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Increased Expression of c-Met is Associated with Chemotherapy-Resistant Breast Cancer and Poor Clinical Outcome

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Funds Collection G

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Background: The relevance of c-Met expression as a prognostic or predictive clinical indicator in chemotherapy-resistant breast cancer remains unknown. The aims of this study were to investigate the expression of c-Met in breast cancer tissues and its association with expression of type II topoisomerase (TOPO II), including in patients who received neoadjuvant chemotherapy (NAC), and to investigate chemotherapy resistance *in vitro* in breast cancer cell lines.

Material/Methods: Tissue samples from 255 patients with breast cancer, with matched adjacent normal breast tissue, were used in tissue microarrays (TMAs). c-Met protein expression levels were determined using immunohistochemistry. Forty-five cases of breast cancer treated with NAC were studied to investigate the association between c-Met and TOPO II expression and clinical outcome. Chemotherapy resistance was evaluated *in vitro* in the MCF-7 and MDA-MB-231 breast cancer cell lines.

Results: Expression of c-Met protein was increased in breast cancer tissue compared with normal breast tissue. In breast cancer tissue samples, increased c-Met expression was significantly associated with increased Ki-67 expression, tumor size, tumor stage, and TOPO II expression, and with reduced overall survival (OS) rates. Increased c-Met expression and reduced TOPO II expression were associated with chemotherapy resistance. In breast cancer cell lines, knockdown of c-Met expression induced TOPO II expression and increased tumor cell sensitivity to chemotherapy.

Conclusions: The findings of this study support a role for c-Met as a clinical prognostic marker and for c-Met and TOPO II as predictive markers for response to chemotherapy in patients with breast cancer.

MeSH Keywords: Antineoplastic Agents • Breast Neoplasms, Male • Proto-Oncogene Proteins c-met

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Background

In the United States, breast cancer is the second leading cause of death from malignancy in women, after lung cancer [1]. Worldwide, the incidence of breast cancer continues to increase, and the occurrence of breast cancer is associated with genetic and environmental factors and with lifestyle factors that include nutrition and obesity [2–5]. Primary prevention of breast cancer and cancer screening programs aim to reduce mortality from breast cancer. Currently, the detection rates and survival rates from early breast cancer have been improved, and mortality rates have been reduced because of advances in the early diagnosis of breast cancer [3-5]. However, the incidence of advanced breast cancer at initial diagnosis is more common in developing countries, accounting for between 30–60% of cases, and in cases of triple-negative breast cancer, the incidence of local lymph node metastases at diagnosis has been reported to be as high as 90% [6].

Neoadjuvant chemotherapy (NAC) refers to chemotherapy administered before surgery in patients with breast cancer who do not have metastases [7]. NAC aims to reduce the extent of surgery, reduce tumor stage, and improve patient survival [8]. A clinical study found that patients treated with NAC could achieve a complete pathological response and a complete clinical response, resulting in improve disease-free survival (DFS) and overall survival (OS) [9]. However, other studies have shown that NAC is not effective for all patients with breast cancer, and the side effects of chemotherapy and other delays caused by NAC can result in adverse clinical effects [8,10].

With improvements in molecular biology and genomics, the importance of analysis of breast cancer tumors at the molecular level for gene and protein expression, prior to administering chemotherapy, has become increasingly recognized [11,12]. The cell surface receptor tyrosine kinase, c-Met, belongs to the MET gene family, and its ligand is hepatocyte growth factor (HGF), both of which play important roles in cell proliferation, cell differentiation, tissue regeneration, cell survival, and tumor invasion [13]. Abnormal activation of c-Met can occur through several mechanisms, including mutation, amplification, and upregulation of transcription of the c-Met gene [14]. Increased expression of c-Met has been shown to occur in several types of solid tumor arising in the brain [15], the breast [16], the colon and rectum [17], the stomach [18], head and neck [19], the lung [20], the liver [21], skin [22], prostate [23], and soft tissue [24].

Currently, the mechanism of drug resistance in breast cancer remains unclear, and the relevance of c-Met expression as a prognostic or predictive clinical indicator in chemotherapy-resistant breast cancer remains unknown. Therefore, the aims of this study were to investigate the expression of c-Met in breast cancer tissues and its association with expression of type II topoisomerase (TOPO II), including in patients who received neoadjuvant chemotherapy (NAC), and to investigate chemotherapy resistance *in vitro* in breast cancer cell lines.

Material and Methods

Ethical approval, patient consent, and patient confidentiality

This study and the study protocol were approved by the Research Ethics Committee of Nanjing First Hospital, China. All patients provided informed consent before surgery. Patient confidentiality was maintained by de-identification of patient data and all data obtained during the study was confidential. The study and its findings did not affect the clinical management or treatment plan for each patient. According to the study protocol, patients were not involved in the study design, were not informed of the study findings, and were not contacted on completion of the study.

