Original Article
Correlation of expression of WWOX and JNK with clinicopathologic features in human breast carcinoma

Sai Huang, Chenggang Wang, Yanrong Lv, Ying Liu, Jintao Ma, Xiao Wang

Department of Breast Surgery, Qilu Hospital of Shandong University, Jinan, China

Abstract: The aim of our study was to compare the expression levels of c-Jun N-terminal kinase (JNK) and WW domain-containing oxidoreductase (WWOX) in human breast carcinoma, to analyze the correlation between the expression of WWOX and JNK with the clinicopathologic features of human breast carcinoma, and to explore the potential mechanism of their antitumor effects. The mRNA and protein levels of WWOX and JNK in forty paired breast carcinoma tissues and the adjacent normal tissues were detected by real-time quantitative polymerase chain reaction (RT-PCR) and Western blot analysis. Protein expression was further confirmed by immunohistochemistry (IHC). The mRNA expression levels of both JNK and WWOX were downregulated in carcinoma tissues relative to those in the adjacent normal tissues, as determined by Western blot analysis and IHC (*P*<0.01). JNK expression was positively correlated with WWOX expression (r=0.47, *P*=0.002). Both WWOX and JNK play important roles in breast cancer. Therefore, the antitumor ability of WWOX and JNK could supply significant information for therapeutic strategy.

Keywords: JNK, WWOX, western blot, RT-PCR, breast cancer

Introduction

The WW domain-containing oxidoreductase (WWOX) gene, which is located at the fragile site FRA16D, encodes a 46 kDa protein containing two functional WW domains in the NH2-terminal region and a short-chain dehydrogenase domain (SDR). The WW domains are correlated with the protein-protein interactions [1]. The SDR domain of the WWOX protein exhibits dehydrogenase activity in the presence of selected steroid substrates [2]. Itcan be demonstrated that WWOX plays an important role in steroid-related regulated tissues, such as prostate, ovary, and breast tissues. The presence of WWOX as a tumor suppressor gene has already been reported in osteosarcoma [3], breast cancer [4], ovarian cancer [5] and other cancers. However, the functional mechanisms involved remain unclear. The previous studies provided evidence that the interaction of WWOX is associated with the genes mediating apoptosis or inducing signal transduction, such as BRCA1 [6], SMAD4 [7], and ATM [8].

c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase super-family. The JNK family, consisting of three isoforms (JNK1, JNK2, and JNK3), is encoded by three separate genesthat are alternatively spliced to create at least ten variants of 46 and 55 kDa [9]. JNK1 and JNK2 are expressed in most tissues, whereas JNK3 expression is mainly restricted to expression in brain, heart, and testis tissues [10]. The JNK signal transduction pathway participates inmany physiological processes, including inflammatory responses, morphogenesis, cell proliferation, differentiation, survival, and death. JNK is also widely acknowledged to be involved in cancer development and progression.

JNK hasbeen proven to physically interact with WWOX and inhibit apoptosis in human hepatocellular carcinoma (HCC) [11]. It was demonstrated that JNK had completely opposite functions in liver cancer [12] and breast cancer [13]. However, the relevance of WWOX and JNK in breast cancer and HCC has remained unclear.

In our current study, we measured the expression levels of WWOX and JNK in breast carcinoma patients. We also calculated the correlationof WWOX and JNK levels with the clinico-

pathologic features of the disease. The correlation of WWOX and JNK in breast carcinoma was also examined.

Materials and methods

Patients and tissue samples

A total of forty paired breast carcinoma and adjacent normal tissues (an adjacent normal tissue was defined as that being located at least 5 cm from the tumor edge) were obtained from female patients who were surgically treated in Qilu Hospital of Shandong University (Jinan, China) between February 2016 and January 2017. The samples were examined histologically for the presence of tumor cells. Each tumor sample was considered suitable for this study when the proportion of tumor cells was more than 60%. The median age of the patients was 52 years (range: 37-77 years). The patients met the following criteria: primary unilateral non-metastatic breast tumors and no radiotherapy, chemotherapy or immunotherapy before surgery. Samples were snap frozen in liquid nitrogen and were maintained at -80°C until further analysis. Tissues used for IHC were fixed in 10% neutral-buffered formalin andembedded in paraffin blocks. The medical records of the patients were reviewed, and histologic parameters were evaluated based on hematoxylin/eosin-stained slides and the original pathology reports. Tumor stage and grade were assessed according to the 7th Edition of the UICC/AJCC TNM Staging System. The Ki-67 index was assigned a positive score when 14% or more of the tumor cells were immunostained according to the guidelines of the St. Gallen International Expert Consensus [14]. This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. Each patient provided a written informed consent for the use of their clinical samples.

