Association between the methylation status of *PCDH17* and the efficacy of neoadjuvant chemotherapy in triple-negative breast cancer

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Abstract. The present study aimed to assess whether the methylation status of the protocadherin 17 gene (*PCDH17*) in triple-negative breast cancer (TNBC) tissues was associated with the efficacy of neoadjuvant chemotherapy (NAC). The present study included 280 patients diagnosed with TNBC using core needle biopsy. Tumor pathological diagnosis was determined via hematoxylin and eosin staining. Immunohistochemical staining was used to determine estrogen receptor, progesterone receptor, human epidermal growth factor receptor-2 and Ki-67 status. *PCDH17* methylation status was analyzed using methylation-specific PCR. χ^2 tests were performed to analyze differences between *PCDH17* methylation status and TNBC clinicopathological features. Univariate and multivariate logistic regressions were used to analyze whether *PCDH17* methylation status predicted a curative effect of NAC. The multivariate analysis included factors with P<0.2 from the univariate analysis and those that were clinically associated with NAC. A total of 228 patients were positive for *PCDH17* methylation, while the remainder 52 were negative. Additionally, 107 patients achieved pathological complete response (pCR) after NAC. The pCR rate was 67.3% among the 52 patients negative for *PCDH17* methylation and 31.6% among the 228 patients positive for *PCDH17* methylation. Patients who were negative for *PCDH17* methylation and had high *Ki67* expression exhibited significantly higher pCR rates than their counterparts. The present results demonstrate that *PCDH17* methylation status may predict the response to NAC in patients with TNBC. Therefore, this epigenetic characteristic may serve as an indicator of treatment efficacy.

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Introduction

Triple-negative breast cancer (TNBC) is a type of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2). TNBC accounts for 15-20% of all cases of breast cancer and has more aggressive biological characteristics (1,2). For locally advanced TNBC, the standard treatment is neoadjuvant chemotherapy (NAC) followed by surgery (3). The primary goal of NAC is to achieve a pathological complete response (pCR), as most patients with pCR have a good survival outcome (4). The pCR rate for patients with TNBC who receive NAC is 30-40% (5). Increasing evidence has revealed that TNBC exhibits higher chemosensitivity compared with other breast cancer subtypes; thus, patients with TNBC are more likely to achieve a pCR following NAC (6). However, whether NAC can prolong the survival of patients remains uncertain. Studies have demonstrated that patients who had achieved pCR after receiving NAC displayed a significantly improved prognosis. However, subgroup analysis revealed that the survival rates of patients who did not achieve pCR were lower compared with those of patients who achieved pCR, even when compared with the adjuvant chemotherapy group (7-9). This may be due to the increased risk of recurrence caused by residual lesions in patients who received NAC but did not achieve pCR (10). TNBC is a heterogeneous disease, which comprises subtypes with different biological behaviors and clinical outcomes (11). Therefore, it is important to develop strategies that can accurately predict the efficacy of NAC for TNBC to select candidates with an improved predicted prognosis.

Several molecular markers have been identified to predict responses to NAC, including p53 (12), cytokeratin ck5/6 (13), epidermal growth factor receptor (14), Ki67 (15), and methylation of Ras association domain family 1 isoform A (*RASSF1A*) and WNT inhibitory factor-1 (*WIF-1*) (16) in serum. However, biomarkers that can accurately predict the response to NAC in clinical settings remain limited. Protocadherin 17 (*PCDH17*) is a member of the cadherin superfamily located at 13q21.2 and it is primarily involved in intracellular signaling and cell-to-cell interactions (17). As a tumor suppressor gene, *PCDH17* exerts

