by logistic regression. Survival time was defined as the interval between the date of surgery and the date of death from any cause or the final follow-up (December 2020). Alive patients at the end of the follow-up were censored. Survival curves are displayed using the Kaplan–Meier method and compared by the log-rank test. Cox proportional hazard regression was performed to obtain independent prognostic factors. Statistical significance was considered at p-value<0.05. All analyses were conducted using the R program (v.4.0.3; R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinicopathological characteristics

A total of 253 eligible cases with tissue blocks were available for immunohistochemistry assessment. Two patients died, and two were lost to follow-up after the first round of chemotherapy, leaving 249 cases for the analysis.

The clinicopathological characteristics and protein expression of all patients classified by CMT response are presented in Table 1. A CMT response was achieved in 82.3% of the patients. CA125 levels were categorized into low and high based on the median value (368 U/mL). The information on CA125 level before surgery was missing in 26 patients, however the response rate was not significantly different between patients with available and missing data (82.1% versus 84.6%, respectively, p-value=1.0). The study found that all major histological types had a relatively even distribution. The proportion distributions of CA125, FIGO stage, result of surgery and lymphovascular invasion between CMT responders and non-responders were significantly different. The responders had higher proportions of lower CA125 level, lower FIGO stage, optimal surgery, and absence of lymphovascular invasion.

Protein expression

The immunostaining of representative samples is illustrated in Figure 1. Low BRCA1 expression was found in 62.2% of the cases. The median immunoreactive scores (interquartile range [IQR] for PARP1, XRCC1, and ERCC1 were 120 (IQR 50–215), 165.25 (IQR 104–200), and 220 (IQR: 142.5–294.8), respectively. These three proteins were stratified into low and high expression levels using the median score as a cut-off value. CMT responders and non-responders showed no significant differences in the frequencies of low/high expression of all four proteins (Table 1).

Association of variables with CMT response

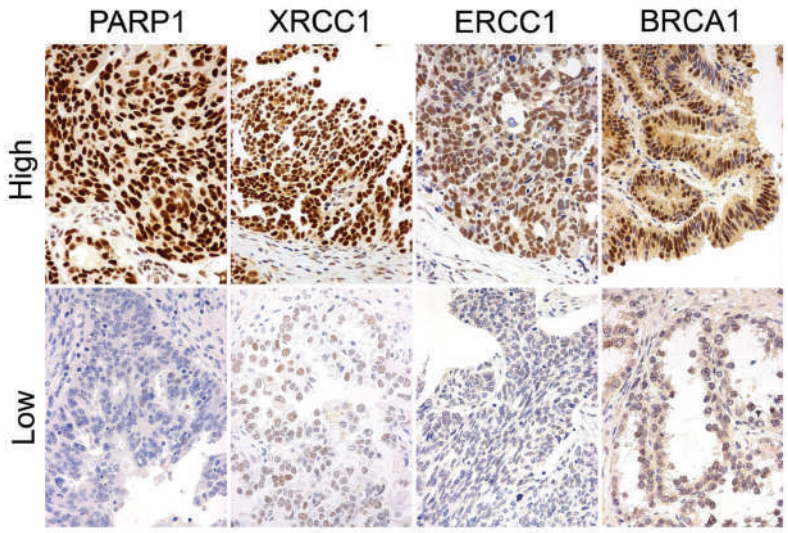
Table 2 presents the odds ratios (OR) with a 95% confidence interval (CI) from the univariate and multivariate logistic regression used to determine the associations of variables with CMT response. An OR value >1 indicates response and <1 indicates non-response. CA125 level, FIGO stage, the result of surgery, and lymphovascular invasion were significantly associated with CMT response according to both univariate and multivariate models. Age was included in the multivariate model as it is an important biological factor. BRCA1 was not a significant factor in univariate analysis but it appeared significant in

the multivariate model (OR 5.01, 95% CI 1.78–14.1). This is likely due to the suppressive effect of CA125 on BRCA1, as the significant effect of BRCA1 appeared when CA125 was present in the model and the CA125 level was inversely associated with BRCA1 level, i.e patients with a high CA125 level more frequently had tumors with low BRCA1 expression (78/112=69.6%) compared to patients with low CA125 (60/111=54.1%, p-value=0.017, data not shown). The other three proteins showed no significant associations with CMT response. For combined protein expression, low PARP1/low BRCA1 (OR 7.62, 95% CI 1.69–34.31) and low ERCC1/low BRCA1 (OR 6.98, 95% CI 1.5–32.52) additively increased the CMT response (Table 3).

