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Pre-Treatment Tumor Expression of ERCC1 in Women with Advanced Stage Epithelial Ovarian Cancer is Not Predictive of Clinical Outcomes:

A Gynecologic Oncology Group Study

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Abstract

Objective—Excision repair cross-complementation group 1 (ERCC1) is required for the repair of platinum-induced DNA damage. This study sought to assess the prognostic value of ERCC1 expression, measured by immunohistochemistry (IHC) using a highly specific antibody, in advanced epithelial ovarian cancer (EOC) patients treated with platinum-based chemotherapy.

Methods—Formalin-fixed, paraffin-embedded tumors were collected from two GOG phase III trials (GOG-172 and GOG-182) of patients with stage III/IV EOC treated with platinum-based

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CONFLICT OF INTEREST

The authors have no conflicts to disclose.

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chemotherapy. ERCC1 was detected by (IHC) using FL297 polyclonal antibody and tumors were categorized as negative or positive, based on nuclear staining of tumor cells. ERCC1 genotyping was performed as previously reported. Associations between ERCC1 expression and clinical characteristics, platinum responsiveness, progression-free survival (PFS) or overall survival (OS) were evaluated.

Results—Of 408 eligible patients, 27% had tumors that were ERCC1 positive. ERCC1 expression was not associated with clinical characteristics or platinum -responsiveness. Women with ERCC1-positive versus -negative tumors had similar median PFS (17.9 months versus 17.5 months, respectively, $p=0.59$), median OS (52.0 months versus 47.0 months, respectively, $p=0.30$), risk of disease progression (adjusted hazard ratio [HR]=0.90, 95% confidence interval [CI]: 0.71–1.15, $p=0.41$), and risk of death (adjusted HR=0.81, 95% CI: 0.61–1.07, $p=0.14$). ERCC1 expression, as measured by IHC, was not associated with single nucleotide polymorphisms (SNPs), in codon 118 and *C8092A*, of the ERCC1 gene.

Conclusions—ERCC1 expression, measured by IHC in pre-treatment tumor specimens, using a highly specific antibody, has limited clinical value in patients with advanced EOC treated with platinum and taxane based chemotherapy.

Keywords

ovarian cancer; biomarker; DNA repair; platinum-resistance; prognosis

INTRODUCTION

Epithelial ovarian cancer (EOC) affects approximately 21,990 patients each year, resulting in approximately 15,460 deaths [1]. Patients typically present with advanced stage disease that receive multimodal therapy, including aggressive surgical cytoreductive surgery to achieve maximal tumor resection followed by combination platinum-based chemotherapy. Aggressive surgery and chemotherapy have yielded 5-year survivals ranging from 30–50% [2–6], with the majority of patients relapsing after an initial response to platinum-based chemotherapy [7]. Patients who achieve optimal cytoreductive surgery generally experience longer progression free (PFS) and overall survival (OS) compared to patients with suboptimal residual disease [2–5]. In addition, other clinical and biologic factors, including genetics, environmental exposure, tumor histology, patient age, performance status and baseline organ function may influence long-term outcomes in patients with advanced EOC [7].

Response to primary and secondary platinum-based chemotherapy remains a critical determinant of survival for EOC patients, and is limited by the development of platinum resistance. Possible mechanisms of platinum resistance include decreased uptake/increased efflux of chemotherapeutic drugs, increased metabolism/inactivation of platinum drugs, or increased repair of platinum-induced DNA damage. The correlation between intracellular influx/efflux of platinum-based therapies, aberrant copper transporter expression, and increased glutathione expression have been studied extensively but have led to little advancement in combating platinum drug resistance [8]. Of note, loss of BRCA1/2 function has been correlated with platinum sensitivity and has increased interest in the relationship between DNA repair mechanisms and platinum drug resistance. In some cases, EOC tumors from *BRCA* mutation carriers who become resistant to platinum-based therapy were discovered to have secondary mutations in the same gene, restoring BRCA function [9]. BRCA1 and BRCA2 are involved in homologous-recombination, a DNA repair mechanism required for repair of double-strand breaks and interstrand crosslinks [10].