an inhibitory effect on tumor growth during normal physiological processes (18). However, its tumor suppressor function is lost as a result of hypermethylation in numerous types of cancer, such as esophageal cancer (17), renal cell carcinoma (19), bladder cancer (20), gastric cancer (21) and breast cancer (22). A previous study indicated that *PCDH17* may be an antagonist of the Wnt/β-catenin signaling pathway in breast cancer. In breast cancer cells transfected with *PCDH17*, the expression levels of active β-catenin and its downstream target genes c-myc and cyclin D1 are decreased (22). In the cytoplasm and nucleus of breast cancer cells, the Wnt/β-catenin receptor Frizzled-1 decreases β-catenin protein expression and increases inhibition of the Wnt/β-catenin signaling pathway, thus decreasing drug resistance in chemotherapy (23). *PCDH17* expression in breast cancer cells is often inhibited or silenced upon *PCDH17* methylation, and drug-induced demethylation can reactivate *PCDH17* expression (22). An association between *PCDH17* methylation and decreased *PCDH17* expression has been observed in most breast cancer cases (22). It was hypothesized that *PCDH17* methylation would reduce its inhibitory effect on the Wnt/β-catenin signaling pathway, leading to elevated chemoresistance; therefore, the present study investigated the predictive value of *PCDH17* methylation status for NAC efficacy in TNBC.

In the present study, methylation-specific PCR (MSP) was used to detect the methylation status of *PCDH17* in TNBC specimens prior to NAC. The objective of the study was to assess whether the methylation status of *PCDH17* in TNBC tissues was associated with the response to NAC.

Materials and methods

Patients and samples. The present study included 280 female patients (mean age, 50.7 years; range, 27-69 years) with breast cancer who were treated at the Jining No.1 People's Hospital and the Shandong Provincial Qianfoshan Hospital Affiliated to Shandong University (Jining, China) between January 2016 and June 2019. The inclusion criteria were as follows: i) TNBC (stage IIB or III) diagnosed via ultrasound-guided core needle biopsy (16G Bard biopsy needle) performed prior to chemotherapy and confirmed by two experienced independent pathologists (discordant interpretations were confirmed by a third pathologist); ii) available paraffin specimens obtained from biopsy prior to NAC in which tissue DNA could be successfully extracted at concentrations >50 ng/μl; iii) no contraindications for chemotherapy and NAC; and iv) no prior history of chemotherapy, radiotherapy, endocrine therapy or molecular targeted therapy. The exclusion criteria were: i) Other subtypes of breast cancer or inflammatory breast cancer; ii) distant metastases detected via ultrasound, computed tomography or bone scan; iii) response to chemotherapy could not be assessed because surgery was not conducted; and iv) incomplete medical record. After biopsy, all patients received six cycles of TAC regimen (docetaxel + epirubicin + cyclophosphamide), followed by surgery. The present study was approved by the Ethics Committee of the Jining No.1 People's Hospital [institutional review board approval no. Lun Shenyan No. 2017 (011)]. The study was conducted in accordance with the provisions of the Declaration of Helsinki and local regulations. Informed written consent was obtained from all individuals who participated in the present study.

Clinical staging was performed according to the 7th edition of the TNM method from the American Joint Commission on Cancer (24). Physical examination was combined with molybdenum b-ultrasound and magnetic resonance imaging results to determine tumor T staging and regional lymph-node status. Imaging results (and axillary lymph node biopsy when necessary) determined N staging.

Immunostaining. All patients underwent biopsy gun puncture (C.R. Bard, Inc.) before surgery, resulting in four tumor-tissue samples (1.5-2.0 cm in length) per patient. Samples were immediately fixed in 10% formalin at room temperature for 24 h and embedded in paraffin. Some paraffin-embedded tissue blocks were cut into 4-μm-thick continuous slices for hematoxylin and eosin staining (for the pathological diagnosis of the tumor) and for immunohistochemistry (to determine ER, PR, HER-2, and Ki67 status). Hematoxylin and eosin staining was this routinely performed by the hospital as previously described (25). The streptavidin-perosidase staining method was also used for immunohistochemistry as previously described (26).

Monoclonal antibodies against ER (pre-diluted clone 611; cat. no. ORG-8871) and PR (pre-diluted clone 16; cat no. ORG-8721) (both Leica Microsystems, Inc.) were used to determine the ER and PR statuses, following previously published procedures (27,28). HER-2 expression was determined with the HercepTest kit according to the manufacturer's protocol (Agilent Technologies, Inc.) (29). For Ki67, a rabbit monoclonal antibody at a 1:200 dilution was used (RMA-0542, Fuzhou Maixin Biotech Co., Ltd.), following previously published procedures (30).