Patient specimens

Breast tissue samples from surgical excision specimens from 255 patients with breast cancer were obtained from the Department of Pathology, Nanjing First Hospital, China between 2008–2012. Matched adjacent normal breast tissues were also obtained for 43 of these 255 cases. Clinical information for the study participants included age, location, tumor size, TNM stage, and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2) status, Ki-67 status, histologic grade, lymph node status, the presence of metastases, and overall survival (OS). Also, 45 paired tissue samples of breast cancer tissue and adjacent normal breast tissue were obtained from patients who received neoadjuvant chemotherapy (NAC). Tissue samples were obtained from the Department of Pathology, Nanjing First Hospital, and all tissues used underwent histopathology review to confirm the presence of breast cancer, tumor type and grade, and to identify normal breast tissue to confirm that it was free from tumor.

Construction of tissue microarrays (TMAs) and immunohistochemistry (IHC)

Formalin-fixed, paraffin-embedded tissue sections from 255 breast cancer tissue samples and 43 matched adjacent normal breast tissues were used to construct the tissue microarrays (TMAs). The TMAs were constructed in the Department of Pathology, Nanjing First Hospital, using the QuickRay® Manual Tissue Microarrayer (Unitma Co Ltd., Seoul, Korea). Cores of tissue, measuring 2 mm in diameter, were sampled from individual blocks of paraffin-embedded breast tissue samples

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and placed into new paraffin blocks that contained multiple cores. The immunohistochemical method used followed the routine diagnostic immunohistochemistry method used in the Department of Pathology, Nanjing First Hospital. Using light microscopy, a visual scoring system was used to quantify the degree of immunopositivity. A primary rabbit monoclonal antibody to c-Met (Abcam, Cambridge, MA, USA) (dilution 1: 100) and a primary monoclonal anti-human E-cadherin antibody (LSBio, Seattle, WA, USA) (dilution 1: 100) were used.

Cell lines, cell culture, and cell transfection with short interfering RNA (siRNA)

The human breast cancer cell lines MCF-7 and MDA-MB-231, and the normal human breast epithelial cell line MCF-10A were cultured. Cell lines were maintained at 37°C in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were cultured in a humidified atmosphere containing 5% CO_2. Cells were seeded into six-well plates and were transfected with 10 nM short interfering RNA (siRNA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The following siRNA sequences were used:

si1, 5'-CUAGACUUCUCCUUGGAAA-3'; and si2, 5'-UUGAACAGCGAGCUAAAUA-3'.

Quantitative real-time polymerase chain reaction (qRT-PCR) for c-Met and topoisomerase II (TOPO II)

Total RNA was isolated from breast cancer cell lines using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was reverse transcribed into cDNA using M-MuLV reverse transcriptase (Takara Bio, Otsu, Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the Cobas z 480 detection system (Roche, Basel, Switzerland) and SYBR PrimeScript RT-PCR kit (Takara Bio, Otsu, Japan). The following PCR primers were used (Genscript. Nanjing, China):
human c-Met (forward): AGCAATGGGGAGTGTAAAGAGGR;
human c-Met (reverse): CCCAGTCTTGTACTCAGCAAC;
TOPO II (forward): 5'-AGCCATTGACGCAGTTCATGT-3';
TOPO II (reverse): 5'-GCCTGGCACAAAGGTAACCTCC -3';
GAPDH (forward): 5' -GAAGGTGAAGGTCGGAGTC-3'; and
GAPDH (reverse): 5' -GAAGATGGTGATGGGATTTC-3'.

Results were normalized to respective internal controls. The Ct-value for each sample was calculated using the ΔΔCt method, and results were expressed as $2^{-\Delta\Delta Ct}$.

Protein extraction and Western blot

Intracellular proteins were extracted using RIPA lysis and extraction buffer (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. Protein concentrations were determined using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of protein were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked for one hour and then incubated with primary antibodies overnight at 4°C. After washing three times with phosphate-buffered saline containing Tween (PBST), the membrane was incubated for one hour with secondary antibodies at room temperature. The membrane was washed with PBST and incubated with SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA) for 5 minutes. The specific bands underwent automated image capture and analysis using the ChemiDoc XRS+ System (Bio-Rad, Hercules, CA, USA). GAPDH was used as the internal control.

Cell Counting Kit-8 (CCK-8) cell proliferation assay

Cells were seeded at 5×10^3 cells/well into a 96-well plate. Cell proliferation and viability were measured using the Cell Counting Kit-8 (CCK-8) assay (Beyotime Biotechnology, Shanghai, China). Absorbance was measured at 450 nm using an ELx800 Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA).

Statistical analysis

Statistical analysis was performed using the SPSS version 19.0 statistical software package (SPSS Inc., Chicago, IL, USA). The unpaired Student's t-test was used to compare groups. Statistically significant differences between groups were determined using the Student's t-test and the chi-squared (χ^2) test. Kaplan-Meier analysis was performed to determine the cumulative patient overall survival (OS). Survival curves were compared using the log-rank test. Differences between groups were analyzed using the Student-Newman-Keuls (SNK) method. A P-value <0.05 was considered to be statistically significant.

