Estrogen receptor-α promoter methylation is a biomarker for outcome prediction of cisplatin resistance in triple-negative breast cancer

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Abstract. A number of previous studies have indicated the presence of a link between estrogen receptor-α (ERα) methylation and triple-negative breast cancer (TNBC). However, the association between ERα methylation and drug resistance during the treatment of TNBC remains unclear. Methylation-specific polymerase chain reaction was used to investigate the methylation of ERα in the genomic DNA of 35 patients with TNBC who were defined as cisplatin-based chemotherapy-resistant using chemosensitivity testing. Survival probabilities by covariates were assessed using Kaplan-Meier estimator survival analysis and Cox's proportional hazards models, adjusting for age, menopausal status, tumor size, lymph node metastasis and ERα promoter DNA methylation. Of the 35 patients with TNBC analyzed, 8 exhibited ERα promoter DNA methylation. Cisplatin resistance was confirmed to be overwhelmingly associated with ERα methylation by univariate and multivariate analysis. Even in a limited analysis in patients with ERα methylation, the results generated from methylated tumor tissue and unmethylated tumor tissue revealed that expression of breast cancer type 1/2 susceptibility proteins was increased in ERα-methylated breast tumor tissue compared with in unmethylated tissue. The ERα methylation group tended to have significantly shorter progression-free (P=0.010) and overall (P=0.023) survival times compared with those in the unmethylated group. Similarly, shorter progression-free (P=0.024) and overall (P=0.018) survival times were observed in the cisplatin-resistant group compared with the cisplatin-non-resistant group. ERα methylation predicts a poor clinical outcome for patients with TNBC. The results of the present study indicated that ERα methylation may be a candidate surrogate biomarker for outcome prediction and cisplatin resistance in TNBC. Further investigation is required to identify potential biomarkers in a larger cohort in a prospective study.

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Introduction

Breast cancer is the most common type of cancer among women and the fifth most common cause of mortality from cancer in China (1). Triple-negative breast cancer (TNBC) accounts for between 10 and 17% of cases of breast cancer (2,3). TNBC is an immunohistochemical description of breast cancer characterized by negative staining for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) (4). TNBC is associated with younger women and with an increased risk of visceral and/or central nervous system metastases during the first 1-3 years of follow-up following diagnosis (5). Owing to the underlying biological heterogeneity within TNBC and lack of special target therapy, the concept of a standard approach to TNBC treatment is inappropriate (6). TNBC is always more shared in individuals harboring mutations of breast cancer type 1/2 susceptibility proteins (BRCA1/2), and aberrations in BRCA1/2 may sensitize breast cancer cells to cisplatin (7). The addition of cisplatin significantly improved survival times in unselected patients in 1988, with other studies confirming activity for cisplatin when used as first-line chemotherapy in TNBC (8-10). Results indicated that the combination of gemcitabine and cisplatin gave a favorable clinical response and managed toxicity as a first-line chemotherapeutic agent in patients with metastatic TNBC, in particular patients with the basal-like disease subtype (8). However, the molecular mechanisms underlying cisplatin sensitivity and cisplatin resistance in TNBC are unclear. Previous studies have demonstrated that platinum-based combination chemotherapy may represent an optimum treatment in TNBC, particularly when patients received anthracycline and/or taxanes, or exhibit homogeneous DNA damage repair defections (9,11). Although promising, treatment with cisplatin-based combination therapies faces certain hurdles,

including the mechanism of platinum resistance and lack of biomarkers to predict responses to cisplatin (12,13).

Methylation of the ERα promoter has previously been demonstrated to be associated with sporadic TNBC, ranging between 30 and 40% in breast cancer samples. It has been demonstrated that methylation of the ERα1, ERα3, ERα4 or ERα5 promoters is associated with basal-like breast cancer (14). ERα and BRCA1 promoter methylation may contribute to poor disease-free survival (DFS) and overall survival (OS) times. Fanconi anemia complementation group F (FANCF) promoter methylation in its CpG islands caused the inactivation of FANCF and acquired cisplatin resistance during tumor progression in ovarian tumors (15). It is not clear whether the ERα methylation would have a similar effect to that in FANCF, causing primary cisplatin resistance in TNBC. To investigate this hypothesis, ERα methylation in the same panel of primary or recurrent breast tumor samples from patients with breast cancer was measured; subsequently, a cisplatin sensitivity test was conducted and the association between ERα methylation and cisplatin resistance was evaluated. To the best of our knowledge, the present study is the first to be designed to identify ERα methylation markers of resistance in patients with TNBC.

