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Original Article

WIP1 is relevant to tumor malignancy and metastasis in breast cancer

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Abstract: Wild-type p53-induced phosphatase 1 (WIP1) is a serine/threonine protein phosphatase that has been shown to be correlated with tumor proliferation, differentiation and anti-apoptotic processes in several malignant tumors. However, the significance of WIP1 expression in breast cancer is still far from clear. To evaluate the clinical significance of WIP1 oncogene in breast cancer, the expression of WIP1 was investigated in 120 breast cancer biopsies and adjacent breast tissues by immunohistochemistry. The correlation between WIP1 expression and postoperative survival rate was also analyzed. WIP1 was up-regulated in breast cancer tissues (96/120). Down-regulation of WIP1 in MCF-7 breast cancer cells was established using Lentivirus-mediated infection. The absence of WIP1 resulted in dramatic decrease of cell proliferation, invasion, and metastasis ability as well as increase cell apoptosis. Subsequent investigations revealed that, p53 protein expression was significantly higher in WIP1-infected cells than in normal tumor cells. Our founding indicates that WIP1 ameliorated the malignancy of MCF-7 cells, which is probably achieved via regulating p53 protein expression. Taken together, WIP1 may be a useful regulator in breast cancer malignancy and metastasis in breast cancer and may involved in proliferation, apoptosis, migration and invasion of breast cancer cells by regulating p53 protein expression.

Keywords: Wip1, breast cancer, clinical significance, tumor malignancy, metastasis

Introduction

Breast cancer is one of the most commonly diagnosed tumor in Chinese women and is the leading cause of death in female cancers. According to the statistics, the number of Chinese women with breast cancer account for 12.2% of all newly diagnosed breast cancers and 9.6% of deaths from all deaths in the worldwide [1]. Therefore, breast cancer has become a severe public health burden in China. The exploration of new biomarkers and technologies that are capable of achieving early diagnosis, tumor grade determination as well as morbidity evaluation has become an important and urgent demand in breast cancer research. Elucidation of the clinical, biological, and pathological characteristics of breast cancer is expected to facilitate the discovery of new therapeutic treatments for breast cancer.

Wild-type p53-induced phosphatase PPM1D (or WIP1) is a serine/threonine protein phosphatase, which gene located in 17q23/q24 region of human chromosome. Accumulated studies have proved that abnormal expression of WIP1 is often involved in tumor cell proliferation, differentiation and anti-apoptotic processes, indicating that WIP1 is strongly contributes to the occurrence and development of various types of cancer [2-5]. In the late 1990s, the discovery of WIP1 in a genetic screening study opened a new era of genetic-related research. Thereafter, WIP1 was identified as a novel oncogene and overexpression of WIP1 was proved to be associated with human ovarian cancer, breast cancer, medulloblastoma and neuroblastoma tumors [6-11]. These characteristics of WIP1 have aroused great interest in cancer researchers.

Most recently, the pathogenetic mechanism of WIP1 in human malignancies became a research hotspot. For instance, Kim's research group reported that overexpression of WIP1 in

Table 1. Clinicohistopathologic characteristics of the breast cancer patients involved in this study

Groups	Cases
Age	
≤ 50	48
> 50	72
Types of breast cancer	
Ductal carcinoma	66
Lobular carcinoma	38
Other	16
TNM stage	
I	59
II	42
III	19
Size of tumor (mm)	
< 50	104
> 50	16
Postoperative complications	
Bleeding	3
Seroma	42
Skin flap necrosis	6
First-degree upper extremity edema	15
Second-degree edema	3
No postoperative complications	51

medulloblastoma negatively regulating p53 by abrogating the activity of p53 [2]. The significance of WIP1 in clinical application was also investigated in various cancers. Liang reported that the metastasis and prognosis of adenoid cystic carcinoma patients were closely associated with WIP1 expression [12]. These studies have opened up a new genetic target for future clinical treatment. However, the significance of WIP1 expression in breast cancer is still far from clear.

In present study, we tested WIP1 expression level in 120 paired breast cancer and adjacent non-cancer tissues, analyzed its relation with clinicopathological characteristics and evaluated the impact of WIP1 expression level on breast cancer patients' survival rate. In addition, the correlation of WIP1 expression with breast cancer malignancy and metastasis and its clinical significance was determined. These results should help to gain an enhanced understanding of the etiology of breast cancer.