Triple-negative status (31) was defined as ER and PR levels <1%, as well as HER-2-negative (HercepTest score 0/1+ or gene amplification ratio <2.2 after *in situ* hybridization). Ki67-positive cells were defined as those with yellow nuclei (32). The percentage of Ki67-positive cells from 500 cells was calculated after selection under five different high-power microscope fields. A light microscope (magnification, x200) was used to capture the images. The threshold for Ki67 expression was 20% (high expression, ≥20%; low expression, <20%). Two experienced independent pathologists performed all pathological diagnoses. Consultation with a third pathologist ruled out inter-observer differences.

NAC and surgery. Written informed consent for chemotherapy treatment was obtained from all patients with confirmed diagnosis of TNBC detected by inpatient biopsy. All patients were tested for liver function(serum enzymology test) (33), electrocardiogram and echocardiography to evaluate their tolerance to chemotherapy. Oral dexamethasone tablets were given 3 days prior to NAC. Treatment with TAC regimen (days per cycle; 6 cycles) consisted of: Day 1, epirubicin hydrochloride at 75 mg/m^2 i.v.; day 1, cyclophosphamide at 500 mg/m^2 i.v.; and day 2, docetaxel at 75 mg/m^2 i.v. Omeprazole and ondansetron were prescribed as supportive care (for gastroprotection and antiemesis, respectively). Upon completion of chemotherapy, appropriate granulocyte colony-stimulating factor therapy was given to increase the number of blood cells based on blood test results. All patients underwent modified radical mastectomy after chemotherapy.

Table I. Primers used for M and U sequences in *PCDH17*.

Primer name	Sequence (5'-3')	Length, bp	T_m, ˚C	Base no.
H-*PCDH17* (M)-F	GGAGAGAAGTTTTTGTTCGCGG	108	63.0	22
H-*PCDH17* (M)-R	AATAAATCTTCGCCTCTATTCGTAAA		60.3	26
H-*PCDH17* (U)-F	TGTTTGGAGAGAAGTTTTTGTTTGTG	112	62.2	26
H-*PCDH17* (U)-R	ATAAATCTTCACCTCTATTCATAAAACACAC		61.5	31

PCDH17, protocadherin 17; M, methylated; U, unmethylated; length, the number of base pair that can be amplified; H, human; F, forward; R, reverse; Tm, melting temperature.

Pathological assessment after NAC completion. The efficacy of chemotherapy was evaluated as previously reported (34). Pathological evaluation of the postoperative tissue sections of all patients who received NAC was performed according to the Miller-Payne grading criteria for pathological response (35): Grade 1, no reduction in the overall cellularity of tumor cells; grade 2, loss of tumor cells <30%; grade 3, loss of tumor cells between 30 and 90%; grade 4, >90% loss of tumor cells; and grade 5, no residual invasive cancer observed under microscope, but ductal carcinoma *in situ* may be present. In the present study, grades 1-4 were defined as non-pCR, and grade 5 was defined as pCR.

Methylation detection using MSP. DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissues was performed in strict accordance with the manufacturer's protocol of the FFPE DNA extraction kit (Omega Bio-Tek, Inc.; cat. no. D3399-01). If the resulting DNA concentration detected using NanoDrop 2000 (Thermo Fisher Scientific, Inc.) was <50 ng/μl, extraction was repeated to ensure the DNA concentration was >50 ng/μl. Bisulfite treatment and DNA purification was performed according to the manufacturer's protocol of the Methylation-Gold kit [Zymo Research Corp.; cat. no. D5005S (10)]. The obtained DNA samples were stored at -20˚C until further use. The treated DNA was amplified using methylated and unmethylated PCR primers. According to the principle of MSP, two pairs of primers, one pair for the unmethylated DNA strand (U primers) and one pair for the methylated DNA strand (M primers), were designed using the software Methyl Primer Express v1.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers (Table I) were synthesized by Wuhan Servicebio Technology Co., Ltd. The 25 μl MSP system contained 2.5 μl 10X PCR buffer, 2.5 μl 2.5 mM dNTPs, 2 μl 10 μM primers, 3 μl genomic DNA, 0.1 μl of 5 U/μl Takara Taq Hot Start DNA polymerase (Takara Biotechnology Co., Ltd.; cat. no. R007A), and 14.9 μl double-distilled H_2O.