Materials and methods

Patient materials. Between March 2013 and July 2015, 35 women (median age, 47 years; range, 27-69 years) with TNBC were enrolled in the present study. Primary breast tumor tissues or recurrent breast tumor tissues were obtained by surgical resection at the Department of Breast Surgery or Department of Medical Oncology, Liaoning Cancer Hospital and Institute (Shenyang, China). Tissue sections containing >30% tumor cells were selected to detect drug sensitivity including more than eight protocols, based on the National Comprehensive Cancer Network guidelines (16), including cisplatin-based chemotherapy with MTT methods. The association between cisplatin resistance *in vitro* and clinicopathological characteristics was analyzed in patients with TNBC. Tumor tissues immunohistochemically identified as TNBC were identified as ER-negative (threshold value, 1%) (sc-542, Santa Cruz, 1:200), PR-negative (sc-539, Santa Cruz, 1:250) and HER2-negative [immunohistochemistry (IHC, sc-08, Santa Cruz, 1:500) 0/1+, or IHC 2+/fluorescence *in situ* hybridization (Hercep TestTM, DAKP A/S, Glostrup, Denmark) non-amplified from the archived pathological reports in the Liaoning Cancer Hospital and Institute]. The present study used research protocols approved by the Liaoning Cancer Hospital and Institute. All samples were obtained with the patient's informed consent. Diagnoses were confirmed by review of clinicopathological features; the clinical data collected included age, family histology, tumor grade, hormone receptor status, lymph node status and tumor size.

Methylation-specific polymerase chain reaction (PCR) (MSP). DNA extract of ERα1, ERα3, ERα4 and ERα5 was isolated from tumor tissues using phenol/chloroform extraction and ethanol precipitation in high-solubility SDS/proteinase K solution. DNA concentration was qualified by determination of optical density $(OD)_{260/280}$ and amplified with specific unmethylated and methylated sequences primers using MSP. Sodium bisulfite-treated DNA was amplified using methylation- and unmethylation-specific primers (presented in Table I) and designated M label and U label, respectively. A total of 2 μg DNA was denatured using NaOH (final concentration, 0.2 M) for 10 min at 37˚C. For samples with 2 μg DNA, salmon sperm DNA (Sigma Aldrich; Merck KgaA, Darmstadt, Germany) was added as carrier prior to modification. A total of 30 μl of 10 mM hydroquinone (Sigma Aldrich; Merck KGaA) and 3 M sodium bisulfite (Sigma Aldrich; Merck KGaA) at pH 5 were added and mixed, and samples were incubated under mineral oil at 50˚C for 16 h. ERα1, ERα3, ERα4 and ERα5 for MSP using the six primer pairs as described previously (14) and purified using the Promega Wizard Genomic DNA Purification kit (A1120, Promega Corporation, Madison, WI, USA). The positive control consisted of alleles from healthy volunteers methylated with SssI methyltransferase (New England Biolabs, Inc., Ipswich, MA, USA), and the negative control was modified using RNA-free water. PCR amplification was performed with the reaction mixtures including 12.5 μl Premix Taq with 1 μl (20 μM) of each primer and 100 ng bisulfite-modified DNA template, with a final volume of 25 μl. The thermocycling conditions were: Denaturation by heating to 95˚C for 10 min, followed by 14 amplification cycles of 94˚C for 30 sec, 62˚C (ERα1) or 59˚C (ERα3, ERα4 and ERα5) for 45 sec (-0.5˚C decreased/cycle) and 72˚C for 45 sec, ending with a final extension of 72˚C for 10 min. The PCR products were separated on a 1% agarose gel stained with GeneFinder™ and images captured by Fluorchem 5500 (ProteinSimple, San Jose, CA, USA). Methylation was considered to be present if the methylated label was detected.