Results

Clinicopathologic features of patients with breast cancer

The clinicopathologic characteristics of patients with breast cancer are shown in Table 1. All patients were women with a median age of 59.12 years (range, 25–86 years). The distribution of TNM stage included 47 patients who had stage 1 breast cancer, 147 patients with stage 2 breast cancer, and

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Table 1. Associations between high c-Met expression and clinicopathologic characteristics in BC patients.

Characteristic	n	c-Met expression (%)				χ^2	p
		Low or no		High			
Total							
Tumor location						0.59	0.898
Left	154	71	(46.10)	83	(53.90)		
Right	101	45	(44.60)	56	(55.40)		
Age						3.123	0.090
Premenopausal	110	57	(51.80)	53	(48.20)		
Postmenopausal	145	59	(40.70)	86	(59.30)		
Pathology stage						0.057	0.972
1	47	21	(44.70)	26	(55.30)		
2	159	72	(45.30)	87	(54.70)		
3	49	23	(46.90)	26	(53.10)		
Tumor size						4.560	0.037
≤2 cm	59	34	(57.60)	25	(42.40)		
>2 cm	195	82	(41.80)	114	(48.20)		
ER						1.402	0.247
Positive	98	40	(40.80)	58	(59.20)		
Negative	157	76	(48.40)	81	(51.60)		
PR						0.944	0.379
Positive	125	53	(42.40)	72	(57.60)		
Negative	130	63	(48.50)	67	(51.50)		
HER2						0.033	0.896
Positive	93	43	(46.20)	50	(53.80)		
Negative	162	73	(45.10)	89	(54.90)		
Ki-67						4.736	0.024
Positive	136	65	(47.80)	71	(52.2)		
Negative	119	45	(37.80)	74	(62.20)		
Lymph node status						1.306	0.257
N0	111	55	(49.50)	56	(50.50)		
N1+2+3	144	61	(42.40)	83	(57.60)		
Metastasis							
M0	64	29	(45.30)	35	(54.70)	0.001	0.545
M1	191	87	(45.50)	104	(54.50)		
TNM stage						5.084	0.025
1	90	48	(53.30)	42	(46.70)		
2	97	44	(45.40)	53	(54.60)		
3	68	24	(35.30)	44	(64.70)		
TOPO II						10.129	0.001
Positive	136	71	(52.50)	65	(47.80)		
Negative	119	45	(37.80)	74	(62.20)		

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Table 2. c-Met expression in breast cancer tissues compared with matched tumor neighbor.

Characteristic	n	c-Met expression (%)		χ^2	*p*
		Low or no	High		
Breast tissues				3.489	0.043
Cancer	255	116 (45.49)	139 (54.51)		
Matched tumor neighbor	43	30 (69.77)	13 (30.23)		