Association of protein expressions with overall survival

The 5-year OS was 57.5% (95% CI 51–64.8). The median OS was not reached in 12 years (Figure 2). Kaplan-Meier curves of low and high protein expressions are shown in Figure 3. Patients with low BRCA1 expression had higher 5-year OSs compared to patients with high BRCA1 (54.5% versus 59.2%, respectively) although the difference was not statistically significant (p-value=0.3). Low and high expression of PRAP1, XRCC1 and ERCC1 also show no survival difference by the log-rank test (p-value=0.68, 0.66, 072, respectively).

Table 4 shows the results of Cox regression analysis for OS. CA125 level, FIGO stage, result of surgery, histologic type and lymphovascular invasion were significant factors in the univariate model. However, in the multivariate model, only FIGO stage and result of surgery remained significant. In addition, age and BRCA1 expression turned out to be significant prognostic factors. Low BRCA1 expression was significantly associated with favorable OS (hazard ratio [HR] 0.61, 95% CI 0.38–0.98) while the other three proteins showed no prognostic significance. All pairs of protein combinations also showed no significant association with OS (data not shown).



PRARP1=poly(ADP-ribose) polymerase 1, XRCC1=X-ray repair cross-complementing 1, ERCC1=.excision repair cross-complementation group 1, BRCA1=breast cancer type 1

Figure 1 Immunostaining of representative cases showing high (upper panel) and low expression (lower panel) of PARP1, XRCC1, ERCC1, and BRCA1. Original magnification, 400x

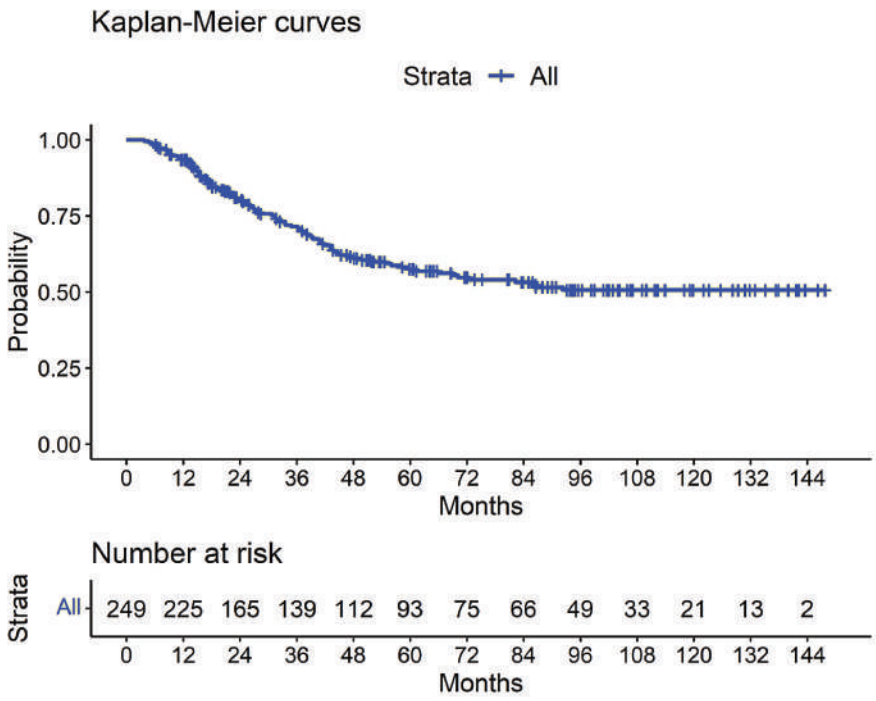
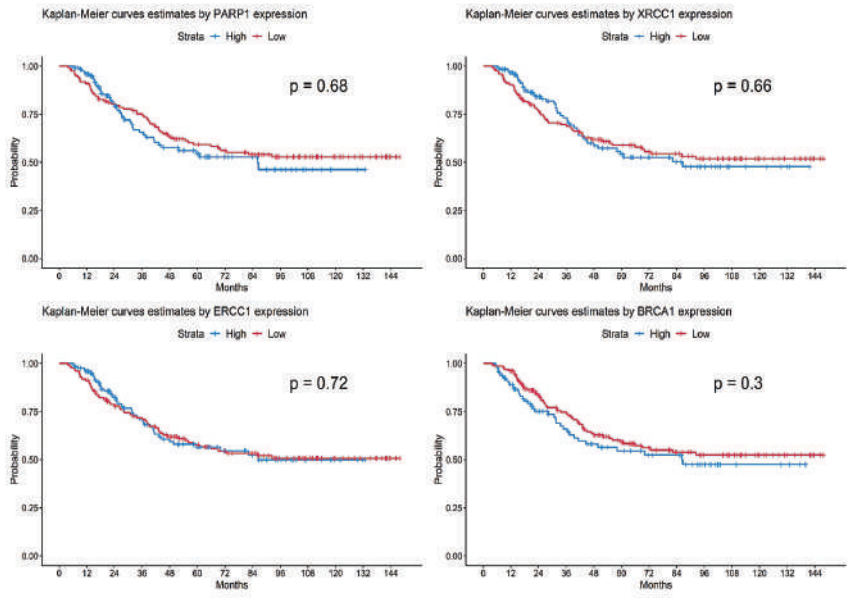