PCR amplification conditions were as follows: Pre-denaturation at 95˚C for 5 min, 40 cycles of denaturation at 95˚C for 30 sec, annealing at 55˚C for 30 sec and extension at 72˚C for 30 sec, and final extension at 72˚C for 5 min. The PCR products were separated by electrophoresis on a 2% agarose gel. After separation, the gel was placed in a gel imager in which the bands were observed and imaged. Placental DNA treated with SssI methyltransferase (New England Biolabs, Inc.) was subjected to bisulfite treatment and served as a positive control for methylated DNA, whereas placental DNA not treated with methyltransferase was used as a positive control for unmethylated DNA (36). A blank control group was included, in which distilled water was used as the template for the MSP. The status of methylation was defined as positive if only the M band was amplified (single pattern) or if both U and M bands were simultaneously amplified (mixed pattern). The sample was deemed to be unmethylated if only the U band was amplified. All methylation assays were repeated twice to ensure reproducibility.

Statistical analysis. Data were presented as numbers and percentages and were analyzed using SPSS 17.0 (SPSS, Inc.). Patients were divided into negative *PCDH17* methylation or positive *PCDH17* methylation groups. The difference of clinicopathological parameters between the two groups were analyzed using a χ^2 test. The association between *PCDH17* methylation status and efficacy of NAC was analyzed using univariate and multivariate logistic regression. The selection or rejection of variables for the multivariate analysis was based on evidence from the literature. Factors with P<0.2 in the univariate logistic regression and factors in the literature clinically considered to be closely associated with the efficacy of neoadjuvant chemotherapy were included in the multivariate analysis model. Multivariate logistic regression analysis applies the forward method. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. All 280 patients with TNBC received NAC and surgery. Of these participants, 30.4% (85/280) were at stage IIB, while 69.6% (195/280) were at stage III (Table II). A total of 250 cases of invasive ductal carcinoma were observed (89.3%); the remaining 30 samples (10.7%) were mucinous adenocarcinoma (n=7), myeloid carcinoma (n=13), poorly differentiated adenocarcinoma (n=4) and chemoplastic carcinoma (n=6). Immunohistochemical examination (Fig. 1) revealed 215 samples (76.8%) with high Ki67 expression and 65 samples (23.2%) with low Ki67 expression (Table II).

Associations between PCDH17 methylation and clinicopathological parameters. DNA was successfully extracted from the tissues of all 280 patients. The concentrations of all DNA samples were >50 ng/μl. Fig. 2 includes the methylated

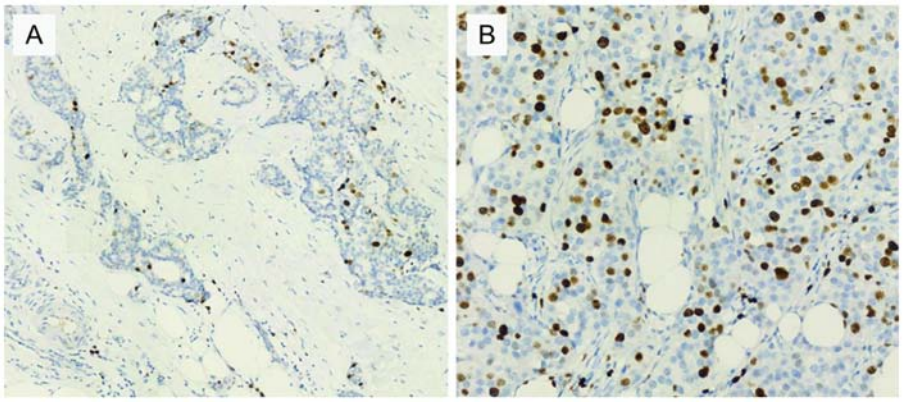

Figure 1. Immunohistochemical analysis of Ki67 expression in human triple-negative breast cancer. (A) Low Ki67 expression analyzed using streptavidin-perosidase staining; (magnification, x200). (B) High Ki67 expression (SP staining; magnification, x200).