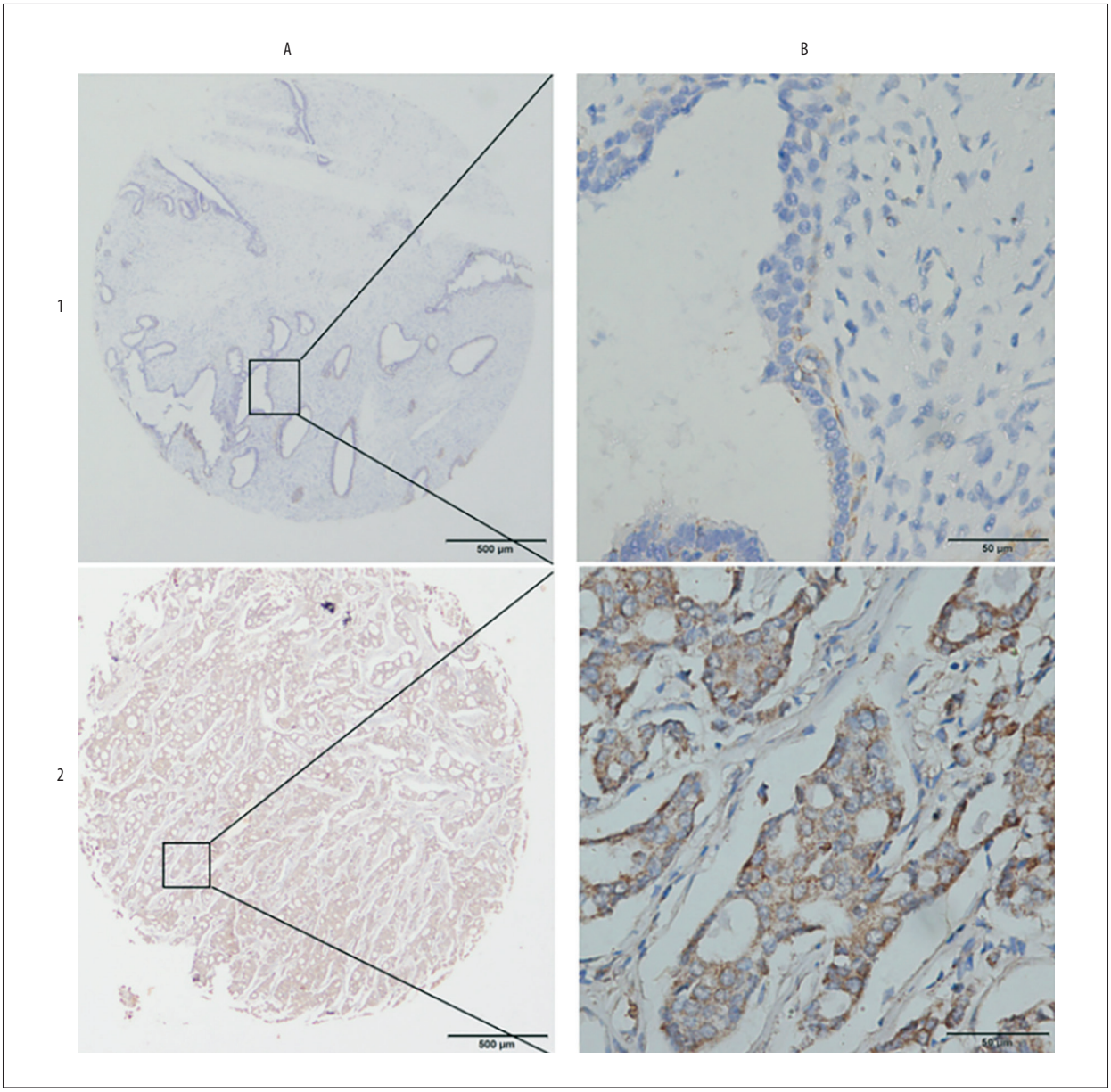

Figure 1. Representative photomicrographs of c-Met protein expression using immunohistochemistry (IHC) in tissue microarrays (TMAs) containing sections of breast tissue. 1. Matched adjacent normal breast tissue with low levels of c-Met expression. 2. Breast tissue containing invasive ductal carcinoma shows high levels of c-Met expression; Row A shows c-Met immunostaining at a magnification of ×4 (bar=500 mm). Row B shows c-Met immunostaining at a magnification of ×40 (bar=50 mm).

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Table 3. Univariate and multivariate analysis of prognostic biomarkers for OS in BC patients.

	Univariate analysis			Multivariate analysis		
	HR	*p*>|z|	95%CI	HR	*p*>|z|	95%CI
c-Met expression						
High *vs.* Low or no	2.388	0.004	1.197–4.766	2.018	0.015	1.012–3.023
Location						
left *vs.* right	0.81	0.540	0.412–1.591			
Age						
Premenopausal *vs.* postmenopausal	0.964	0.914	0.491–1.890			
Pathology stage						
1 *vs.* 2 *vs.* 3	0.752	0.308	0.434–1.301			
Tumor size						
≤2 cm *vs.* >2 cm	1.891	0.034	1.049–3.408			
ER						
Positive *vs.* negative	1.030	0.932	0.519–2.044			
PR						
Positive *vs.* negative	0.783	0.475	0.399–1.533			
HER2						
Positive *vs.* negative	1.006	0.987	0.503–2.013			
Ki-67						
Positive *vs.* negative	0.716	0.326	0.366–1.400			
Lymph node status						
N0 *vs.* N1+2+3	1.018	0.958	0.519–1.997			
Metastasis						
M0 *vs.* M1	1.365	0.044	1.137–2.974			
TNM stage						
1 *vs.* 2 vs. 3	3.442	<0.001	1.746–3.988	2.903	<0.001	1.423–2.998
TOPO II						
Positive *vs.* negative	2.482	0.012	1.085–2.321	2.034	0.011	1.213–3.002

49 patients with stage 3 breast cancer. The histological tumor grading showed 47 patients had grade 1 (low-grade) breast cancer, 159 patients had grade 2 (medium grade) breast cancer, and 49 patients had grade 3 (high-grade) breast cancer. Clinical biomarkers evaluated on the breast cancer tissue sections included and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), Ki-67, and topoisomerase II (TOPO II).