Materials and methods

Patients and cell culture

120 breast tumor tissues and paired adjacent non-cancer tissues (5 cm distant from the tumor margin) were collected from breast cancer patients with confirmed case at Tangshan Gongren Hospital between January 2005 and January 2010. All of the patients voluntarily participated in this study gave written informed consent before the using of residual samples and this study was approved by Tangshan Gongren Hospital's research ethics committee. The mammary gland tumors in this study were staged according to tumor node metastasis (TNM) classification system. The pathology classification, demographic status and postoperative complications are detailed in **Table 1**. The resected breast tumor tissues and adjacent breast tissues were paraffin-embedded and defined as the cancer group and normal control group, respectively. All breast cancer cases were female, and sporadic without family history.

Breast cancer cells used in this study were obtained from Chinese Academy of Sciences Cancer Hospital and cultured in Phenol Red-free RPMI media replenished with antibiotics and fetal bovine serum (10%).

Immunohistochemical staining

The breast tumor tissue and paired adjacent non-cancer tissues' immunohistochemical staining of WIP1 was carried out as our previous reports [13]. In short, tissue samples were stained with diaminobenzidine (DAB) and visualized by hematoxylin. WIP1 expression level was determined by the staining intensity and the total score of the percentage of positive cells. Positive cells percentage was classified into four groups: 0) ≤ 5%, 1) 5-25%, 2) 25-50%, and 3) > 50%. The results of immunohistochemical staining were evaluated semiquantitavely on the basis of a four-point scale: 0) no staining, 1) weak staining (pale yellow), 2) moderate staining (brown), and 3) strong staining (dark brown). The total score was classified as following: 0, negative (-); 1-2, weakly positive (+); 3-4, medium positive (++); 5-6, strongly positive (+++). A result of (-) or (+) was defined

as low expression, and a result of (++) or (+++) was defined as high expression. Negative controls for the staining were treated in the same manner but without the primary antibody.

Lentivirus-mediated WIP1 infection of MCF-7 cells

MCF-7 cells were seeded at a density of 10^5 cells per well in 24-well plates and incubated for 24 hr. When the cell population reached 80%, 1 mL lentivirus containing WIP1 short hairpin RNA (RNA) or negative control (NC)-shRNA plasmid was added to the cell culture. After infecting for 12 hr, the virus was aspirated and l mL Dulbecco's modified Eagle medium (DMEM) was added. The green fluorescent clones were selected after 48 hr infection under a fluorescence microscope (BX43, Olympus).

Western blot

For western blot analyze, the experiment was carried out as our conventional reported with slight modification [13]. In brief, tissue samples were lysis, homogenized and centrifugated for 20 min (13,400 g) at 4°C. Protein concentration was measured with a bicinchoninic acid (BCA) protein quantification kit (Fluoro Profile, Sigma, USA). Electrophoretic analysis of the protein was carried out by sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the membranes were blocked in 5% skim milk, incubated with the primary antibody as well as horseradish peroxidase-labeled goat anti-mouse IgG respectively. The membranes were developed with electrochemiluminescence, scanned with a FUJI Mini-4000 scanner, and analyzed with LabWorks 4.5 software. All of the experiments were conducted in triplicate.

WIP1 expression in MCF-7 cells before and after infection was detected in a similar manner and referenced to GAPDH.

Reverse transcription quantitative polymerase chain reaction (qPCR)

Total RNA of tissue samples and cell lines was extracted and purified according to our previous report and manufacturer's instructions [13]. For the breast cancer tissues, the reaction conditions were 42°C for 50 min, and terminated at 95°C for 5 min. Primers for WIP1 (forward: 5'-GGCCAAATGAAAGCCCAAGAAAT-3'), (reverse: 5'-CAGAGTTCTTTCGCTGTGAGGTTGT-3') and β-actin were (forward: 5'-ACTTAGTTGCGTTACACCCTT-3'), (reverse: 5'-GTCACCTTCACCGT-TCCA-3') synthesized by Shanghai Biological Engineering Technology Services Limited. β-actin served as the internal control, and relative WIP1 expression was calculated using the $2^{-\Delta\Delta CT}$ method.

To determine WIP1 expression level of shRNA-infected MCF-7 cells, the primers were designed as follows: 5'-TTCCCCATGTTCTACACCACCAG-3' (WIP1 upstream), 5'-TGAGGGTATGACTACACCTTGGAC-3' (WIP1 downstream); 5'-GTCTCCTCTGACTTCAACAGCG-3' (GAPDH upstream), 5'-ACCACCCTGTTGCTGTAGCC-3' (GAPDH downstream). PCR conditions were 95°C for 60 s, 95°C for 15 s, 60°C for 60 s, and 72°C for 45 s for 40 cycles. The GAPDH expression served as the internal control. WIP1 expression was calculated as the ratio of grey band intensity relative to that of GAPDH.