Real-time PCR

Total RNA was extracted using TRIzol Reagent (Life Technologies Co., Ltd., USA) in accordance with the instructions by the manufacturer. RNA was quantified using the DS-11 spectrophotometer (DeNovix Co., Ltd, USA) and its purity was assessed by the A260/A280 and A260/230 ratios (>1.8). Total RNA (1 μg) was reverse-transcribed in a total volume of 10 μL, including 2 μL of RT buffer, 0.5 μL of RT Enzyme Mix, and 0.5 μL of Primer Mix (FSQ-101, Toyobo Co., Ltd., Japan). Reactions were conducted at 37°C for 15 minutes and then at 98°C for 5 minutes. Complementary DNA was stored at -20°C until further use. PCR was performed using the sequences for JNK, as follows: forward, 5'-TGTGT-GGAATCAAGCACCTTC-3'; reverse, 5'-AGGCGTC-ATCATAAAACTCGTTC-3'; WWOX: forward, 5'-TA-GGCACTTTCACCGC CTTC-3'; reverse, 5'-TCCA-CTCGCTCAGGAACTCA-3'; GAPDH forward, 5'-GCACCGTCAAGGCTGAGAAC-3'; reverse, 5'-TG-GTGAAGACGCCAGTGGA-3'. RT-PCR reactions were conducted on a C1000 Touch Thermal Cycler (Bio-Rad, Co., Ltd., USA) and SYBR Green Real-time PCR Master Mix (QPK-201T, Toyobo Co., Ltd., Japan) under the following conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 s, 64°C for 15 s, and 72°C for 45 s. PCR amplification was performed in triplicate. Real-time PCR results were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Total protein was extracted and determined using the BCA Protein Assay Kit in accordance with the instructions by the manufacturer. Equivalent amounts of protein for each sample were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the isolated proteins were subsequently transferred to polyvinylidene fluoride membranes (PVDF; Millipore, Billerica, MA, USA). The membranes were blocked with 5% fat-free milk in Tris-buffered saline containing 1% Tween 20 for 1 h at room temperature. Primary antibodies (p-JNK, 1:500, CST, USA; WWOX, 1:1000, Abcam, USA; GAPDH, 1:1000, Santa, USA) were used to incubate the blocked membranes overnight at 4°C. The membranes were then washed three times with TBST for 5 minutes each, and incubated for 40 minutes with rabbit anti-goat immunoglobulin (IgG)-horseradish peroxidase (HRP) secondary antibody (1:3000; Beijing Golden Bridge Biotechnology). Protein bands were detected using the FluorChem E chemiluminescent Western blot imaging system (Amersham Imager 600, GE Amersham, USA) and quantified using the Quantity One software.

Immunohistochemistry

Paraffin-embedded breast cancer tissues were cut into 4 μm thick sections and then stained
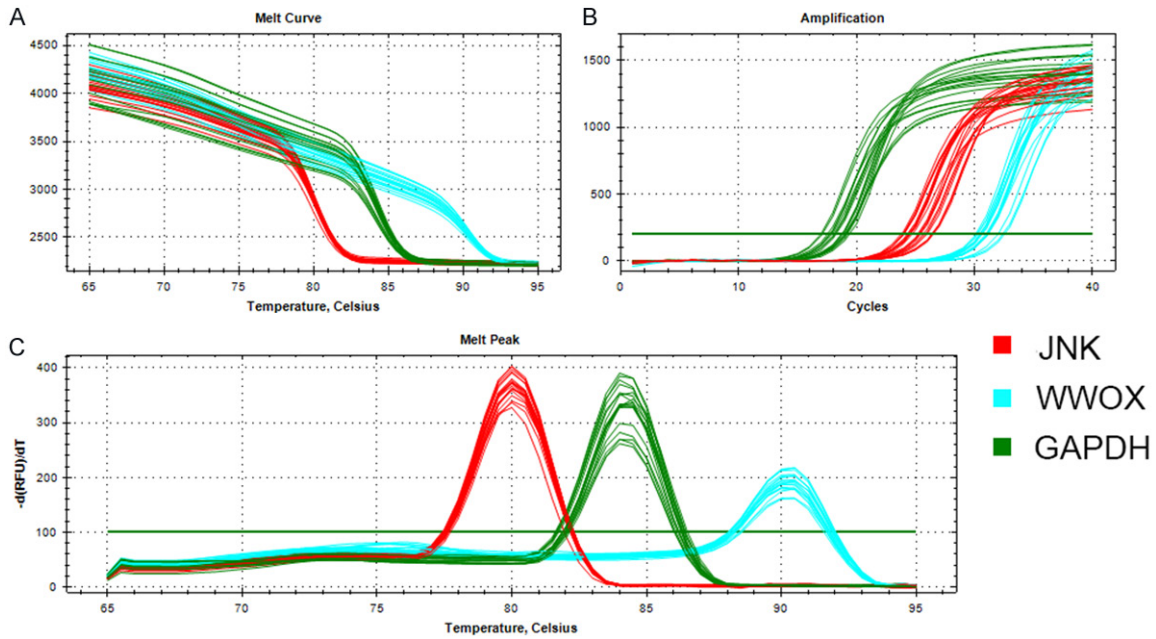
Figure 1. Meltingand amplification curve analyses of different gene RT-PCR products. A. RT-PCR melt curve for JNK, WWOX, and GAPDH; B. RT-PCR amplification curve for different genes. The gene expression levels of JNK, WWOX, and GAPDH were quantified by detecting the threshold cycle (CT) values with real-time quantitative PCR; C. RT-PCR melt peak for JNK, WWOX, and GAPDH with the corresponding melting temperatures of specific target amplicons.