Drug-sensitivity test in vitro. The drug sensitivity of surgical biopsy or surgical excision tissues was assessed using an MTT assay. The primary or recurrent breast cancer tissues were collected from each patient immediately following surgical removal. The cancer cells were digested from tissues and cultured in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (SH30084.03, Hyclone, Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2, and were used in the exponential phase of growth. Cultured cells were treated with cisplatin (A14200156601, QILU Pharmaceutical) at a final concentration of 0.2 mg/ml, for 24 or 48 h. Drug sensitivity was evaluated using an MTT assay. A total of 150 μl MTT solution (Sigma Aldrich; Merck KGaA) at concentration of 5 mg/ml was added for 2 h at 37˚C. After removing the supernatant, 150 ml DMSO (Sigma Aldrich; Merck KGaA) was added to each well for absorbance reading at a wavelength of 490 nm using a plate reader (Tecan Sunrise™, Tecan Group Ltd., Männedorf, Switzerland).

Western blotting. The proteins from breast cancer tissues were extracted using a Tissue or Cell Total Protein Extraction Kit (C510003; Sangon Biotech Co., Ltd., Shanghai, China). Protein content was determined by the Lowry method using bovine serum albumin as the standard. The samples containing 100 μg proteins were separated via SDS-PAGE (10% gel). Following transfer to polyvinylidene fluoride membranes, the samples were blocked using 5% skim milk powder in TBS-T (30 mM Tris-HCl, 125 mM NaCl, 0.1% Tween 20) for 1 h at room

Table I. Primer pair sequences of ERα1, ERα3, ERα4 and ERα5.

Name	Primer pair sequences	Size, bp	Site[a], bp
ERα1 U	5'-TTTTGGGATTGTATTTGTTTTTGTTG-3'	192	+44
	5'-AAACAAAATACAAACCATATCCCCA-3'		
ERα1 M	5'-TTTTGGGATTGTATTTGTTTTCGTC-3'	192	+236
	5'-AACAAAATACAAACCGTATCCCCG-3'		
ERα3 U	5'-GGATATGGTTTGTATTTTGTTTGT-3'	120	+225
	5'-ACAAACAATTCAAAAACTCCAACT-3'		
ERα3 M	5'-GATACGGTTTGTATTTTGTTCGC-3'	130	+345
	5'-CGAACGATTCAAAAACTCCAACT-3'		
ERα4 U	5'-ATGAGTTGGAGTTTTTGAATTGTTT-3'	158	+310
	5'-ATAAACCTACACATTAACAACAACCA-3'		
ERα4 M	5'-CGAGTTGGAGTTTTTGAATCGTTC-3'	151	+468
	5'-CTACGCGTTAACGACGACCG-3'		
ERα5 U	5'-GGTGTATTTGGATAGTAGTAAGTTTGT-3'	120	+375
	5'-CCATAAAAAAAAACCAATCTAACCA-3'		
ERα5 M	5'-GTGTATTTGGATAGTAGTAAGTTCGTC-3'	118	+495
	5'-CGTAAAAAAAAACCGATCTAACCG-3'		

[a]Relative to transcription start site. ERα, estrogen receptor-α; U, unmethylated; M, methylated.

temperature. The PVDF membranes were incubated with the primary antibody, specific to either P-glycoprotein (P-gp; cat no. sc-55510; 1:1,500; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or BRCA1 (cat no. ab9141; 1:500; Abcam, Cambridge, UK) overnight at 4˚C. After washing, the blots were incubated with peroxidase-conjugated affinity-purified goat anti-mouse or goat anti-rabbit secondary antibody (1:1,500; cat nos. sc-395758 and sc-45101, respectively; Santa Cruz Biotechnology, Inc.), as appropriate. Band density of P-gp or BRCA1 was scanned and measured using Fluorchem 5500 software (Alpha Innotech Co., San Leandro, CA, USA). The column ratios were determined through scanned β-actin as calibration.