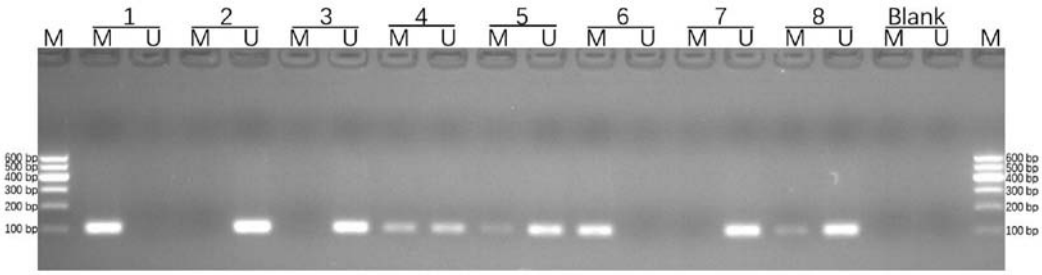

Figure 2. Methylation status of protocadherin 17 in triple-negative breast cancer tissues detected via methylation-specific PCR. 1, Positive control for methylation; 2, Positive control for unmethylation; 3-8, triple-negative breast cancer samples (3 and 7 were unmethylated; 4, 5, 6 and 8 were methylated); Blank, distilled water as blank control.

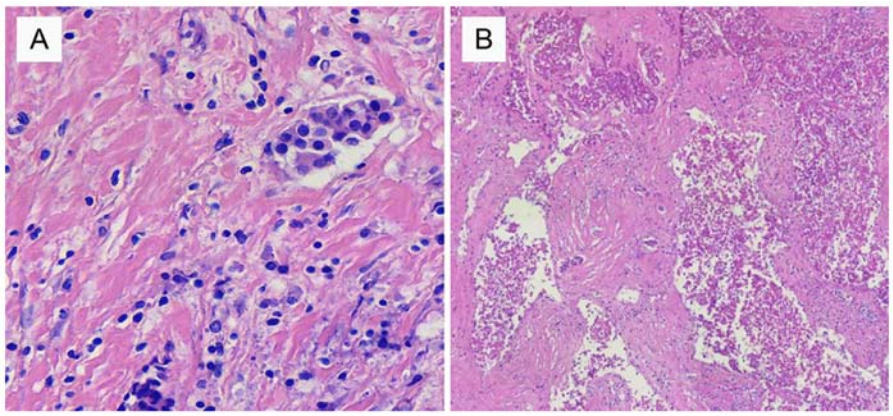

Figure 3. Pathological response after neoadjuvant chemotherapy in human triple-negative breast cancer. (A) Non-pathological complete response (H&E staining; magnification, x200). (B) Pathological complete response (H&E staining; magnification, x200). H&E, hematoxylin and eosin.

positive control, the demethylated positive control, the blank control and methylation status of protocadherin 17 in triple-negative breast cancer tissues detected via methylation-specific PCR (3 and 7 were unmethylated; 4, 5, 6 and 8 were methylated). Methylation analyses by MSP revealed that of the 280 patients with TNBC, 228 (81.4%) were positive for *PCDH17* methylation and 52 (18.6%) were negative for *PCDH17* methylation. The results of the associations between methylation status of *PCDH17* and clinicopathological features are shown in Table II. Clinicopathological parameters, such as age, menopausal status, histological tumor grade, T stage, TNM stage, pathological tumor type, lymph node metastasis, radiotherapy and Ki67 status, were not significantly different in patients with different *PCDH17* methylation status (P>0.05; Table II).

Pathological response after NAC. According to the pathological response criteria, 107 patients achieved pCR and 173 patients did not achieve pCR. Fig. 3 shows the representative images of pCR and non-pcr. The pCR rate was 38.2%. Among the 52 patients negative for *PCDH17* methylation, 35 achieved pCR, resulting in a pCR rate of 67.3%. Among the 228 patients positive for *PCDH17* methylation, 72 achieved pCR, resulting in a pCR rate of 31.6%, the significant result

Table II. Associations between negative (n=52) and positive (n=228) *PCDH17* methylation and clinicopathological parameters in 280 patients with triple-negative breast cancer.