Excision repair cross-complementation group 1 (ERCC1) is one subunit of the DNA repair endonuclease ERCC1-XPF. The enzyme was discovered for its essential role in nucleotide excision repair (NER) [11], the repair pathway that removes helix-distorting lesions affecting one strand of DNA such as cisplatin-induced monoadducts and intrastrand crosslinks [12]. ERCC1-XPF is also essential for the repair of DNA interstrand crosslinks (ICL) [12, 13], highly cytotoxic lesions induced by bifunctional genotoxins like cisplatin [14]. ERCC1-XPF is the only enzyme required for both NER and ICL-repair and, therefore, removal of all types of platinum-induced DNA damage [12–14]. Hence, ERCC1 has been the focus of much research aimed at discovering mechanisms of tumor resistance to platinum-based therapy [13, 14]. Elevated ERCC1 expression has been implicated in the development of platinum resistant disease in head and neck, non-small cell lung and gastric cancer, as well as ovarian carcinoma [15–19]. However, in many tumor types the results are variable, showing positive or no correlation between ERCC1 expression and various clinical endpoints [20, 21]. Part of this variability may arise from the fact that an antibody commonly used in clinical trials (8F-1) to measure ERCC1 protein expression by immunohistochemistry (IHC) is not specific for ERCC1 [22]. Bhagwat et al evaluated six commercially available antibodies for specificity detecting ERCC1 by western blot, immunoprecipitation, immunofluorescence, and IHC. Conclusions demonstrated that the FL297 antibody was specific for ERCC1 when using IHC [22]. Herein, we sought to test the hypothesis that increased ERCC1 protein expression, as detected by IHC, predicts poor response to platinum based therapy in EOC, using an antibody that was previously characterized and demonstrated to be specific for ERCC1 [22].

MATERIALS AND METHODS

Patients

Patients who participated in GOG-172 and GOG-182 and provided tumor samples for translational research were included in this study. GOG-172 was a randomized trial of IV versus IP cisplatin + paclitaxel with optimally resected stage III EOC or primary peritoneal carcinoma (defined as 1 cm maximal diameter of residual tumor) [23]. GOG-182 was a randomized trial of primary triplet and sequential doublet chemotherapy compared to standard IV carboplatin + paclitaxel in women with previously untreated, histologically-confirmed stage III or IV EOC with either optimal or suboptimal residual disease after primary cytoreduction [24]. Please see Supplemental Data for regimen details. Women on both protocols were required to have adequate hematologic and vital organ function as previously described but could not have a borderline tumor with low malignant potential [23,24]. Pathology was centrally reviewed and confirmed by the Gynecology Oncology Group (GOG) pathology committee. All women provided written informed consent for treatment and translational research, and participating institutions were required to obtain annual Institutional Review Board (IRB) approval consistent with federal, state, and local requirements. In addition, Magee-Womens Hospital of the University of Pittsburgh Medical Center provided IRB approval for this study of ERCC1 in EOC.

Clinical End-Points

PFS was calculated as the time in months from study enrollment to disease progression or death, or to the date of last contact for women who were alive with no evidence of disease progression. OS was calculated as the time from enrollment to death or to the date of last contact for those who were still alive. Platinum-sensitive disease was defined as PFS ≥ 12 months, platinum-resistant as PFS between 6 to 11 months and platinum-refractory as PFS < 6 months.

Tumor Specimens and Immunohistochemistry for ERCC1

Formalin-fixed and paraffin-embedded primary tumor specimens were collected during primary cytoreductive surgery and prior to the initiation of any first-line chemotherapy from women. Immunohistochemistry (IHC) was performed on 5-micrometer sections. Antigen retrieval was completed at pH 6 using DAKO Target retrieval solution (DakoCytomation, Carpinteria, CA) at 95°C for 20 minutes. Specimens were then blocked with 3% hydrogen peroxide for 10 minutes, rinsed with deionized water and Tris buffered saline (TBS), followed by CAS blocking solution (Invitrogen Corporation, Carlsbad, CA) for 5 minutes. Slides were incubated with FL297 (anti-ERCC1 rabbit polyclonal antibody 1:250, Santa Cruz Biotechnology, Santa Cruz, CA) for 60 minutes at room temperature. Primary antibody signal was then detected using biotinylated anti-mouse secondary antibody for 30 minutes and DAB+ for 5 minutes at room temperature (Vectastain ABC kit; Vector Laboratories). Hematoxylin was used for counterstaining. Human tonsil and tumor tissue incubated with IgG were utilized as positive and negative controls for each processing run, respectively. ERCC1 expression was evaluated (RD and JMR) in a binary fashion where any nuclei staining in a specimen was defined as positive and lack of any nuclei staining as negative (Figure 1). The reviewers were masked to clinical outcomes and discrepancies were simultaneously reviewed a second time for consensus.

Isolation of DNA and Genotyping

DNA was extracted from white blood cells from whole blood and processed by polymerase chain reaction (PCR) for *ERCC1* codons 118 and *C8092A* as described previously [25].