Table 1. RT-PCR analysis of WWOX and JNK mRNA expression in breast cancer tissues compared with that in adjacent non-tumorous tissues

	Sample	N	Mean ± SD	T	P
JNK	T	40	0.60±0.32	8.3	<0.01[a]
	N	40	1±0		
WWOX	T	40	0.34±0.28	15.04	<0.01[b]
	N	40	1±0		

a, Mean JNK values were significantly lower in breast cancer tissues than in adjacent non-tumorous tissues.
b, Mean WWOX values were significantly lower in breast cancer tissues than in adjacent non-tumorous tissues.

by IHC. The slides were baked for 1 h at 65°C, deparaffinized with xylene, and rehydrated using a series of graded ethanol and distilled water. Antigen retrieval was conducted by heating sections using a microwave oven for 20 minutes in 0.01 mol/L sodium citrate buffer (pH 6.0). Subsequently, 3% hydrogen peroxide solution was added to block endogenous peroxidase activity at room temperature for 10 minutes, and the slides were immersed twice in phosphate-buffered saline. The sections were incubated with primary antibody (mouse anti-JNK 1:200, Santa, USA; mouse anti-WWOX 1:300, Santa, USA) overnight at 4°C. The sections were then incubated with Polink-2 plus Polymer HRP Detection System (PV-9002, Beijing Golden Bridge Biotechnology Co., Ltd., Beijing, China) in accordance with the instructions by the manufacturer. The 3,3'-diaminobenzidine reagent was used to detect the signal from the antigen-antibody reaction. All sections were counterstained with hematoxylin. The slides were visualized on an IX71-SIF type microscope (Olympus, Japan).