Parameter	n (%)	Negative *PCDH17* methylation	Positive *PCDH17* methylation	χ^2 value	P-value
Age, years					
<50	118 (42.1)	23 (44.2)	95 (41.7)	0.114	0.735
≥50	162 (57.9)	29 (55.8)	133 (58.3)		
Menopausal status					
Pre	136 (48.6)	28 (53.8)	108 (47.4)	0.711	0.399
Post	144 (51.4)	24 (46.2)	120 (52.6)		
T stage					
T2	68 (24.3)	7 (13.4)	61 (26.8)	5.894	0.052
T3	174 (62.1)	34 (65.4)	140 (61.4)		
T4	38 (13.6)	11 (21.2)	27 (11.8)		
Lymph node metastasis					
No	52 (18.6)	12 (23.1)	40 (17.5)	0.857	0.355
Yes	228 (81.4)	40 (76.9)	188 (82.5)		
TNM stage					
IIB	85 (30.4)	10 (19.2)	75 (32.9)	3.739	0.053
III	195 (69.6)	42 (80.8)	153 (67.1)		
Histological grade					
I	57 (20.4)	11(21.2)	46 (20.2)	1.494	0.474
II	133 (47.5)	21 (40.4)	112 (49.1)		
III	90 (32.1)	20 (38.5)	70 (30.7)		
Pathological type					
Other types	30 (10.7)	4 (7.7)	26 (11.4)	0.610	0.435
IDC	250 (89.3)	48 (92.3)	202 (88.6)		
Ki67					
Low expression	65 (23.2)	10 (19.2)	55 (24.1)	0.568	0.451
High expression	215 (76.8)	42 (80.8)	173 (75.9)		
Radiotherapy					
No	43 (15.4)	7 (13.5)	36 (15.8)	0.177	0.674
Yes	237 (84.6)	45 (86.5)	192 (84.2)		
pCR					
No	173 (61.8)	17 (32.7)	156 (68.4)	22.893	0.001
Yes	107 (38.2)	35 (67.3)	72 (31.6)		

PCDH17, protocadherin 17; IDC, invasive ductal carcinoma; pCR, pathological complete response.

presented in Table II for the pCR being significantly different depending on the *PCDH17* methylation status.

Univariate regression analysis across both groups of patients revealed that patients who were negative for *PCDH17* methylation and those with a high Ki67 expression had significantly higher pCR rates [odds ratio (OR), 4.46, P=0.001; OR=2.78, P=0.002, respectively; Table III]. Factors with P<0.2 in univariate regression analysis were included in multivariate logistic regression analysis. In addition to these factors, age and lymph node metastasis, which are considered clinically relevant to neoadjuvant chemotherapy, were also included in the multivariate regression analysis (32,37). The results revealed that patients who were negative for *PCDH17* methylation and those with high Ki67 expression had significantly higher pCR rates (OR=4.48, P=0.001; OR=3.04, P=0.001, respectively; Table III).

Discussion

The efficacy of NAC in the treatment of breast cancer has been widely recognized in recent years. NAC is able to reduce micrometastases and improve long-term survival, as well as increase the chance of breast-conserving surgery (38). However, numerous urgent problems remain to be solved in the clinical application of NAC for breast cancer. For example, as breast cancer is highly heterogeneous at the molecular level,

Table III. Univariate and multivariate logistic regressions of factors associated with pathological complete response.