Statistical Methods

The PFS or OS probability was estimated using the Kaplan-Meier method, and the log-rank test was employed to compare the difference between ERCC1 positive and negative patients. The association of ERCC1 expression with PFS or OS was further analyzed using a Cox model by controlling for important clinical factors including tumor cell type (clear cell + mucinous versus other), treatment regimen (seven regimes as defined in Table 1) and an aggregate of tumor stage by residual disease status (optimally-resected stage III, suboptimally-resected stage III, stage IV). The Chi-square or Fisher-exact test was used to assess the association of ERCC1 expression with clinical covariates. All reported P values are two-sided and $p < 0.05$ was considered statistically significant.

RESULTS

A total of 408 patients were included for this analysis, including 212 patients from GOG-172 and 196 patients from GOG-182. The median age of this population was 58 years, 91% were Caucasians, 79% had serous adenocarcinoma, 53% were poorly differentiated and 76% had stage III disease with optimal residual (Table 1). As of this analysis, 268 women had died, 67 were alive with no evidence of disease and 73 were alive with disease recurrence. Median follow-up for those women who were still alive was 71 months (75 months for GOG-172 and 65 months for GOG-182).

Of 408 patients, 108 (27%) were defined as ERCC1 positive and the remaining 300 (73.5%) as negative by IHC. Representative sections of negative and positive ERCC1 staining are shown in Figure 1. There is no evidence that ERCC1 expression was associated with patient clinical characteristics (Table 2). Based on all 408 patients from two protocols, the median PFS was 17.9 months for ERCC1 positive vs. 17.5 months for ERCC1 negative patients, without showing a difference ($p = 0.586$) (Figure 2A). The result was consistent across multivariate analysis after controlling for histology, stage, residual disease and treatment regimen (HR: 0.90, 95% CI: 0.71–1.15, $p = 0.405$) (Table 3). The analyses were also

conducted for each protocol separately, and there was no indication that IHC detection of ERCC1 was predictive of PFS, either in GOG-172 (cisplatin-based chemotherapy) or GOG 182 (carboplatin-based chemotherapy) (Figure 2B–2C and Table 3). The results on OS were similar. The median OS were 52 months and 47 months, respectively, for ERCC1 positive and negative patients ($p=0.304$) and the HR adjusted for clinical covariates was 0.81 (95% CI: 0.61–1.07, $p=0.140$) (Figure 3A and Table 3). Of note, the analysis from GOG-182 suggested a trend toward decreased risk of death among patients with ERCC1 positive (HR: 0.70, 95% CI: 0.46–1.04, $p=0.080$) (Table 3 and Figure 3C). There is no association between ERCC1 protein expression and two common, *ERCC1* SNPs (codon 118 and *C8092A*) as shown in Figure 4.

DISCUSSION

Platinum resistance in advanced EOC is a difficult problem to manage clinically, contributing to the high case-fatality ratio associated with advanced stage disease. Establishing a reliable biomarker of platinum resistance would enhance patient management by limiting toxicity from ineffective chemotherapy and triaging patients towards treatments that may have an increased response rates. Even though there is much interest in ERCC1 as a potential biomarker of platinum-resistance, our findings demonstrate that ERCC1 protein expression, as assessed by IHC in pre-treatment tumor specimens utilizing a well-characterized, highly specific antibody, is not associated with PFS or OS in patients with advanced stage EOC. It is possible that expression of ERCC1 may change following primary chemotherapy. However, most patients do not undergo repeat biopsies at the time of recurrence, and additional specimens were not available for patients enrolled on these phase III trials.

Early *in vitro* investigation provided the basis for the interest in ERCC1 protein expression as a predictor of platinum resistance in ovarian cancer. Exposing ovarian tumor cell lines to cisplatin in culture increases ERCC1 protein expression in a time- and dose-dependent fashion [26]. Furthermore, cells with a two-fold increased expression of ERCC1, determined by immunoblot, demonstrate increased removal of cisplatin DNA adducts, as measured by atomic absorption spectrometry [27]. These results were consistent with, but not definitive proof of, the hypothesis that ERCC1 expression is inducible in response to genotoxic stress and rate limiting for DNA repair.