Statistical analysis. All statistical data are presented as the mean ± standard deviation and the data analyses were performed using SPSS for Windows (version 13.0; SPSS, Inc., Chicago, IL, USA). A χ^2 test and Fisher's exact test were used for binary variable comparisons. PFS and OS curves were constructed using the Kaplan-Meier method and a log-rank test was used to perform comparisons between patients with or without ER-α promoter methylation and cisplatin resistance. Factors influencing cell inhibition by cisplatin, including ER-α promoter methylation and other prognostic factors, were investigated through univariate logistic regression and Cox's proportional hazards regression model with hazards ratio (HR) calculation with 95% confidence interval (95% CI). P<0.05 was considered to indicate a statistically significant difference. OS time was calculated from initiation of treatment to mortality, and individuals alive were censored at the time of last follow-up.

Results

Cisplatin resistance in drug-sensitivity test and association with clinicopathological characteristics. Between 1 March 2013 and 30 July 2015, 35 women with TNBC, with a median age of 47 years (range, 27-69 years), were enrolled. Baseline characteristics of the patients were well-balanced between the drug-sensitivity groups. The drug-sensitivity test including assessment of sensitivity to cisplatin alone and in combination with gemcitabine or capecitabine in all 35 patients with TNBC. Primary cancer cells from patients with TNBC were exposed to various concentrations of cisplatin (final concentration, 0.2 μg/ml) for 48 h. On the basis of the threshold of the majority of drugs in the antitumor drug-sensitivity test *in vitro*, cells whose proliferation was decreased by ≤30% were defined as cisplatin-resistant. Using this definition, the results *in vitro* indicated that 23 individuals exhibited cisplatin sensitivity (inhibition ratio of breast cancer cell, >30%) and 12 patients exhibited cisplatin resistance. The inhibition rate of primary breast cancer cells from premenopausal patients was 47.12% and that of primary breast cancer cells from postmenopausal patients was 44.79%. Cisplatin resistance *in vitro* occurred more often in cells from postmenopausal patients, patients negative for lymph node metastasis (cell inhibition ratio, 51.24 vs. 40.78% for lymph node metastasis-positive and -negative tissues, respectively) and larger tumor size group (cell inhibition ratio, 49.15 vs. 43.91% for tumor sizes <2 cm and >2 cm, respectively). However, the differences in tumor size, lymph node metastasis status, age and menopausal status were not significant (Table II). All of the patients with ERα methylation (n=8) exhibited cisplatin insensitivity *in vitro*. Cell inhibition ratios were 20.25% in the ERα-methylated group and 53.44% in the ERα-unmethylated group. The multivariate Cox's model yielded results for the following: Arm 1, age <40 years; age >40 years [hazard ratio (HR), 0.715; 95% confidence interval (CI), 0.30-1.70; P=0.715]; arm 2, premenopausal vs. postmenopausal (HR, 0.850; 95% CI, 0.37-1.97; P=0.711); arm 3, tumor size <2 cm vs. tumor size >2 cm (HR, 0.850; 95% CI, 0.38-1.93; P=0.698); arm 4, lymph node metastasis-positive vs. lymph node

Table II. Univariate and multivariate model for cell inhibition by cisplatin in TNBC.

		Univariate Cell inhibition ratio		Multivariate Cell inhibition ratio		
Factor	Patients, n	Mean, %	P-value	OR	95% CI	P-value
Age, years			0.594			0.715
<40	20	40.40		0.715	0.30-1.70	
≥40	15	53.13				
Menopausal status			0.693			0.711
Premenopausal	19	47.12		0.85	0.37-1.97	
Postmenopausal	16	44.79				
Tumor size, cm			0.970			0.698
<2	13	49.15		0.85	0.38-1.93	
≥2	22	43.91				
LNM			0.182			0.155
Positive	18	51.24		1.66	0.83-3.33	
Negative	17	40.78				
ERα methylation			0.000			<0.001
Positive	8	20.25		19.41	4.86-77.53	
Negative	27	53.44				