Figure 2 Kaplan–Meier curves for overall survival of the entire cohort



PRARP1=poly(ADP–ribose) polymerase 1, XRCC1=X–ray repair cross–complementing 1, ERCC1=excision repair cross–complementation group 1, BRCA1=breast cancer type 1

Figure 3 Kaplan–Meier curves for overall survival of low and high protein expressions with a p value of log–rank test

Table 1 Clinicopathological characteristics and protein expressions classified by status of chemotherapeutic response

Variable	Number (%)			p-value
	Total (N=249)	Responders (N=205)	Non-responders (N=44)	
Age (years)				0.491
<60	191 (76.7)	159 (77.6)	32 (72.7)	
≥60	58 (23.3)	46 (24.4)	12 (27.3)	
CA125 level (U/mL) (n=223)				0.002
<368	111 (49.8)	100 (54.6)	11 (27.5)	
≥368	112 (50.2)	83 (45.4)	29 (72.5)	
FIGO stage				<0.001
I	73 (29.3)	69 (33.7)	4 (9.1)	
II	73 (29.3)	64 (31.2)	9 (20.5)	
III	93 (37.3)	67 (32.7)	26 (59.1)	
IV	10 (4.0)	5 (2.4)	5 (11.4)	
Result of surgery				<0.001
Optimal	183 (73.5)	164 (80)	19 (43.2)	
Sub-optimal	66 (26.5)	41 (20)	25 (56.8)	
Histological type				0.508
Endometrioid	49 (19.7)	44 (21.5)	5 (11.4)	
High grade serous	72 (28.9)	60 (29.3)	12 (27.3)	
Clear cell	63 (25.3)	48 (23.4)	15 (34.1)	
Mucinous	39 (15.7))	31 (15.1)	8 (18.2)	
Low grade serous	4 (1.6)	4 (2.0)	0 (0)	
Mixed	22 (8.8)	18 (8.8)	4 (9.1)	
Lymphovascular invasion				0.021
Absence	162 (65.1)	140 (68.3)	22 (50)	
Presence	87 (34.9)	65 (31.7)	22 (50)	
PARP1 expression				0.632
High	127 (51.0)	106 (51.7)	21 (47.7)	
Low	122 (49.0)	99 (48.3)	23 (52.3)	
XRCC1 expression				0.103
High	124 (49.8)	107 (52.2)	17 (38.6)	
Low	125 (50.2)	98 (47.8)	27 (61.4)	
ERCC1 expression				0.488
High	125 (50.2)	105 (51.2)	20 (45.5)	
Low	124 (49.8)	100 (48.8)	24 (54.5)	
BRCA1 expression				
High	94 (37.8)	73 (35.6)	21 (47.7)	0.132
Low	155 (62.2)	132 (64.4)	23 (52.3)	

BRCA1=breast cancer type 1, CI=confidence interval, ERCC1=excision repair cross-complementation group 1, OR=odds ratio, PARP1=poly(ADP-ribose) polymerase 1, XRCC1=X-ray repair cross-complementing 1

Table 2 Association of clinicopathological variables and protein expressions with chemotherapeutic response in patients with epithelial ovarian cancer by logistic regression