The IHC slides were stored independently by two pathologists without knowledge of patient clinical information and in accordance with previously described methodology [15]. Each IHC slide was assessed by the staining intensity (I) and the proportion/extent (E) of the stained tumor cells; and graded as follows: 0= no staining, 1= weak, 2= moderate, and 3= strong. A proportion score was assigned to represent the estimated proportion of positively stained tumor cells: 1=0%-25%, 2=25%-50%, 3=50%-75%, and 4=75%-100%. Total score was calculated by multiplying the intensity scores and extent. A staining score higher than 2 for WWOX or P-JNK expression was considered positive, whereas a lower score was considered negative.
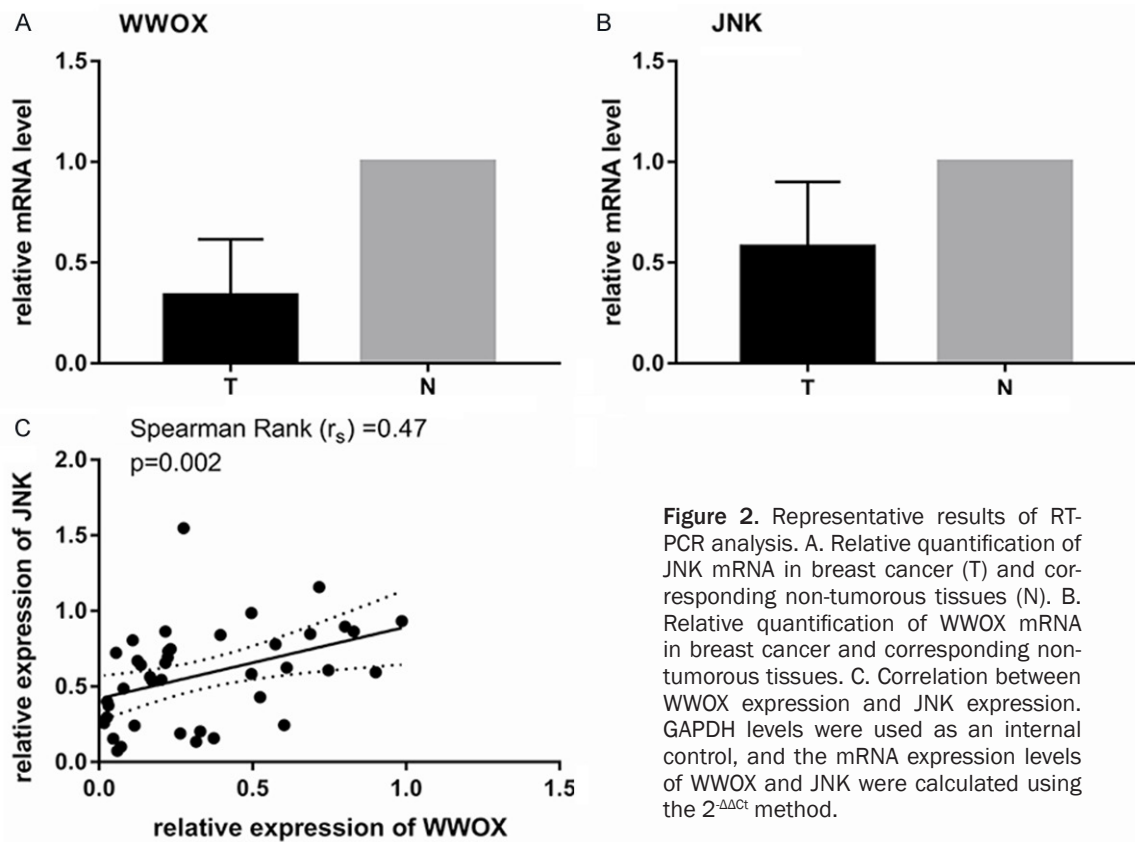
Figure 2. Representative results of RT-PCR analysis. A. Relative quantification of JNK mRNA in breast cancer (T) and corresponding non-tumorous tissues (N). B. Relative quantification of WWOX mRNA in breast cancer and corresponding non-tumorous tissues. C. Correlation between WWOX expression and JNK expression. GAPDH levels were used as an internal control, and the mRNA expression levels of WWOX and JNK were calculated using the $2^{-\Delta\Delta Ct}$ method.
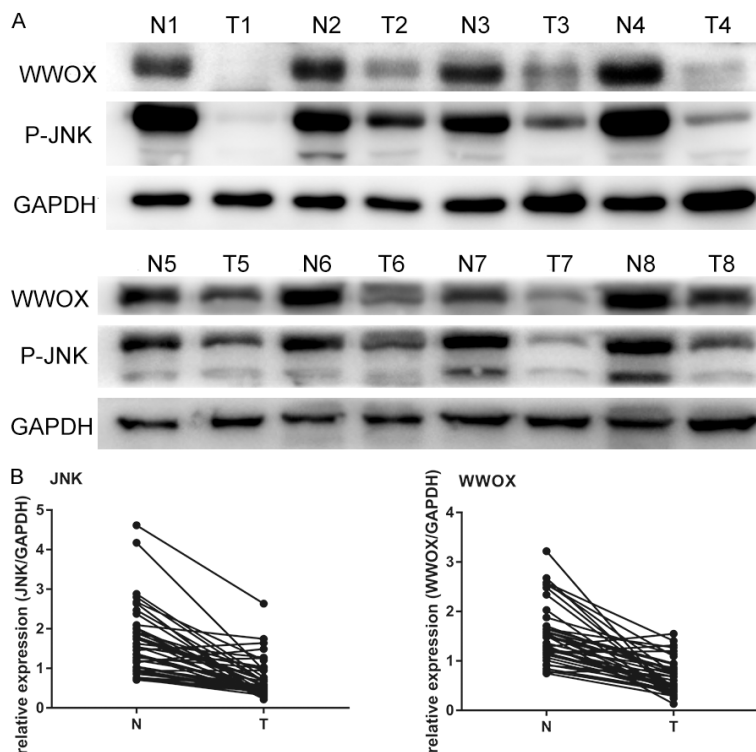
Figure 3. Expression of WWOX and JNK in breast carcinoma (T) and corresponding non-tumorous tissues (N). A. Representative results of Western blot analysis. GAPDH demonstrates the same amount of protein in the gel. B. Relative expression of WWOX and JNK band normalized to GAPDH in matched non-tumorous tissues.

Statistical analysis

Frequency tables were analyzed using the χ^2 test. The correlation of the expression levels of WWOX and JNK with the clinicopathologic characteristics was assessed using the Spearman rank correlation. All statistical tests were two-sided. Statistical significance was set at *P*<0.05. All data were analyzed using SPSS 23.0.

Results

Expression of WWOX and JNK in breast carcinoma tissues

The mRNA and proteinexpression levels of WWOX and JNK were quantitated inall fortypaired breast carcinomaand adjacent normal tissues. The RT-PCR assay results indicated that the melting curve of JNK, WWOX, and internal reference gene GAPDH products showed a single peak and a

Table 2. Western blot analysis of p-WWOX and JNK protein expression in breast cancer tissues compared with that in adjacent non-tumorous tissues

	Sample	N	Mean ± SD	T	P
JNK	T	40	0.74±0.49	7.63	<0.01[a]
	N	40	1.73±0.88		
WWOX	T	40	0.67±0.36	7.57	<0.01[b]
	N	40	1.56±0.65		

a, Mean JNK values were significantly lower in breast cancer tissues than in adjacent non-tumorous tissues.
b, Mean WWOX values were significantly lower in breast cancer tissues than in adjacent non-tumorous tissues.