MTT assay

WIP1-shRNA- and NC-shRNA-infected MCF-7 cells at the logarithmic growth phase (70-80% confluence) were seeded in 96-well plates at a density of 5000 cells/well (200 μL media/well). Cell growth was terminated after 1, 2, 3, and 4 days, respectively, and 20 mL of 5 mg/mL MTT solution was added 4 hr before the termination of culture. After incubated with MTT for 4 hr, the medium in each well was discarded and 200 mL/well dimethyl sulfoxide was used to dissolve the internalized purple formazan crystals. The absorbance value (D) was detected on an automatic microplate reader at wavelength of 490 nm and reference wavelength of 620 nm. Cell survival was calculated as follow:

$$\text{Survival rate (\%)} = (D_{\text{experimental group}}/D_{\text{control group}}) \times 100$$

Flow cytometry

WIP1-shRNA- and NC-shRNA-infected MCF-7 cells at the logarithmic growth phase (70-80% confluence) were digested, collected, washed twice with 4°C PBS and resuspended in 1 mL binding buffer with a concentration of 1×10^6 cells/mL. 100 μL cell suspension was transferred to a 5 mL flow tube and 10 μL propidium iodide was added. Cells were incubated in the dark for 15 min and the number of apoptotic cells was detected using flow cytometry. For

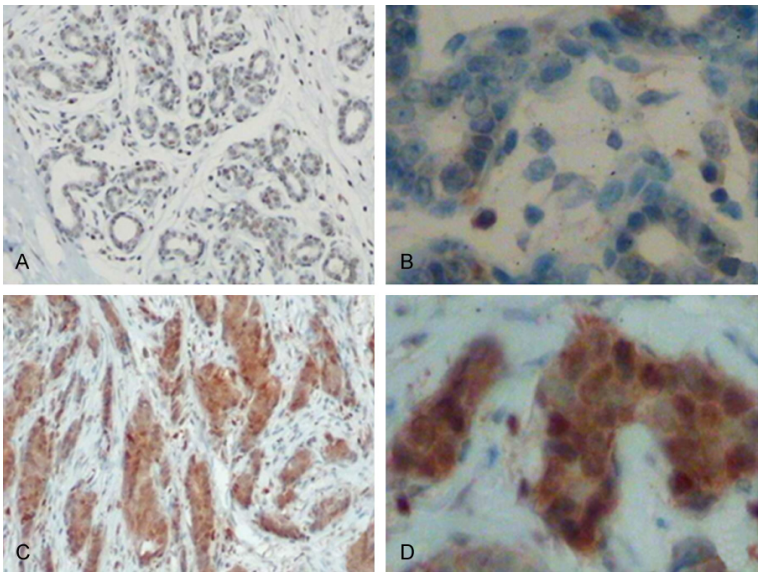

Figure 1. Immunohistochemical staining result of WIP1 protein expression in adjacent normal tissues (A, B) and breast tumor tissues (C, D). (A and C) SP ×100; (B and D) SP ×400.

Table 2. The expression scores of WIP1 in breast tumor tissues and adjacent normal breast tissues

Groups	Cases	WIP1 Protein Expression					
		-	+	++	+++	Z	*P**
Cancer tissues	120	24	19	43	34	-9.561	0.000
Adjacent tissues	120	88	24	8	0		

Student t-test was used to analyze the statistical significance of comparison between two groups. **P* < 0.05.

measurement of the cell cycle, 195 μL of the same cell suspension was transferred to a 5 mL flow tube, and 5 μL Annexin V-FITC and 10 μL propidium iodide were added to each tube. The tubes were incubated for 15 min in the dark and the cell cycle was detected by flow cytometry.

Transwell invasion assay

Cell invasion assay was conducted with a polycarbonate microporous membrane and was capped with or without 50 μL Matrigel (8.4 g/L). WIP1-shRNA- and NC-shRNA-infected MCF-7 cells were suspended in serum-free medium with a density of 1×10^6 cells/mL. 50 μL cell suspensions were transferred to the upper chamber and 800 μL DMEM (10%) was added to the lower chamber. After cultured for 18 hr, cells on the surface of the upper chamber were removed by scraping with a cotton swab. Cells on the lower filter surface were kept in 4% paraformaldehyde and stained with 0.1% crystal violet for 20 min. Cells were counted in 5 random portions of each film and the average number of invading tumor cells was calculated.

Statistical analysis

Data were analyzed with SPSS16.0 statistical software. The Chi-square test was performed for comparisons of patient characteristics between two groups. The Student t-test was performed for comparisons between two groups. Differences between the WIP1-shRNA and NC-shRNA groups were compared by an independent samples t-test or one-way ANOVE analysis of variance. The Kaplan-Meier method was used to estimate survival rates, and differences were compared with the two-sided log-rank test. $P < 0.05$ was considered statistically significant.