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (years)				
<60	(Ref)		(Ref)	
≥60	0.77 (0.37–1.62)	0.492	0.76 (0.27–2.15)	0.601
CA125 level (U/mL)				
<368	(Ref)		(Ref)	
≥368	0.31 (0.15–0.67)	0.003	0.17 (0.05–0.55)	0.003
FIGO stage				
I	(Ref)		(Ref)	
II	0.41 (0.12–1.4)	0.157	0.54 (0.13–2.24)	0.394
III	0.15 (0.05–0.45)	<0.001	0.49 (0.11–2.09)	0.333
IV	0.06 (0.01–0.29)	<0.001	0.13 (0.02–0.89)	0.037
Result of surgery				
Optimal	(Ref)		(Ref)	
Sub-optimal	0.19 (0.1–0.38)	<0.001	0.19 (0.07–0.53)	0.001
Histological type				
Endometrioid	(Ref)		(Ref)	
High grade serous	0.57 (0.19–1.73)	0.32	1.63 (0.38–7.04)	0.51
Clear cell	0.36 (0.12–1.08)	0.069	0.11 (0.02–0.53)	0.006
Mucinous	0.44 (0.13–1.47)	0.183	0.19 (0.03–1.13)	0.067
Low grade serous	0 (0–Inf)	0.99	0 (0–Inf)	0.991
Mixed	0.51 (0.12–2.13)	0.356	0.73 (0.1–5.09)	0.751
Lymphovascular invasion				
Absence	(Ref)		(Ref)	
Presence	0.46 (0.24–0.9)	0.023	0.37 (0.14–0.95)	0.038
PARP1 expression				
High	(Ref)		(Ref)	
Low	0.85 (0.44–1.64)	0.632	1.5 (0.51–4.42)	0.46
XRCC1 expression				
High	(Ref)		(Ref)	
Low	0.58 (0.3–1.12)	0.105	0.32 (0.1–1.08)	0.066
ERCC1 expression				
High	(Ref)		(Ref)	
Low	0.79 (0.41–1.53)	0.488	1.34 (0.47–3.86)	0.582
BRCA1 expression				
High	(Ref)		(Ref)	
Low	1.65 (0.86–3.18)	0.135	5.01 (1.78–14.1)	0.002

BRCA1=breast cancer type 1, CI=confidence interval, ERCC1=excision repair cross-complementation group 1, OR=odds ratio, PARP1=poly(ADP-ribose) polymerase 1, XRCC1=X-ray repair cross-complementing 1

Table 3 Association of combined protein expressions with chemotherapeutic response in patients with epithelial ovarian cancer by logistic regression

Variable	Univariate analysis		Multivariate analysis†	
	OR (95% CI)	p-value	OR (95% CI)	p-value
PARP1/BRCA1				
High/high	(Ref)		(Ref)	
High/low	1.69 (0.65–4.41)	0.285	5.29 (1.36–20.61)	0.016
Low/high	0.66 (0.24–1.81)	0.417	1.62 (0.33–8)	0.556
Low/low	1.3 (0.57–2.96)	0.531	7.62 (1.69–34.31)	0.008
XRCC1/BRCA1				
High/high	(Ref)		(Ref)	
High/low	3.31 (1.01–10.81)	0.047	5.48 (1.24–24.17)	0.025
Low/high	0.61 (0.22–1.7)	0.347	0.35 (0.07–1.69)	0.19
Low/low	1.007 (0.46–2.21)	0.986	1.63 (0.43–6.09)	0.471
ERCC1/BRCA1				
High/high	(Ref)		(Ref)	
High/low	2.21 (0.81–5.97)	0.12	6.96 (1.77–27.3)	0.005
Low/high	0.85 (0.31–2.31)	0.745	1.98 (0.46–8.57)	0.362
Low/low	1.27 (0.56–2.87)	0.567	6.98 (1.5–32.52)	0.013
PARP1/XRCC1				
High/high	(Ref)		(Ref)	
High/low	0.52 (0.19–1.45)	0.215	0.31 (0.08–1.25)	0.1
Low/high	1.28 (0.34–4.83)	0.718	1.43 (0.18–11.64)	0.738
Low/low	0.63 (0.3–1.34)	0.233	0.48 (0.14–1.61)	0.235
PARP1/ERCC1				
High/high	(Ref)		(Ref)	
High/low	0.81 (0.29–2.31)	0.697	1.88 (0.45–7.94)	0.39
Low/high	0.94 (0.31–2.84)	0.909	2.3 (0.43–12.34)	0.33
Low/low	0.77 (0.36–1.64)	0.499	2.13 (0.54–8.35)	0.28
XRCC1/ERCC1				
High/high	(Ref)		(Ref)	
High/low	0.52 (0.19–1.45)	0.215	0.35 (0.09–1.31)	0.12
Low/high	1.28 (0.34–4.83)	0.718	1.48 (0.19–11.8)	0.712
Low/low	0.63 (0.3–1.34)	0.233	0.57 (0.2–1.6)	0.289