sharp peak, respectively. In addition, the melting temperature was uniform (**Figure 1**). The curves showed no extra waves. The corresponding melting temperatures were as follows: JNK, 80.0°C; WWOX, 90.5°C, and GAPDH, 84.0°C. The data suggested that the amplification products of JNK, WWOX, and GAPDH gene were specific and the performance of the respective primer pairs was efficient. The expression of JNK mRNA in breast carcinoma samples was 0.60±0.32, which is significantly lower than that of the corresponding adjacent non-tumorous tissues ($P<0.01$, **Table 1**; **Figure 2A**). In addition, the result demonstrated that most of tumor tissues expressed lower mRNA level of WWOX compared with adjacent normal tissues ($P<0.01$, **Table 1**; **Figure 2B**).

The representative bands of WWOX and JNK protein expression detected by Western blot analysis are shown in **Figure 3A**, and GAPDH was used as a loading control. The protein expression levels of WWOX and JNK in breast cancer were significantly lower compared with those in the matched adjacent non-cancerous tissues ($P<0.01$, **Table 2**; **Figure 3B**).

Correlation of JNK expression with WWOX expression

The relative mRNA expression of JNK was higher than that of WWOX (0.60±0.32 vs. 0.34±0.28, **Table 1**). In addition, a highly significant positive correlation was observed between the mRNA expression levels of WWOX and JNK in the cancer tissues (r=0.47, $P=0.002$, **Figure 2C**). The relative protein expression levels of WWOX and JNK detected by Western blot analysis (0.74±0.49 vs. 0.67±0.36, **Table 2**, Spearman test, r=0.603, $P<0.01$) were consistent with the mRNA expression results.

The IHC assay showed that expression of WWOX was significantly reduced compared with that in the normal breast tissue, with a corresponding decrease of P-JNK activity. A highly significant positive correlation was observed between the expression levels of WWOX and P-JNK in the cancer tissues (r=0.38, $P=0.029$, **Table 4**).

Patient clinicopathologic features

There were four patients assessed with tumor grade 1, two patients with grade 2, and nine patients with grade 3. A total of nineteen (47.5%) cases were classified as Stage I, sixteen (40.0%) cases were classified as Stage II, and five (12.5%) cases were classified as Stage III. A total of twenty-two patients had lymph node metastases (**Table 3**). IHC analysis of WWOX expression was detectable in nine (22.5%) of the forty breast cancer tissues and thirty-five (87.5%) of the forty matched non-tumorous tissues. P-JNK was detectable in ten (25%) of the forty breast cancers and thirty-two (80%) of the forty matched non-tumorous tissues.

Correlation of WWOX and JNK protein expression with clinicopathologic features

The absence of WWOX and P-JNK expression was observed more frequently in cancer than in matched non-tumorous tissues ($P<0.01$). The most typical WWOX and P-JNK staining observed was cytoplasmic and there was no nuclear staining in any tissue. Representative immunostaining results for WWOX and PJNK are shown in **Figure 4**.

To elucidate the potential roles of WWOX and JNK in the development and progression of breast carcinoma, the correlation of expression levels of WWOX and JNK with other clinicopathologic features in forty breast carcinoma tissues were investigated. The lymph node metastases were more common in cancers without WWOX expression than in those with WWOX expression (67.7% versus 11.1%, $P=0.01$). However, no correlation was determined between WWOX expression and other clinicopathologic features such as age, ER status, PR status, P53 status, Ki67 status, or TNM stage. No correlation was found between JNK expression and other clinicopathologic features.

Table 3. Correlation between the WWOX and JNK expression levels and clinicopathologic features of breast carcinoma

Characteristics	n	JNK (%)		P	WWOX (%)		P
		Positive n=10	Negative n=30		Positive n=9	Negative n=31	
Age (y)							
>50	25	7 (70)	18 (60)	0.57	7 (77.8)	18 (58.1)	0.28
≤50	15	3 (30)	12 (40)		2 (22.2)	13 (41.9)	
Stage							
Ita	35	8 (80)	27 (90)	0.41	5 (55.6)	30 (96.8)	0.001
III	5	2 (20)	3 (10)		4 (44.4)	1 (3.2)	
ER							
Positive	32	8 (80)	24 (80)	1.00	6 (66.7)	26 (83.9)	0.26
Negative	8	2 (20)	6 (20)		3 (33.3)	5 (16.1)	
PR							
Positive	28	6 (60)	22 (73.3)	0.43	5 (55.6)	23 (74.2)	0.28
Negative	12	4 (40)	8 (26.7)		4 (44.4)	8 (25.8)	
Her-2							
Positive	21	6 (60)	15 (50)	0.58	7 (77.8)	14 (45.2)	0.09
Negative	19	4 (40)	15 (50)		2 (22.2)	17 (54.8)	
P53							
Positive	14	3 (30)	11 (36.7)	0.70	3 (33.3)	11 (35.5)	0.91
Negative	26	7 (70)	19 (63.3)		6 (66.7)	20 (64.5)	
Ki67							
Positive	33	8 (80)	25 (83.3)	0.81	8 (88.9)	25 (80.6)	0.57
Negative	7	2 (20)	5 (16.7)		1 (11.1)	6 (19.4)	
Lymphnode metastasis							
Yes	22	5 (50)	13 (43.3)	0.71	1 (11.1)	21 (67.7)	0.01
No	18	5 (50)	17 (56.7)		8 (88.9)	10 (32.3)	
Tumor grade							
1	4	0 (0.0)	4 (13.3)	0.43	0 (0.0)	4 (12.9)	0.27
2	27	7 (70.0)	20 (66.7)		8 (88.9)	19 (61.3)	
3	9	3 (30.0)	6 (20)		1 (11.1)	8 (25.8)	