Increased c-Met protein expression in breast cancer samples was detected using immunohistochemistry (IHC)

Immunohistochemical analysis of c-Met expression in 255 cases of breast cancer and 43 matched adjacent normal breast tissues showed that c-Met expression was significantly increased in breast cancer tissues (139/255, 54.51%) compared with matched adjacent normal breast tissues (13/43, 30.23%) (Table 2). The c-Met protein was mainly localized to the cytoplasm and cell membrane (Figure 1). The X-tile software

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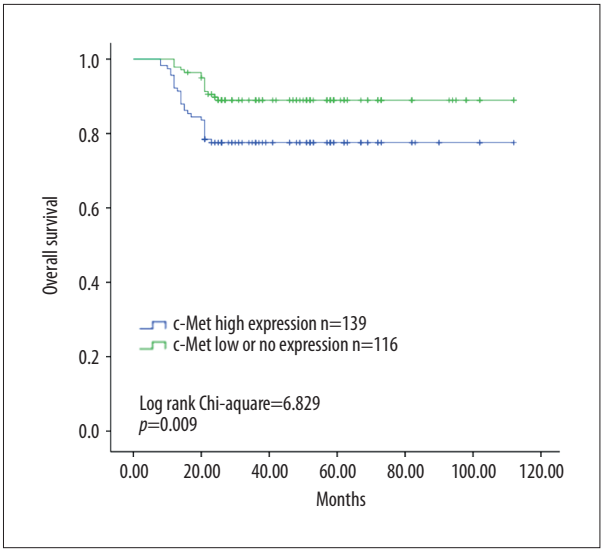

Figure 2. Overall survival (OS) curves for patients with breast cancer using the Kaplan-Meier method and the log-rank test. Overall survival (OS) curves for patients with breast cancer with high c-Met expression (blue line, 1) and patients with low or no c-Met expression (green line, 2).

program for TMA data analysis was used (*http://www.tissuearray.org/rimmlab*). A cut-off value of 150 was selected to define high, low, or no c-Met expression and values between 0–150 were considered as low or no c-Met expression, while values between 151–300 was considered to represent high c-Met expression.

Table 4. Compared c-Met and TOPO II expression before and after NAC therapy.

	n	c-Met	+	+	–	–	χ^2	*p*
		TOPO II	–	+	–	+		
	45						21.346	0.011
Before NAC			9 (20.00)	27 (60.00)	1 (2.22)	8 (17.78)		
After NAC			13 (28.89)	4 (8.89)	1 (2.22)	26 (57.78)		

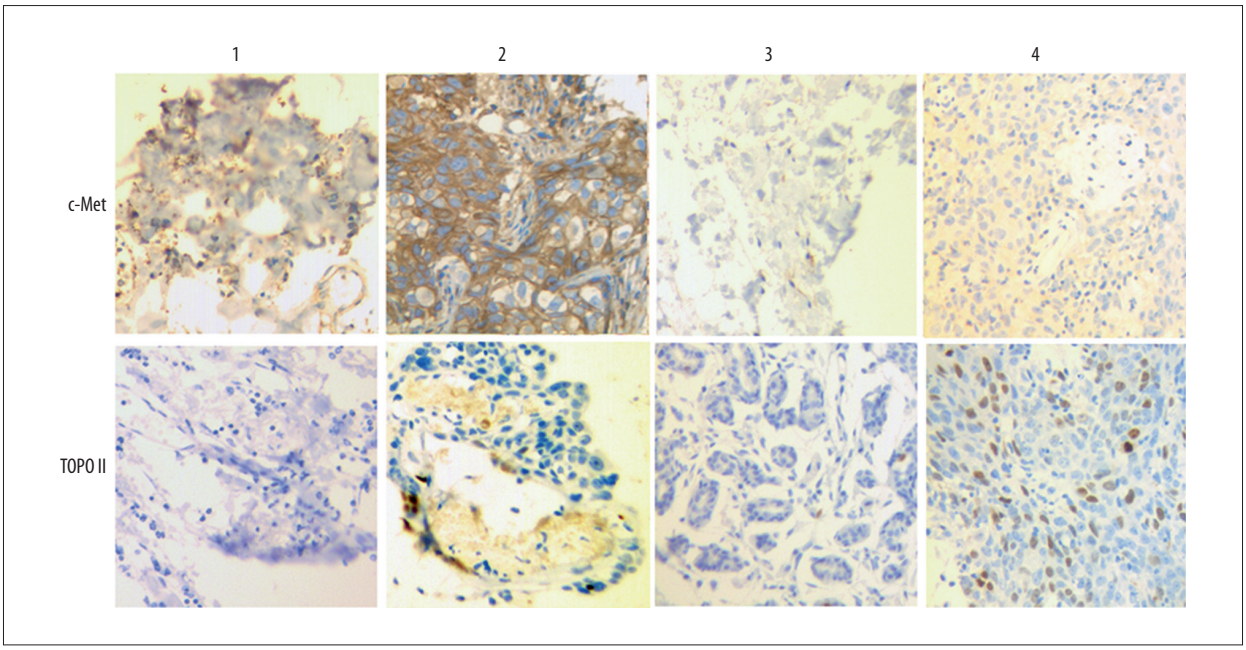

Figure 3. Representative photomicrographs of c-Met and TOPO II protein expression using immunohistochemistry (IHC) in tissue microarrays (TMAs) containing sections of breast tissue. Row 1 shows high levels of c-Met expression or low or negative TOPO II expression. Row 2 shows high levels of c-Met expression or high levels of TOPO II expression. Row 3 shows low or no expression of c-Met or TOPO II. Row 4 shows low or no expression of c-Met and high levels of TOPO II expression. Line 1 shows c-Met immunostaining with magnification ×20. Line 2 shows TOPO II immunostaining with magnification ×20.