†Adjusted for age, CA125 level, FIGO stage, result of surgery, histological type, lymphovascular invasion, and other proteins.
BRCA1=breast cancer type 1, CI=confidence interval, ERCC1=excision repair cross-complementation group 1, OR=odds ratio, PARP1=poly (ADP-ribose) polymerase 1, XRCC1=X-ray repair cross-complementing 1

Table 4 Association of clinicopathological variables and protein expressions with overall survival in patients with epithelial ovarian cancer by Cox regression

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age (years)				
<60	(Ref)		(Ref)	
≥60	0.91 (0.56,1.46)	0.69	0.57 (0.32,1)	0.049
CA125 level (U/mL)				
<368	(Ref)		(Ref)	
≥368	1.66 (1.08,2.54)	0.02	1.34 (0.77,2.33)	0.296
FIGO stage				
I	(Ref)		(Ref)	
II	1.71 (0.9,3.26)	0.102	1.12 (0.54,2.32)	0.764
III	3.43 (1.93,6.08)	<0.001	1.71 (0.83,3.5)	0.143
IV	3.8 (1.48,9.81)	0.006	1.92 (0.7,5.3)	0.207
Result of surgery				
Optimal	(Ref)		(Ref)	
Sub-optimal	2.87 (1.92,4.29)	<0.001	2.5 (1.54,4.06)	<0.001
Histologic type				
Endometrioid	(Ref)		(Ref)	
High grade serous	1.69 (0.89,3.2)	0.108	1.2 (0.58,2.49)	0.622
Clear cell	1.67 (0.86,3.27)	0.133	1.98 (0.92,4.27)	0.082
Mucinous	1.19 (0.54,2.6)	0.67	1.28 (0.51,3.25)	0.596
Low grade serous	1.43 (0.19,10.98)	0.729	1.54 (0.18,12.92)	0.689
Mixed	2.29 (1.06,4.93)	0.035	1.85 (0.78,4.38)	0.162
Lymphovascular invasion				
Absence	(Ref)		(Ref)	
Presence	1.59 (1.06–2.39)	0.024	1.48 (0.91,2.41)	0.116
PARP1				
High	(Ref)		(Ref)	
Low	0.88 (0.59,1.32)	0.549	0.87 (0.51,1.49)	0.62
XRCC1				
High	(Ref)		(Ref)	
Low	1.01 (0.68–1.5)	0.97	1.21 (0.68,2.15)	0.508
ERCC1				
High	(Ref)		(Ref)	
Low	1.03 (0.69,1.54)	0.871	1.06 (0.62,1.79)	0.841
BRCA1				
High	(Ref)		(Ref)	
Low	0.8 (0.53,1.21)	0.297	0.61 (0.38,0.98)	0.042

BRCA1=breast cancer type 1, CI=confidence interval, ERCC1=excision repair cross-complementation group 1, HR=hazard ratio, PARP1=poly(ADP-ribose) polymerase 1, XRCC1=X-ray repair cross-complementing 1

Discussion

In this study, we hypothesized that the expression status of DNA-repair proteins might indicate EOC sensitivity to platinum-based CMT which eventually predicts survival outcomes. The results showed that low BRCA1 expression was a strong predictive factor of CMT response and was an independent prognostic factor for OS. Additionally, combined low expression of PARP1/BRCA1 and ERCC1/BRCA1 additively enhanced CMT response.

Deficiency of BRCA1-specific function, as a result of germline mutation, somatic mutation, or epigenetic silencing, directs cells toward an error-prone repair process that leads to genomic instability and cell death[20]. A previous study found that immunohistochemistry evaluation of BRCA1 had a high negative predictive value (95.4%) and high positive predictive value (87.5%) for detecting BRCA1 loss caused by a variety of mechanisms[19]. We found that 62.2% of the evaluated tumor sections showed absent/low BRCA1 expression, which is consistent with previous studies[15,19,21]. A majority of these studies used an MS110 clone antibody and a cut-off value of <10% positively stained tumor cells as the criteria for absent/low BRCA1 expression. In the present study, we applied a similar antibody and the same cut-off value, allowing a relevant comparison of our results with those of previous studies.