Table 4. Correlation of WWOX and P-JNK expression in breast carcinoma

| P-JNK	WWOX		Total
	Positive	Negative	
Positive	5	5	10
Negative	4	26	30
Total	9	31	40

A highly significant inverse correlation was found between P-JNK expression and WWOX expression (χ^2=5.78, R=0.38, P=0.029).

Discussion

Breast cancer is one of the leading causes of death for women between ages 50 and 60 years. Despite advances in chemotherapy, radiotherapy, and adjuvant hormonal therapy, one third of patients with breast cancer relapse and die of the disease. New therapeutic strategies are needed to improve this outcome [16]. WWOX is also inactivated in breast and lung cancers by regulatory region DNA methylation. Promoter methylation was also detected in tissue adjacent to breast cancer, and methylation in WWOX exon 1 distinguished breast cancer DNA from DNA of adjacent and normal tissue. Interestingly, JNK has also been described as having a tumor-suppressive role, because in some mammary tumor models, the inhibition of the JNK pathway has been described to promote tumorigenesis.
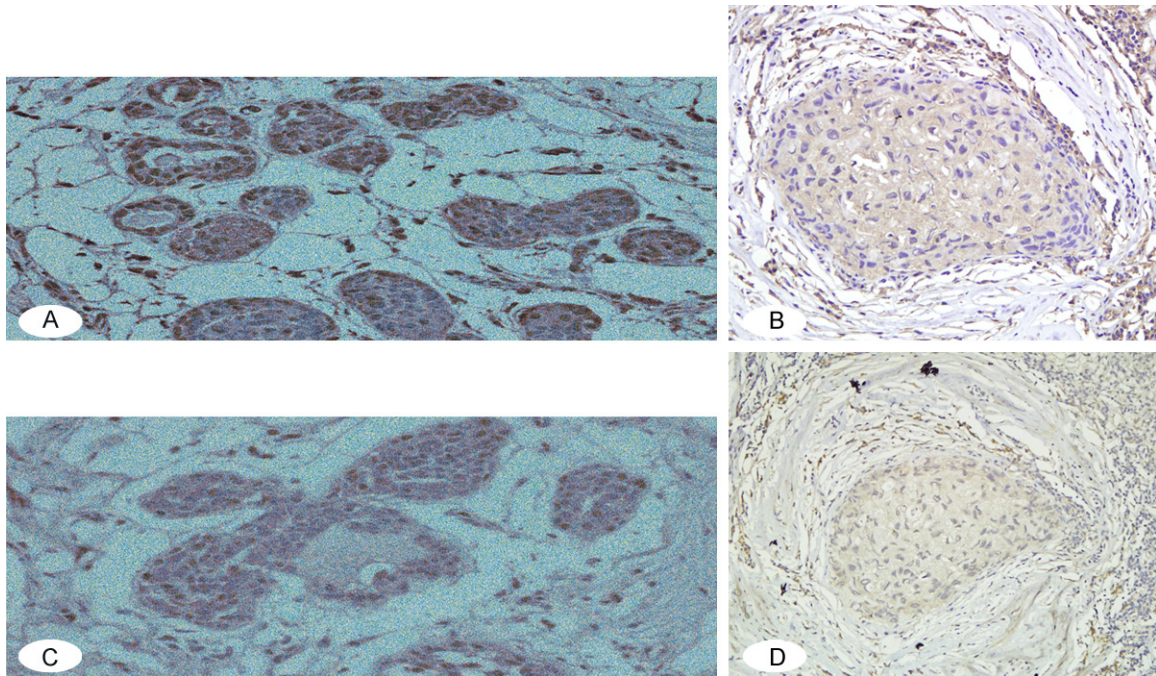
Figure 4. Representative photomicrographs of WWOX and JNK immunohistochemistry results (Ductal carcinoma, Magnification ×200). A. High expression of JNK in adjacent non-tumorous tissues (score=9); B. Low expression of JNK inbreast carcinoma (score=2); C. High expression of WWOX in adjacent non-tumorous tissues (score=6); D. Low expression of WWOX in breast carcinoma (score=2).