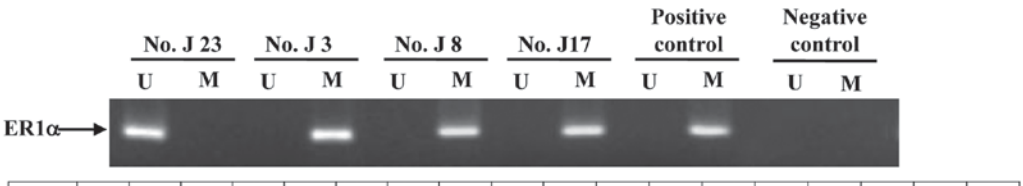
OR, odds ratio; CI, confidence interval; LNM, lymph node metastasis; ERα, estrogen receptor-α.

metastasis-negative (HR, 1.66; 95% CI, 0.83-3.33; P=0.155); arm 5, ERα methylation vs. ERα unmethylation (HR, 19.41; 95% CI, 4.86-77.53; P<0.001).

Analysis of ERα methylation and cisplatin resistance in TNBC. Bisulfite-treated DNA was amplified with specific primers for ERα. If one or more regions were positive for MSP in the methylation labels ERα1, ERα3, ERα4 and ERα5, the tumor tissue was considered to be ERα-methylated. As presented in Fig. 1, neither ERα methylation nor unmethylation were observed in the negative control group; however, the ERα methylation lane was present in positive group. This result demonstrated that MSP had sensitivity and specificity for the present study. In the 35 tumor tissue samples, 8/35 (22.9%) of specimens were ERα-methylated (Fig. 1). The formulation of ERα methylation varied in the samples. ERα1 methylation was positive in four samples (J3, J8, J17 and J34), ER3 methylation was observed in 7/8 positive samples (J1, J3, J5, J19, J23 and J34), ERα4 methylation was positive in three samples (J5, J8 and J23) and ERα5 methylation was observed in three samples (J8, J17 and J23). ERα3 methylation was the most widely shared among these samples. All eight samples with ERα methylation were resistant to cisplatin. The results of this analysis indicated that ERα methylation was significantly associated with cisplatin resistance *in vitro*. However, the formation and degree of methylation was not associated with cell prohibition by cisplatin in the ERα methylation group. ERα methylation may serve an important role in primary cisplatin resistance in TNBC.

ERa methylation promotes cisplatin resistance in TNBC by BRCA overexpression. To determine the contribution of the multidrug-resistance protein P-gp and BRCA1 to primary cisplatin resistance induced by ERα methylation, western blot analysis was performed on primary cancer cells obtained from patients. As presented in Fig. 2A, ERα methylation was associated with an increase in the protein expression of BRCA1, whereas the protein expression level of P-gp was not associated with ERα methylation. These results provide evidence that ERα methylation promotes cisplatin resistance *in vitro* via the overexpression of BRCA, rather than P-gp.

Clinical prognosis of patients in vivo. A total of 35 patients, for whom the ERα methylation characteristics were known, were assessed (Fig. 3). As presented in Fig. 3A and C, a significant decrease in ERα methylation was identified in PFS and OS rates. A similar pattern was observed for cisplatin resistance: A significant decrease in PFS rate was observed in patients with cisplatin resistance for the follow-up duration (Fig. 3B). As presented in Fig. 3D, OS rate with cisplatin resistance gradually increased in the first 12 months of follow-up, however, the curve exhibited a significantly decreased OS rate in the subsequent months. Between the two survival curves there is a marked crossover. The median PFS time for patients with ERα methylation was 3 months, compared with 6 months for those in the ERα-unmethylated arm. The median OS time was similar between the two groups: 14 months in ERα methylation-positive arm and 20.5 months in the ERα methylation-negative arm. The median PFS and OS times of patients with cisplatin resistance were 3 and 16 months, respectively, compared with 7 months and 21.5 months for those in the cisplatin-sensitive arm.