Previous studies consistently found that EOC patients harboring *BRCA1* mutations had better survival outcomes[22,23]. However, only a few studies have evaluated BRCA1 expression with respect to platinum response. The results of the present study, showing that absent/low BRCA1 expression was strongly associated with a better platinum-based CMT response agreed with the results reported by Carser et al.[18] In contrast, Ali et al.[14] did not find the same association; however, they used an immunohistochemical score of <80 to define low/negative BRCA1 expression as compared to the 10% cut-off used in the present study and by Carser et al.[18]; this likely contributed to the differences observed in the results. However, it is of note that a significant portion of the BRCA1-high patients (35.6%) were in the responsive group, therefore, this marker alone cannot be used for treatment selection.

We found that BRCA1 expression was significantly associated with OS, which is consistent with findings reported by Hjortkjær et al.[15] However, other authors identified no prognostic significance in multivariate analysis.[10,25] Notably, high grade serous carcinoma was the major histological type (60%–100% of the samples) used in most previous studies, whereas the present study evaluated all histological subtypes of EOC, with only 30% of these representing high grade serous carcinoma.

We did not detect significant roles for PARP1, XRCC1, or ERCC1 expression in predicting CMT response and OS. These three proteins are primarily responsible for repairing single-strand breaks. One study reported a significant association between PARP1 expression and therapeutic response to platinum-based therapy, although the authors did not perform multivariate analysis[14], whereas other studies reported no significant prognostic role for PARP1 in EOC[10,15,25,26]. Similar conflicting results have been published for ERCC1[12,25,27,28]. However, our results were discordant from those of studies by Abdel-Fatah et al. and Ali et al. that documented significant predictive and prognostic roles of XRCC1[11,14]. Possible explanations for these discrepant outcomes include the antibody used in the immunohistochemistry and the cut-off value applied to determine low/high expression. In addition, many studies did not perform multivariate analysis for adjustment of potential confounders to determine an independent effect where outcomes associated with protein expression were concerned.

Additionally, we found that combined low expressions of PARP1/BRCA1 and ERCC1/BRCA1 additively enhanced CMT response. These results may support the therapeutic option of using a PARP inhibitor not only in hereditary *BRCA1*-mutated ovarian cancer but also in sporadic cancers showing absent or low BRCA1 expression.

Currently, reports on the potential benefits of combined low expression of ERCC1/BRCA1 on tumor sensitivity have not been documented. Nevertheless, we also found a significant association of combined high XRCC1/low BRCA1 (OR 5.48) and high ERCC1/low BRCA1 (OR 6.96) with CMT response. The significant effect was principally due to the effect of low BRCA1 (OR 5.01). However, these results were contradictory to the theoretical expectation and cannot be explained clearly.

The strengths of our study include the large sample size and the study samples covering all major histologic subtypes which ensured the representativeness of EOC and generalizability of the results. Nonetheless, the study had the following limitations. First, the study included patient data and specimens dating from 2008. The stored tissues may have been subject to loss of antigenicity that could affect immunohistochemistry results. We addressed this potential problem by incorporating "year" in the multivariate model but no significant change in the results was observed (data not shown). Second, germline *BRCA1* mutations were not ascertained, which hindered our ability to highlight the role of BRCA1 expression in hereditary and sporadic EOC separately. Lastly, the major limitation relates to the use of TMA in assessing protein expression, which leads to a concern of representativeness with regard to the whole section due to tumor heterogeneity. However, a number of validation studies performed in various cancers including in EOC reported a moderate to high agreement between duplicate or triplicate 0.6–2.0 mm core diameter with whole tissue sections[29–31]. TMA is now widely used in biomarker assessment with the advantages of reducing cost, time, and variability in experimental conditions. In addition, many researchers have used TMA for assessing BRCA1 in EOC as well as in other gynecologic cancers[32–34]. Therefore, the use of TMA in this study was in line with the current practice in oncology research.

Conclusion

In summary, this study provides evidence that EOC with low BRCA1 expression detected by immunohistochemistry is more likely to be responsive to platinum-based therapy and is associated with favorable overall survival compared to tumors with high BRCA1 expression. These findings also indirectly support the targeting of PARP1 as a treatment modality for BRCA1-deficient tumors. Although ERCC1 was also found to be a potential therapeutic target, further clinical and experimental studies are needed for confirmation.

Conflict of interest

All authors declare no conflicts of interest.

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