In this study, we found that the mRNA and protein expression of JNK was higher than those of WWOX in breast carcinoma tissues. Moreover, induced expression of JNK exhibited a significant positive correlation with the downregulation of WWOX. However, other studies showed that JNK inhibits WWOX-mediated apoptosis in HCC [11]. In addition, JNK inhibition enhanced WWOX-mediated apoptosis, and then WWOX suppressed HCC cell proliferation [17], which suggests that JNK can potentially exhibit a negative correlation with WWOX in HCC. The aforementioned conclusion regarding the correlation of WWOX and JNK was totally in contrast with the results of our study in breast cancer. Notably, the role of JNK in HCC differs from that in breast carcinoma. In our study, the result of the analysis of correlation between WWOX and JNK expression revealed that WWOX was correlated with JNK. The correlation between WWOX and JNK leads to the presumption that WWOX may suppress breast carcinoma via JNK.

JNK participates in many physiological processes and performs different roles in different cancers. For instance, JNK acts as a tumor promot-er in lung cancer [18] and pancreatic cancer [19], and as a tumor suppressor in breast cancer [13] and prostate cancer [20]. Opposite functions of JNK in different cancers may be related to the correlation between WWOX and JNK. Yan et al. reported that the WWOX gene may promote apoptosis of ovarian cancer stem cells by upregulating the expression of JNK [5], which was consistent with our viewpoint. However, the reason for the contrasting correlation between WWOX and JNK in different cancers may be complex and has yet to be determined.

Reduced expression in WWOX has been observed in several cancers. WWOX expression is found to be significantly decreased in lung cancer samples relative to their non-malignant bronchioli counterparts [21]. The protein and mRNA expression of WWOX was reduced in gastric cardia adenocarcinoma tissues and was associated with loss of heterozygosity and methylation of the gene [22]. The current study shows that downregulation of WWOX correlates with the clinicopathologic features of breast cancer, including tumor stage and lymph node metastasis. The results obtained in our study are similar to those in other studies, which

observed the loss of WWOX expression in urinary bladder tumors [23]. Other reports indicated that WWOX signaling pathway loss contributes to lymph node metastasis [24], which seems to be consistent with our observation of WWOX expression in breast cancer tissues. Previous studies showed that the fragile *WWOX* gene is inactivated in a significant fraction of breast cancers, mainly due to DNA hypermethylation [25]. DNA hypermethylation can be reversed by DNA methyltransferase inhibitors, such as DAC or zebularine agents that have therapeutic potential for cancers.

A limitation of our study is that the number of samples used in the study is relatively small. The result may not be in accordance with the real situation regarding the correlation of WWOX with the clinicopathologic features of breast cancer. In addition, further studies *in vitro* with a larger sample size are needed to verify the correlation between WWOX and JNK in breast cancer.

In conclusion, it is the only study that has analyzed the correlation of WWOX and JNK with the clinicopathologic features of breast cancer. We have proven that JNK protein expression is correlated with WWOX protein expression in human breast carcinoma, suggesting that both WWOX and JNK play important roles in breast cancer. The role of WWOX and JNK on human breast cancer requires further investigation. Our data may further broaden the potential application of WWOX and JNK and suggeststhat JNK may be a promising therapeutic target for anti-tumor therapy.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiao Wang, Department of Breast Surgery, Qilu Hospital of Shandong University, Jinan 250012, China. Tel: +86 185600-85176; E-mail: wangxiao@sdu.edu.cn

References

[1] Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA and Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. Cancer Res 2000; 60: 2140-2145.

[2] Saluda-Gorgul A, Seta K, Nowakowska M and Bednarek AK. WWOX oxidoreductase--substrate and enzymatic characterization. Z Naturforsch C 2011; 66: 73-82.

[3] Wen J, Xu Z, Li J, Zhang Y, Fan W, Wang Y, Lu M and Li J. Decreased WWOX expression promotes angiogenesis in osteosarcoma. Oncotarget 2017; 8: 60917-60932.

[4] Gothlin Eremo A, Wegman P, Stal O, Nordenskjold B, Fornander T and Wingren S. Wwox expression may predict benefit from adjuvant tamoxifen in randomized breast cancer patients. Oncol Rep 2013; 29: 1467-1474.

[5] Yan H, Tong J, Lin X, Han Q and Huang H. Effect of the WWOX gene on the regulation of the cell cycle and apoptosis in human ovarian cancer stem cells. Mol Med Rep 2015; 12: 1783-1788.

[6] Schrock MS, Batar B, Lee J, Druck T, Ferguson B, Cho JH, Akakpo K, Hagrass H, Heerema NA, Xia F, Parvin JD, Aldaz CM and Huebner K. Wwox-Brca1 interaction: role in DNA repair pathway choice. Oncogene 2017; 36: 2215-2227.