	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14	J15	J16	J17	J18
ERα1			■					■									■	
ERα3	■		■		■			■										
ERα4					■			■										
ERα5								■									■	
CisR	Yes	No	Yes	No	Yes	No	No	Yes	No	Yes	No	No	No	No	No	No	Yes	No
	J19	J20	J21	J22	J23	J24	J25	J26	J27	J28	J29	J30	J31	J32	J33	J34	J35	
ERα1																■		
ERα3	■				■											■		
ERα4					■													
ERα5					■													
CisR	Yes	No	No	No	Yes	No	No	Yes	No	No	Yes	No	Yes	No	No	Yes	No	

U, unmethylation; M, methylation; CisR, Cisplatin resistant; ■Represents methylation of ERα.

Figure 1. Methylation of the ERα gene promoter in TNBC. Methylation of the ERα1, ERα3, ERα4 and ERα5 gene promoters was analyzed by methylation-specific polymerase chain reaction in TNBC. ERα methylation was present in samples shaded dark grey. Negative control and positive control in the figure represent water and positive methylation-treated DNA, respectively. ERα, estrogen receptor-α; U, unmethylated; M, methylated; TNBC, triple-negative breast cancer; CisR, cisplatin-resistant.

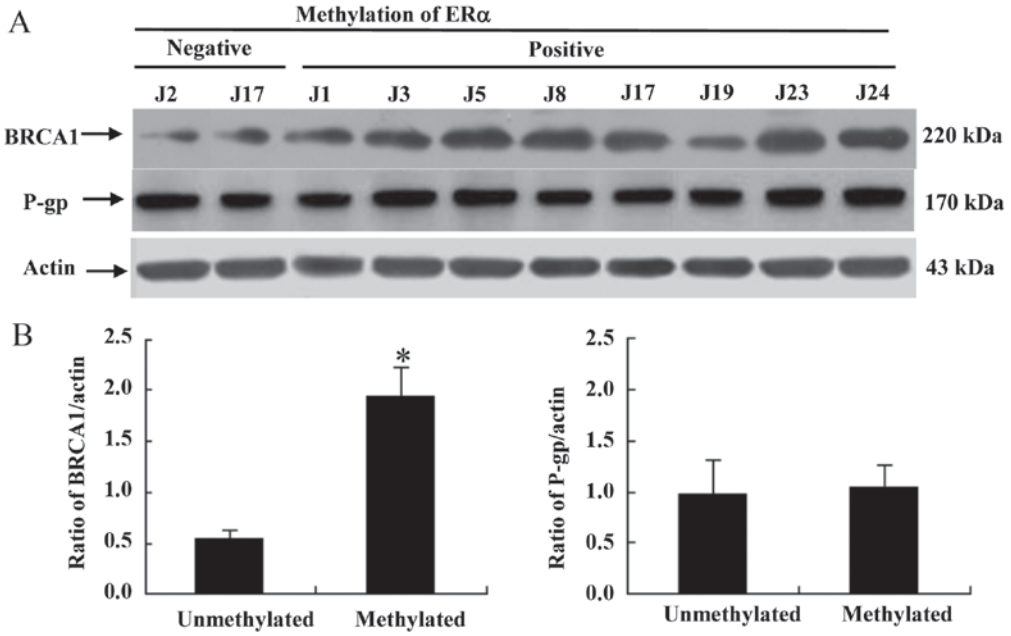


Figure 2. ERα methylation increases the protein expression of BRCA1, but not P-gp, in patients with triple-negative breast cancer. (A) Western blot analysis of BRCA1 and P-gp expression levels in tumor tissues from patients with ERα methylation and ERα unmethylation. Equal amounts of total protein (100 μg) were detected and β-actin protein expression was used as control. (B) Representative results of calculated BRCA1 and P-gp obtained from all the ERα methylation subgroup and ERα unmethylation subgroup. Data are presented as relative to ERα unmethylation. *P<0.05 vs. ERα unmethylation. BRCA1, breast cancer type 1 susceptibility protein; P-gp, P-glycoprotein; ERα, estrogen receptor-α.