Previous studies investigating a relationship between ERCC1 protein expression and clinical endpoints in ovarian carcinoma yielded conflicting results (supplemental data). Originally, Steffensen et al used IHC with the anti-ERCC1 antibody 8F1 to examine 100 tumor specimens [28]. Forty-five percent of specimens were positive which was associated with a significantly poorer response to chemotherapy ($p<0.001$). This did not translate into a difference in OS benefit [28]. However, treatment regimens after disease recurrence are highly varied and may significantly alter OS statistics leading to a recent shift towards increased emphasis on PFS as the primary study endpoint in ovarian cancer. A second study, corroborated these findings by demonstrating that low ERCC1 protein expression was significantly associated with drug sensitivity ($p<0.001$) in 63 patient specimens [29]. A majority of patients in both studies received carboplatin and cyclophosphamide as adjuvant therapy instead of the more standard platinum/taxane combination which has been shown to improve OS [30]. To address the possible effect of different chemotherapy regimens on OS, Steffensen et al repeated the analysis on 101 patients with advanced EOC treated with carboplatin and paclitaxel. They once again found a significant relationship between negative ERCC1 expression and platinum sensitivity ($p=0.0013$) and PFS ($p=0.0012$), but no significant difference in OS ($p=0.099$) [18]. A major difference between this study and ours is the low percent of patients that were scored as ERCC1-positive (14% vs. 27% in the

present study). Furthermore, patients with stable disease were included in the platinum sensitive group in the previous studies. Most importantly, they used the antibody 8F-1, which is not specific for the ERCC1 protein [22]. Bhagwat et al demonstrated that the only commercially available antibody specific for detecting ERCC1 by IHC is the FL297 antibody [22].

In contrast, a separate group conducted an independent analysis utilizing the same, non-specific antibody 8F-1 antibody for IHC, the same scoring method, and on samples from patients (n=80) treated with similar a chemotherapy regimen and found low overall ERCC1 expression (20.3%) and no association between protein expression and platinum responsiveness (p=0.21) [20]. Our result is consistent with this negative result, and is the largest study to date with over 420 primary tumor specimens. Furthermore, our data provide definitive information about the relationship between ERCC1 protein expression and clinical outcomes, because our study utilized an antibody demonstrated to be specific for ERCC1 [22].

While IHC of paraffin embedded tumor sections is a relatively easy modality for measuring, potential prognostic biomarkers, peripheral blood cells are even easier and more rapid to obtain and characterize. In keeping with the hypothesis that ERCC1 expression is a regulator of DNA repair capacity, there has been much interest in analyzing *ERCC1* SNPs for their ability to predict a patient's response to platinum-based therapy. Using ovarian cancer cell lines, it was demonstrated that the wild type codon 118 (C/C) correlated with increased repair of cisplatin-induced DNA damage [31]. Furthermore, in EOC tumor samples, a genotype of C/C at codon 118 was associated with a greater risk of disease progression (HR=3.73, p=0.003) compared with patients with the C/T or T/T genotype [32].

Previously, we demonstrated that the genotype of the *ERCC1* codon 118 was not associated with clinical outcome [25]. However, women with *ERCC1 C8092A* C/A or A/A genotype had a significantly increased risk of disease progression, compared to patients with a C/C genotype [25]. These results were corroborated by Kim et al, who found that C/A or A/A *ERCC1 C8092A* genotype were significantly correlated with poor PFS and OS [33]. Conversely, other investigators have shown no correlation between SNP *C8092A* and survival, but did show correlation with increased rates of renal dysfunction after treatment with cisplatin [34]. Finally, a large pharmacogenetic study with patients enrolled on the Scottish Randomized Trial in Ovarian Cancer (SCOTROC1) failed to show any significant associations between genotype and outcome or toxicity after examining four *ERCC1* polymorphisms including codon 118 and *C8092A* [35]. The current study demonstrates that neither SNP is associated with ERCC1 protein expression as measured by IHC.

Developing sensitive molecular diagnostic tests to determine who will and will not respond to standard chemotherapy regimens is an important component of individualized treatment planning to improve patient outcomes. Utilizing sequential tumor specimens in patients who have been treated with platinum based chemotherapy may allow for the evaluation of induction of specific genes involved in DNA repair. With increased use of neoadjuvant chemotherapy and interval cytoreductive surgery in patients with bulky stage III–IV disease, it may be possible to compare paired tumor specimens obtained pre- and post-chemotherapy. These results may lead to an improved understanding of the development of chemotherapy resistance. In this current study we showed that ERCC1 expression, as detected by IHC, was not a biomarker to predict clinical outcomes or response to therapy in patients with advanced EOC treated with platinum based chemotherapy. It is possible that xeroderma pigmentosum, complementation group f (XPF), the obligate binding partner of ERCC1, is the subunit that may have biomarker potential. Studies investigating XPF protein expression and ovarian cancer have yet to be performed. Further studies to detect subtleties in ERCC1

protein expression may be clinically valuable in determining prognosis or even directed therapeutic options. Utilizing alternative methodologies such as quantitative immunoblot and mass spectrometry are likely to improve sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- ERCC1 tumor expression in advanced EOC is not associated with patient survival
- Common polymorphisms in *ERCC1* were not associated with ERCC1
- immunohistochemical expression

FIGURE 1.