[7] Hsu LJ, Chiang MF, Sze CI, Su WP, Yap YV, Lee IT, Kuo HL and Chang NS. HYAL-2-WWOX-SMAD4 signaling in cell death and anticancer response. Front Cell Dev Biol 2016; 4: 141.

[8] Abu-Odeh M, Salah Z, Herbel C, Hofmann TG and Aqeilan RI. WWOX, the common fragile site FRA16D gene product, regulates ATM activation and the DNA damage response. Proc Natl Acad Sci U S A 2014; 111: E4716-4725.

[9] Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000; 103: 239-252.

[10] Bogoyevitch MA and Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiol Mol Biol Rev 2006; 70: 1061-1095.

[11] Chang NS, Doherty J and Ensign A. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. J Biol Chem 2003; 278: 9195-9202.

[12] Seki E, Brenner DA and Karin M. A liver full of JNK: signaling in regulation of cell function and disease pathogenesis, and clinical approaches. Gastroenterology 2012; 143: 307-320.

[13] Cellurale C, Girnius N, Jiang F, Cavanagh-Kyros J, Lu S, Garlick DS, Mercurio AM and Davis RJ. Role of JNK in mammary gland development and breast cancer. Cancer Res 2011; 72: 472-481.

[14] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B and Senn HJ. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen international expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol 2011; 22: 1736-1747.

[15] Maris P, Blomme A, Palacios AP, Costanza B, Bellahcene A, Bianchi E, Gofflot S, Drion P, Trombino GE, Di Valentin E, Cusumano PG, Maweja S, Jerusalem G, Delvenne P, Lifrange E, Castronovo V and Turtoi A. Asporin is a fibroblast-derived TGF-beta1 inhibitor and a tumor suppressor associated with good prognosis in breast cancer. PLoS Med 2015; 12: e1001871.

[16] Golubnitschaja O, Debald M, Yeghiazaryan K, Kuhn W, Pesta M, Costigliola V and Grech G. Breast cancer epidemic in the early twenty-first century: evaluation of risk factors, cumulative questionnaires and recommendations for preventive measures. Tumour Biol 2016; 37: 12941-12957.

[17] Aderca I, Moser CD, Veerasamy M, Bani-Hani AH, Bonilla-Guerrero R, Ahmed K, Shire A, Cazanave SC, Montoya DP, Mettler TA, Burgart LJ, Nagorney DM, Thibodeau SN, Cunningham JM, Lai JP and Roberts LR. The JNK inhibitor SP600129 enhances apoptosis of HCC cells induced by the tumor suppressor WWOX. J Hepatol 2008; 49: 373-383.

[18] Cellurale C, Sabio G, Kennedy NJ, Das M, Barlow M, Sandy P, Jacks T and Davis RJ. Requirement of c-Jun NH(2)-terminal kinase for Ras-initiated tumor formation. Mol Cell Biol 2011; 31: 1565-1576.

[19] He G, Zhang L, Li Q and Yang L. miR-92a/DUSP10/JNK signalling axis promotes human pancreatic cancer cells proliferation. Biomed Pharmacother 2014; 68: 25-30.

[20] Han I, Yun M, Kim EO, Kim B, Jung MH and Kim SH. Umbilical cord tissue-derived mesenchymal stem cells induce apoptosis in PC-3 prostate cancer cells through activation of JNK and downregulation of PI3K/AKT signaling. Stem Cell Res Ther 2014; 5: 54.

[21] Bleau AM, Freire J, Pajares MJ, Zudaire I, Anton I, Nistal-Villan E, Redrado M, Zandueta CN, Garmendia I, Ajona D, Blanco D, Pio R, Lecanda F, Calvo A and Montuenga LM. New syngeneic inflammatory-related lung cancer metastatic model harboring double KRAS/WWOX alterations. Int J Cancer 2014; 135: 2516-2527.

[22] Guo W, Dong Z, Dong Y, Guo Y, Kuang G and Yang Z. Genetic and epigenetic alterations of WWOX in the development of gastric cardia adenocarcinoma. Environ Mol Mutagen 2013; 54: 112-123.

[23] Ramos D, Abba M, Lopez-Guerrero JA, Rubio J, Solsona E, Almenar S, Llombart-Bosch A and Aldaz CM. Low levels of WWOX protein immunoexpression correlate with tumor grade and a less favorable outcome in patients with urinary bladder tumors. Histopathology 2008; 52: 831-839.

[24] Guler G, Himmetoglu C, Jimenez RE, Geyer SM, Wang WP, Costinean S, Pilarski RT, Morrison C, Suren D, Liu J, Chen J, Kamal J, Shapiro CL and Huebner K. Aberrant expression of DNA damage response proteins is associated with breast cancer subtype and clinical features. Breast Cancer Res Treat 2011; 129: 421-432.

[25] Momparler RL. Epigenetic therapy of cancer with 5-aza-2'-deoxycytidine (decitabine). Semin Oncol 2005; 32: 443-451.