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Table 5. Association of c-Met and TOPO II expression and response to chemotherapy in BC patients.

Response to chemotherapy	n	c-Met	+	+	–	–	χ^2	*p*
		TOPO II	–	+	–	+		
	45						39.85	<0.001
Valid (CR+PR)	28		0 (0.00)	0 (0.00)	2 (7.14)	26 (92.86)		
Invalid (SD+PD)	17		13 (76.47)	3 (17.65)	1 (5.88)	0 (0.00)		

Comparison of expression levels of c-Met protein compared with clinicopathologic characteristics in patients with breast cancer

Clinicopathologic associations between c-Met protein expression levels and clinicopathologic variables in patients with breast cancer were found and are summarized in Table 1. Increased c-Met expression was significantly associated with tumor size (χ^2=4.560; P=0.037), TNM stage (χ^2=5.084; P=0.025), Ki-67 expression (χ^2=4.736; P=0.024), and TOPO II expression (χ^2=10.129; P=0.001). However, there was no significant correlation between c-Met protein expression and other clinicopathologic variables.

Increased expression of c-Met protein correlated with reduced overall survival (OS)

Univariate and multivariate analysis were used to evaluate the relationship between c-Met protein expression and prognostic factors in patients with breast cancer (Table 3). Increased expression of c-Met protein (HR=2.388; 95% CI, 1.197–4.766; P=0.004) was associated with poor overall survival (OS) using univariate analysis, as were other prognostic factors, including tumor size (HR=1.891; 95% CI, 1.049–3.408; P=0.034), metastasis (HR=1.365; 95% CI, 1.137–2.974; P=0.044), TNM stage (HR=3.442; 95% CI, 1.746–3.988; P<0.001), and TOPO II expression (HR=2.482; 95% CI, 1.085–2.321; P=0.012). Using multivariate analysis, increased expression of c-Met (HR=2.018; 95% CI, 1.012–3.023; P=0.015), TNM stage (HR=2.903; 95% CI, 1.423–2.998; P<0.001), and TOPO II expression (HR=2.034; 95% CI, 1.213–3.002; P=0.011) were independently associated with poor prognosis. Kaplan-Meier survival curves were used to compare patients with breast cancer with different expression levels of c-Met. The results showed that increased expression of c-Met protein was correlated with reduced OS in patients with breast cancer (P=0.009) (Figure 2).

Comparison of c-Met and TOPO II expression before and after neoadjuvant chemotherapy (NAC)

Because expression levels of c-Met were found to be associated with TOPO II expression, the possibility that c-Met might play a role in the response to chemotherapy was investigated in an additional 45 pairs of breast cancer tissue samples, which were divided into a pre-chemotherapy group and a post-chemotherapy group. The expression levels of c-Met and TOPO II were determined in these samples and the results were combined with the response to anthracycline-based chemotherapy. Both c-Met and TOPO II were expressed in 60.0% (27/45) of tissue samples from patients with breast cancer before NAC. Following NAC, c-Met, and TOPO II were expressed in 57.78% (26/45) of tissue samples from patients with breast cancer (Table 4, Figure 3). These 45 patients were further divided into two groups, a valid group (responders) with a clinical response and a pathological response (CR + PR) and an invalid group (non-responders) (SD + PD). In the responder group, expression of both c-Met- and TOPO II (c-Met+/TOPO II+) was 92.8% (26/28); in the non-responder group, positive expression of c-Met expression and negative expression of TOPO II (c-Met+/TOPO II-) was 76.47% (13/17) was found (Table 5).

Knockdown of c-Met induced TOPO II expression and drug resistance in MCF-7 and MDA-MB-231 cell lines *in vitro*

To further evaluate the relationship between c-Met and drug resistance, c-Met knockdown was studied in MCF-7 and MDA-MB-231 cell lines, which express c-Met is expressed at higher levels compared with other breast cell lines (Figure 4A). As shown in Figure 4B, the relationship between c-Met and TOPO II expression was evaluated using the Ingenuity Pathway Analysis (IPA) software (STRING Database). The results showed that c-Met could interact with and may regulate the function of topoisomerases, such as TOP 1, TOP IIA, and TOP IIB. The use of quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were used to detect the knockdown efficiency of two short interfering RNA (siRNA) c-Met molecules. Compared with the negative control (siNC), both c-Met mRNA and protein expression levels were significantly reduced in the two siRNA groups, while TOPO II expression levels were increased in the two siRNA groups (Figure 4C1, 4C2).