Parameters	Univariate analysis OR (95% CI)	Multivariate analysis P-value	OR (95% CI)	P-value
Age, years				
≥50 vs. <50	1.07 (0.66-1.75)	0.785	1.20 (0.70-2.03)	0.507
Menopausal status				
Post vs. pre	1.35 (0.83-2.20)	0.222		
T stage		0.227		0.318
T3 vs. T2	1.70 (0.93-3.10)	0.086	1.56 (0.84-3.02)	0.155
T4 vs. T2	1.57 (0.68-3.60)	0.292	1.16 (0.47-2.86)	0.749
Lymph node metastasis				
Yes vs. no	0.81 (0.44-1.50)	0.501	0.86 (0.44-1.68)	0.654
TNM stage				
III vs. IIB	1.39 (0.81-2.37)	0.231		
Histological grade		0.410		0.249
II vs. I	0.65 (0.35-1.23)	0.183	0.57 (0.29-1.13)	0.108
III vs. I	0.72 (0.37-1.42)	0.347	0.59 (0.29-1.24)	0.165
Pathological type				
IDC vs. other types	0.79 (0.37-1.70)	0.542		
Ki67				
High expression vs. low expression	2.78 (1.45-5.32)	0.002	3.04 (1.53-6.03)	0.001
PCDH17 methylation				
No vs. yes	4.46 (2.35-8.49)	0.001	4.48 (2.27-8.81)	0.001

PCDH17, protocadherin 17; IDC, invasive ductal carcinoma; OR, odds ratio.

tumors of the same clinical stage and histomorphology do not exhibit the same molecular changes, leading to differences in tumor response to therapies (39). It has been demonstrated that 20% of locally advanced breast cancer is not sensitive to chemotherapy, and NAC will delay the opportunity for timely local treatment (40). Therefore, it is important to individualize the use of NAC to improve the pCR rate and to predict, monitor and accurately evaluate treatment efficacy.

Epigenetic regulation refers to the regulation of the frequency, speed or levels of gene expression through processes such as DNA methylation and post-translational modifications of chromatin histones without altering the genomic DNA sequence, which ultimately lead to phenotypic changes (41). Epigenetic regulation is closely associated with malignant tumors and can affect the sensitivity of tumors to NAC and thereby influence the resistance to chemotherapy drugs (42). Hu *et al* (16) evaluated the methylation of *RASSF1A* and *WIF-1* in the serum of patients with locally advanced breast cancer, and revealed that the degree of methylation of these genes had significant clinical utility in predicting the efficacy of the NAC regimen TAC. Xie *et al* (43) found that the differentially methylated sites in the promoter regions of *ABCG2* and *DNMT3b* could be the specific methylation sites that promoted the differential expression of the *ABCG2* gene, providing novel targets for reversing the ABCG2-mediated multidrug resistance. The DNA methylation status of a number of genes could therefore help to predict the efficacy of NAC in patients with malignant tumors. The present study aimed to investigate whether the methylation status of *PCDH17* could predict the efficacy of NAC. The present study evaluated 280 patients with TNBC who received NAC and identified that 81.4% of patients were positive for *PCDH17* methylation. Univariate and multivariate regression analysis across both groups of patients with different methylation status of *PCDH17* revealed that patients who were negative for *PCDH17* methylation had significantly higher pCR rates. The current data indicated that the methylation status of *PCDH17* may effectively predict the efficacy of NAC in patients with TNBC.

The present study demonstrated the predictive value of *PCDH17* methylation status for NAC, providing a candidate for reliable screening of treatment success under clinical conditions. Furthermore, the current findings suggested that adjusting *PCDH17* methylation may improve NAC effects and therefore should contribute to efforts aimed at developing novel treatment strategies. The limitations of the present study include small sample sizes and body mass index that could not be taken into account since data regarding this factor were not collected. Based on the results of the present study, more reliable clinical data should be obtained by increasing the sample size and including multicenter collaboration, in which the value of *PCDH17* methylation for predicting the prognosis of patients receiving NAC can be further validated to confirm its clinical utility. In conclusion, the present data indicate that *PCDH17* methylation status may be used to predict the response to NAC in patients with TNBC and may serve as a predictive factor for NAC efficacy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RZF designed the study and analyzed the data. DDK carried out the experiments. LL participated in specimen collection and pathological diagnosis. WW performed the surgery. SBW carried out acquisition of data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Jining No.1 People's Hospital, Jinging, China [institutional review board approval no. Lun Shenyan No. 2017 (011)]. All procedures performed in the present study involving human participants were in accordance with the ethical standards of the Institutional and National Research Committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the present study. When receiving ultrasound-guided breast tumor puncture, patients were informed that tissue samples and relevant data would be used for future research work, and provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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