Discussion

Breast cancer cells accumulate genetic and protein changes that allow them to evade chemotherapeutic drugs and cause increasingly higher risks to patients, particularly in those with TNBC (3,4). In view of effective specific target, compared with endocrine therapy in hormone-positive breast cancer or transtuzumab therapy in HER2-positive breast cancer, there is no preferred standard chemotherapy to prohibit the proliferation of cancer cells and their metastasis in patients with TNBC (8,17-19). Accurate identification of TNBC and adequately powered prospective trials are required to identify

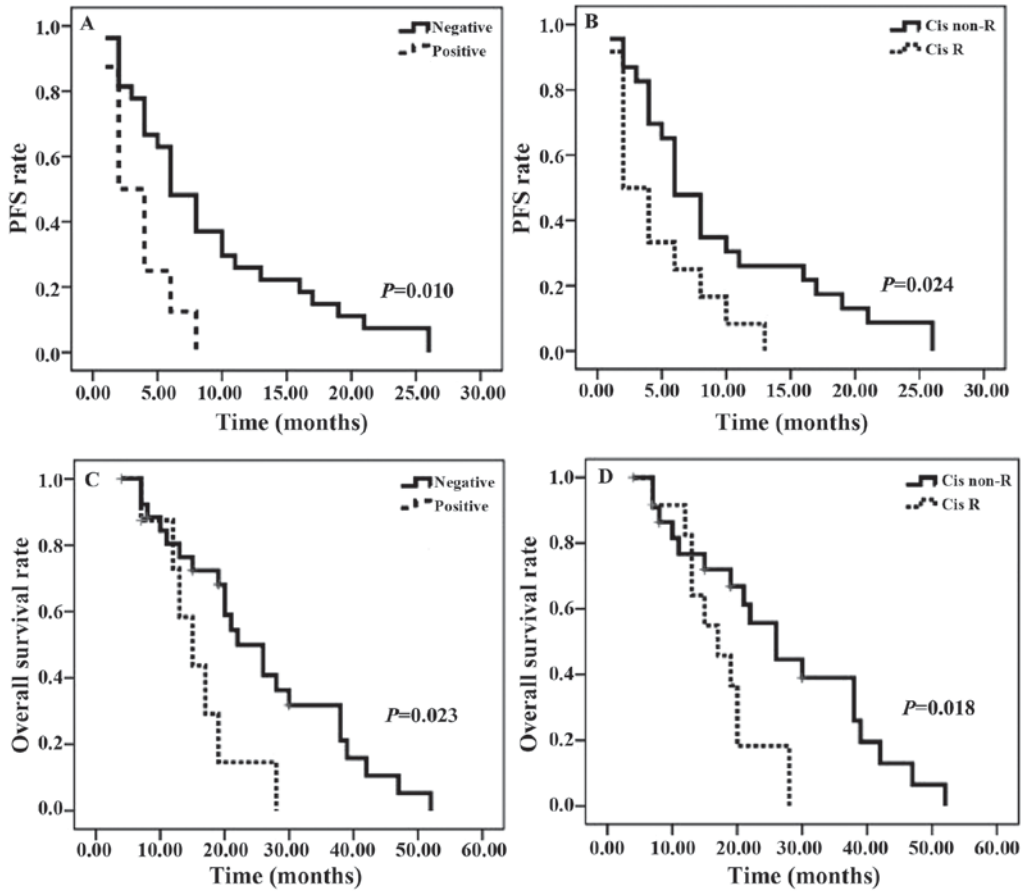


Figure 3. Kaplan-Meier estimator curves for PFS and OS rates in patients with triple-negative breast cancer in the ERα methylation and cisplatin-resistant groups. An event is defined as disease progression for mortality without progression in PFS and as mortality from any cause in OS. (A) PFS ERα methylation and non-methylation groups. (B) PFS cisplatin-resistant and -non-resistant groups. (C) OS ERα methylation and non-methylation groups. (D) OS cisplatin-resistant and -non-resistant groups. In (A) and (C), negative ERα methylation is indicated by a continuous line and positive ERα methylation is indicated by a broken line. In (B) and (D), cisplatin non-resistance is indicated with a continuous line and cisplatin resistance is indicated with a broken line. PFS, progression-free survival; OS, overall survival.

effective chemotherapy regimens and to assess the validity of biomarkers. TNBC accounts for ~15% of all invasive breast cancers with higher histological grade compared with that in other types of molecular cancer (4).