To further explore the effect of c-Met on chemotherapy drug resistance, two siRNAs, si1 and si2, and a negative control (siNC), were transfected into MCF7 and MDA-MB-231 cells, and proliferation was assessed in these two breast cancer cell lines, which were treated with different concentrations

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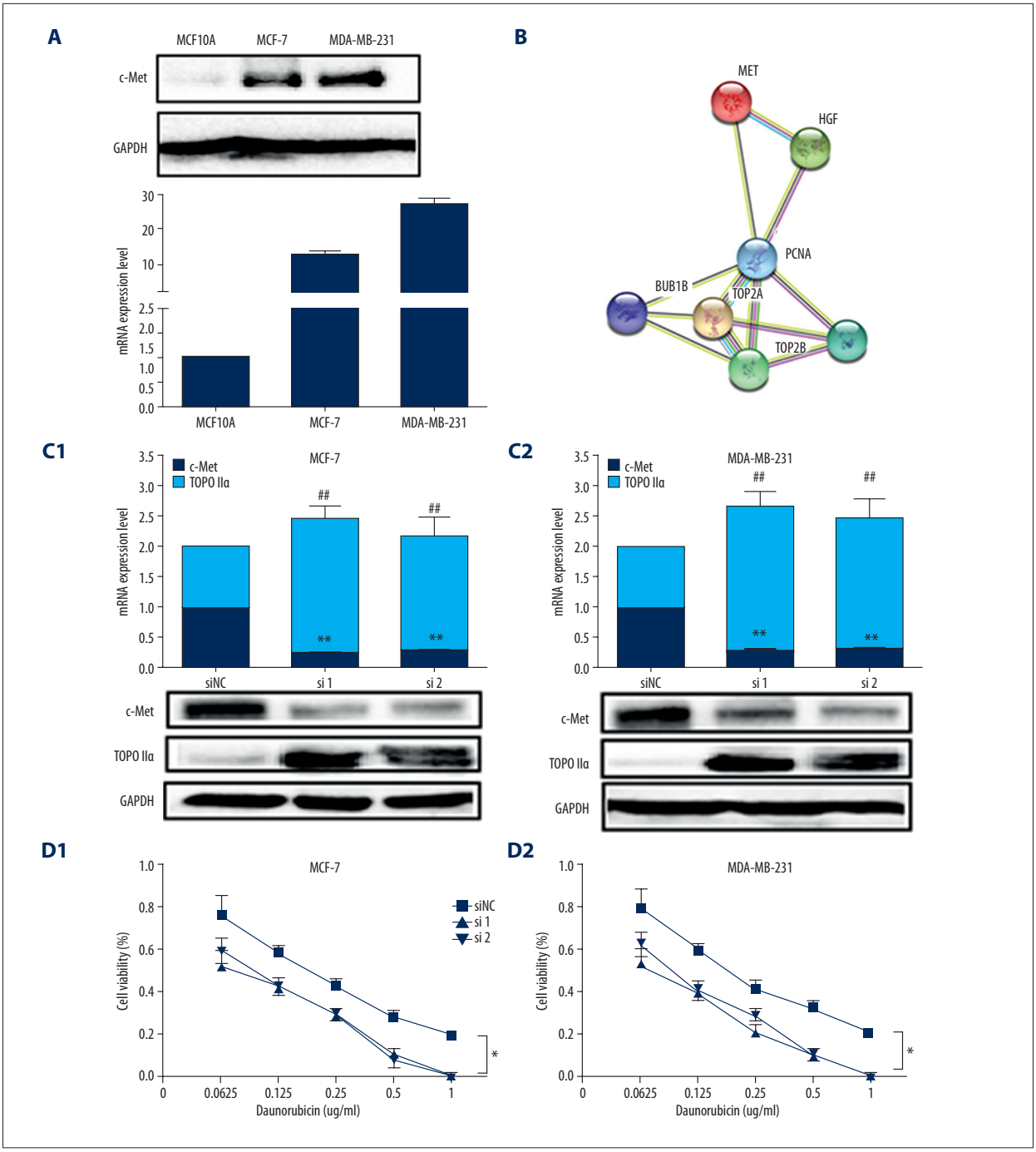

Figure 4. Knockdown of c-Met induced TOPO II expression and drug resistance in breast cancer cell lines, MCF-7 and MDA-MB-231, compared with the normal breast epithelial cell line, MCF10A. (**A**) Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot show c-Met expression in the MCF-7 and MDA-MB-231 breast cancer cell lines, compared with the normal breast epithelial cell line, MCF10A. GAPDH was used as the internal control. (**B**) Ingenuity Pathway Analysis identifies the molecular interaction between c-Met and topoisomerase. (**C**) Western blot and qRT-PCR shows that c-Met protein and mRNA levels in MCF-7 and MDA-MB-231 breast cancer cell lines were significantly reduced after transfection with c-Met short interfering RNA (siRNA) (si1 and si2), knocked down c-Met and induced TOPO II expression in MCF-7 and MDA-MB-231 cells. # The means compared with TOPO II expression of the siNC group, ## P<0.001, * The means compared with c-Met expression of the siNC group, ** P<0.001. (**D**) si1 and si2 c-Met significantly induced the proliferation of the MCF7 and MDA-MB-231 breast cancer cells lines, which was treated by different concentrations of daunorubicin, from 0.0625–1.0 mg/ml. * P<0.05.