ERCC1 expression in ovarian carcinoma. A) Serous ovarian carcinoma with negative immunohistochemical expression of ERCC1 (original magnification 400X). B) Strong nuclear immunohistochemical expression of ERCC1 in tumor cells only, with surrounding stroma negative (original magnification 400X).

FIGURE 2.

Kaplan-Meier estimates of progression-free survival (PFS) between women whose tumors stained positive versus those whose tumors stained negative for ERCC1: (A) analysis from combined data of two protocols; (B) analysis from GOG-172; and (C) analysis from GOG-182.

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FIGURE 3.

Kaplan-Meier estimates of overall survival (OS) between women whose tumors stained positive versus those whose tumors stained negative for ERCC1: (A) analysis from combined data of two protocols; (B) analysis from GOG-172; and (C) analysis from GOG-182.

FIGURE 4.
Associations of ERCC1 Expression and Genetic Polymorphisms (codon118 and C8092A)

Table 1

Patient Characteristics

	GOG-0172 (n=212)	GOG-0182 (n=196)	All patients (n=408)
	No. patients	No. patients	No. patients
Age (years)			
< 55	93	62	155
55 – 64	59	71	130
65	60	63	123
Median (range)	57	60	58
Race			
White	196	176	372
Black	6	14	20
Other *	10	6	16
Performance Status			
0	92	91	183
1	105	95	200
2	15	10	25
Histology			
Serous	167	154	321
Endometrioid	12	14	26
Clear cell	12	5	17
Mucinous	1	1	2
Others †	20	22	42
Tumor Grade			
1	21	15	36
2	88	66	154
3 or clear cell	103	115	218
Stage/debulking			
Stage III-micro	88	33	121
Stage III-optimal	124	68	192
Stage III-suboptimal	-	55	55
Stage IV	-	40	40
Treatment ‡			
Cis+P (IV)	117	-	117
Cis+P (IP)	105	-	95
Cb+P (IV)	-	43	43
Cb+P+G (IV)	-	38	38
Cb+P+D (IV)	-	40	40
Cb+T→Cb+P (IV)	-	42	42
Cb+G→Cb+P (IV)	-	33	33

* Other includes Hispanic, Asian or Pacific Islander.

[†] Other histologic subtypes included: mixed epithelial, undifferentiated, transitional cell and adenocarcinoma, not otherwise specified

[‡] IP: intraperitoneal; IV: Intravenous; Cis: cisplatin; P: paclitaxel; Cb: carboplatin; G:gemcitabine; D: doxil; T: topotecan

Table 2

Association of ERCC1 Expression with Clinical Characteristics

	Positive No.	Negative No.	P value
Age (years)			0.750
Median (range)	58 (24–84)	58 (30–87)	
Race			
White	98	274	0.928
Black	6	14	
Other *	4	12	
Performance Status			
0	49	134	0.970
1	52	148	
2	7	18	
Stage/Debulking			0.936
Stage III – microscopic	31	90	
Stage III – optimal	53	139	
Stage III – suboptimal	13	42	
Stage IV	11	29	
Histology			
Serous	80	241	0.277
Endometrioid	6	20	
Clear cell	7	10	
Mucinous	0	2	
Others †	15	27	
Tumor Grade			
1	11	25	0.671
2	43	111	
3	54	164	

* Other includes Hispanic, Asian or Pacific Islander.

† Other histologic subtypes included: mixed epithelial, undifferentiated, transitional cell and adenocarcinoma, not otherwise specified

Table 3
Progression-free Survival (PFS) and Overall Survival (OS) by ERCC1 Expression

ERCC1 Expression	No.	Adjusted PFS*			Adjusted OS*		
		HR*	95%CI*	P value	HR	95% CI	P value
All Patients							
ERCC1 Negative	300	Referent			Referent		
ERCC1 Positive	108	0.90	0.71–1.15	0.405	0.81	0.61–1.07	0.140
GOG-0172							
ERCC1 Negative	151	Referent			Referent		
ERCC1 Positive	61	0.84	0.59–1.18	0.314	0.93	0.63–1.37	0.704
GOG-0182							
ERCC1 Negative	149	Referent			Referent		
ERCC1 Positive	47	0.98	0.69–1.38	0.891	0.70	0.46–1.04	0.080

*. Progression Free Survival (PFS), Overall Survival, Hazard ratio (HR) and 95% confidence interval (CI) were estimated from Cox model adjusted for stage/debulking, histology and type of chemotherapy.