A number of studies indicate that the weekly addition of paclitaxel to an anthracycline-containing adjuvant therapy may be superior to only anthacycline-containing regimens without paclitaxel; however, the DFS time remained shorter for TNBC compared with non-TNBC (20,21). On the basis of this inhibitory effect on the proliferation of breast cancer cells in mouse models, particularly in the dysfunction of BRCA1 and its pathway, the use of cisplatin to treat TNBC is currently being assessed in clinical trials and certain studies (7-8,22). In neoadjuvant chemotherapy, the use of cisplatin with docetaxel exhibited a higher pathological complete response in patients with BRCA1 mutations (23). In metastatic TNBC, compared with docetaxal, carboplatin exhibited significant improvements in the rate of tumor response (68.0 vs. 33.3%, respectively; P=0.03), median PFS times were 6.8 months (24,25). Similarly, the use of olaprib, an oral poly(ADP-ribose) polymerase (PARP) inhibitor, resulted in tumor regression in <41% in patients carrying BRCA1 mutations (22). The tumors from patients with TNBC tend to carry different tumor protein mutations, and tumor protein p53 (TP53) and BRCA1 mutations have been described as the most dangerous mutant in breast cancer (26-29).

Patients with mutations in BRCA1 exhibit deficiencies in double-stranded DNA break repair; those with germline BRCA1 mutations have a ~20-fold increased risk of breast cancer, with more aggressive carcinogenesis and angiogenesis (30-32). If patients harbor these mutations, the effective response rate to cisplatin was potentially higher compared with that for other chemotherapy regimens (22,33).

Previous results indicated that ERα methylation was associated with tumor stage, lymph node metastasis, nuclear accumulation of p53 and BRCA1 expression in basal-like breast cancer (14,34-37). The mechanism of action of cisplatin in breast cancer cells with DNA repair dysfunctions remains unknown. The present study indicates that ERα methylation was significantly associated with cisplatin resistance. Previous studies have been searching for and identifying other biomarkers and pathways involved in TNBC (38,39). However, non-validated biomarkers were explicated to evaluate chemotherapy efficacy (40). The present study identified that ERα methylation may be a meaningful biomarker for the evaluation of cisplatin resistance. However, it is not clear whether ERα methylation is a biomarker for PARP inhibitors, which, like cisplatin, target DNA repair. No significant difference in

the expression level of P-gp, which serves an important role in multidrug resistance, was identified. However, it should be noted that there was an association between ERα methylation and overexpression of BRCA1. Patients overexpressing BRCA1 exhibit increased DNA repair function (41-44). There are certain possible explanations for the puzzling effect of ERα methylation on the overexpression of BRCA. One may be the effect on ataxia telangiectasia mutated (ATM) protein by ERα methylation: It has been proposed that ATM inhibition by the normal ER may provide a strategy to sensitize tumors to DNA-damaging agents including cisplatin (45). Low ER-expression in patients with ERα methylation may partially counteract ATM inhibition (46,47). In addition, elevated BRCA expression was demonstrated to upregulate ATM-associated DNA double-strand break repair (48,49). Other studies indicated that homogenous repair genes were dysregulated and BRCA was overexpressed in patients with breast cancer (50,51). Notably, BRCA overexpression has been associated with poor outcomes in patients with TNBC (52). Despite this, there was not enough evidence to conclude that ERα methylation directly affects DNA repair in the present study.

The results of the present study require confirmation in clinical trials, as well as in other breast cancer types and different tumors. However, these results may dictate valid therapy. The results of the present study may lead to alteration of the therapy regimens in patients with ERα methylation in TNBC. If clinical trials confirm the results of the present study, patients with TNBC with methylated ERα may benefit from other chemotherapy in place of cisplatin to avoid cisplatin resistance.

In conclusion, the results of the present study indicated that ERα methylation affected not only the sensitivity of breast cancer cells to cisplatin, but also the expression of BRCA1 protein. Further analysis of the mechanism of ERα methylation in cisplatin resistance may aid the development of a novel therapeutic approach to targeting the BRCA1-related signal pathway.

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