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of daunorubicin, between 0.0625–1 μg/ml. The results of the Cell Counting Kit-8 (CCK-8) assay indicated that knockdown of c-Met resulted in increased sensitivity of the breast cancer cell lines to daunorubicin treatment (Figure 4D1, 4D2).

Discussion

Worldwide, breast cancer is one of the most common malignant tumors of women. Therefore, it is essential to identify specific biomarkers that can be used for early diagnosis, guide clinical treatment, and improve patients survival [25]. The c-Met gene is located on chromosome 7q21 and contains 21 exons, and has many regulatory roles in cell growth. Also, c-Met has been shown to have an important role in carcinogenesis through activation of signal transduction pathways. The c-Met protein is expressed at low levels in normal tissues and at high levels in tumor tissues. The ligand of c-Met, hepatocyte growth factor (HGF) can interact with c-Met through paracrine or autocrine signaling, or through c-Met tyramine, which can result in abnormal activation of multiple signal transduction pathways including the PI3K/Akt/mTOR pathway, the PIP2 phospholipase pathway, and the Ras/Raf/MAPK pathway [26–29].

In the present study, immunohistochemistry was used to investigate the expression levels of c-Met protein in breast cancer tissues by constructing tissue microarrays (TMAs) that included 255 samples of breast cancer tissues and 43 matched adjacent normal breast tissue samples. This study also included the analysis of associated clinicopathological characteristics and clinical follow-up data, including these use of neoadjuvant chemotherapy (NAC) and overall survival (OS). Immunohistochemistry showed that c-Met protein was localized to the cell membrane and cytoplasm, and protein expression was increased in breast cancer tissues (54.51%) compared with matched adjacent nontumor breast tissues (30.23%). Also, increased c-Met expression was associated with tumor size, metastasis, TNM stage, Ki-67 expression, and the expression of topoisomerase II (TOPO II). Increased expression of c-Met was a predictor of reduced OS. These findings are supported by those from a previously published study by Jiang et al. [30], who showed that c-Met expression levels were associated with the prognosis of patients with breast cancer and could be used as an independent prognostic biomarker.

Topoisomerase (TOPO) is a nuclear enzyme that catalyzes changes in the superhelical structure of DNA and is divided into two main types, and several subtypes. Topoisomerase II (TOPO II) has an essential role in eukaryotic cells and is an important chemotherapy target in tumors, which is one of the factors implicated in the development of multidrug resistance (MDR) [31]. In the present study, c-Met was expressed in breast cancer tissues and was associated with TOPO II expression. Further investigation of c-Met and TOPO II expression levels in breast cancer samples from patients who had received NAC showed that both c-Met and TOPO II (c-Met+ and TOPO II+) were expressed in the tumor tissues of patients with breast cancer before NAC. Following NAC, c-Met expression was negative and TOPO II expression was positive (c-Met- and TOPO II+). Therefore, the expression of c-Met and TOPO II in breast cancer might be associated with chemotherapeutic efficacy. The findings of the present study showed that patients with breast cancer that was c-Met-negative and TOPO II-positive were more likely to be responsive to chemotherapy, and patients with breast cancer that was c-Met-positive and TOPO II-positive were more likely to be chemotherapy-resistant.

Anthracyclines are used in combination with other forms of chemotherapy in breast cancer. Daunorubicin is an anthracycline. The findings from this study have shown that therapeutic effect of daunorubicin in breast cancer was influenced by the protein expression level of TOPO II. Knockdown of c-Met induced TOPO II mRNA and protein expression, further increasing the sensitivity of breast cancer cell lines to chemotherapy drugs. These results indicated that c-Met could increase the resistance of breast cancer cells to the chemotherapeutic agent.

Conclusions

Currently, the mechanism of chemotherapy resistance of breast cancer is unclear. Therefore, the aims of this study were to explore the relationship between c-Met expression, clinical characteristics, and overall survival (OS) in patients with breast cancer. The relationship between c-Met and TOPO II expression in breast cancer tissue samples from patients who had been treated with neoadjuvant chemotherapy (NAC) and the role of c-Met in chemotherapy resistance in breast cancer cell lines were studied. The findings of this study support a role for c-Met as a clinical prognostic marker and for c-Met and TOPO II as predictive markers for response to chemotherapy in patients with breast cancer. Further large-scale, controlled studies are required to validate these findings and further explore the role and mechanism of action of c-Met in chemotherapy-resistant breast cancer.

Conflict of interest

None.

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