Results

The expression of WIP1 was elevated in breast cancer tissue

To evaluate the WIP1 protein expression in breast cancer, immunostaining was performed as previously described [13] in 120 breast cancer and adjacent non-cancer tissues. Immunohistochemical staining result showed that WIP1 immunohistochemical staining was pale yellow to dark brown in the breast cancer tissues and was weak or even negative in the adjacent breast tissues (**Figure 1**). The expression scores of WIP1 are summarized in **Table 2**. In general, the prevalence of positive WIP1 protein expression (++ and +++) in breast cancer tissues was 80% (96/120), which was obviously higher ($P < 0.05$) than the adjacent tissues (26.7%, 32/120).

Western blot and q-PCR were performed for quantitively confirm WIP1 protein and mRNA

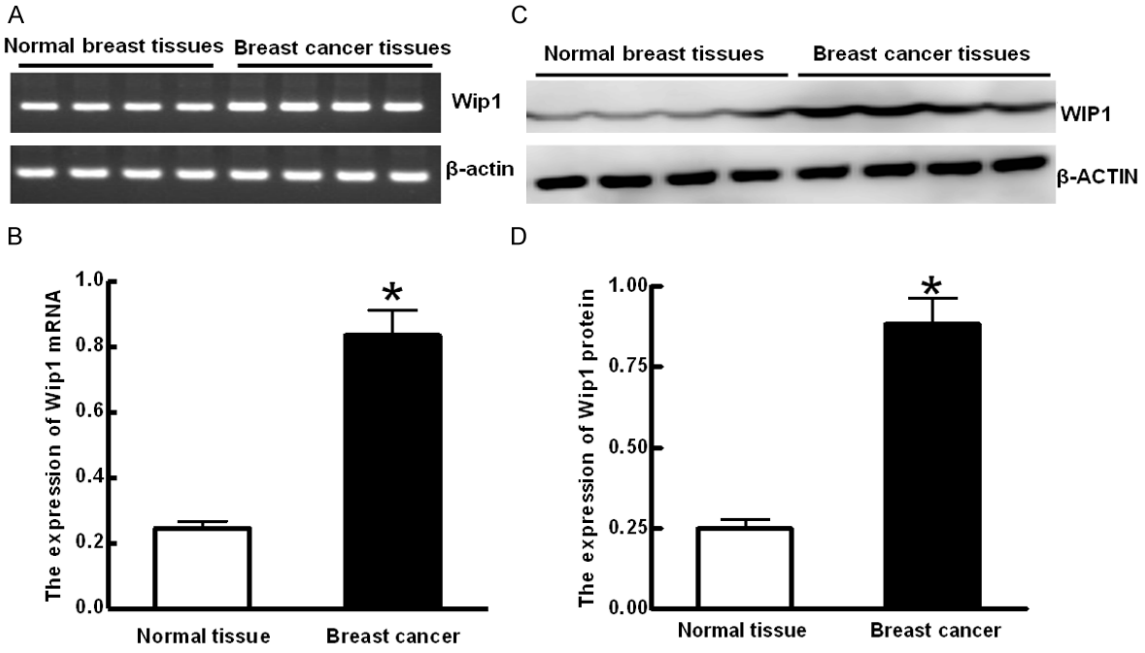

Figure 2. q-PCR and Western blot analysis of Wip1 miRNA (A, B) and protein (C, D) in breast tumor tissue and normal tissues. Data are shown as mean ± SD ($n = 4$). One asterisk indicates statisticalsignificance. ($^*P < 0.05$ breast tumor tissue versus normal tissue).

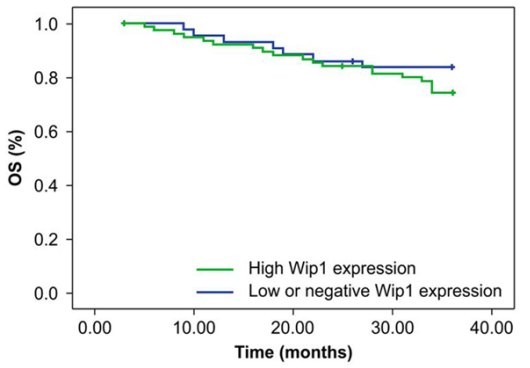

Figure 3. Kaplan-Meier analyses for overall survival (OS) associated with WIP1 expression in breast tumor tissues.

expression respectively and the results indicated that both WIP1 protein (0.885 ± 0.079 vs. 0.251 ± 0.027, $P < 0.001$) and mRNA (0.835 ± 0.076 vs. 0.245 ± 0.021, $P < 0.001$) expression in breast cancer tissues were also obviously higher than that in the adjacent normal tissues (**Figure 2**).

The results of the above experiment demonstrated that WIP1 expression is much higher in human breast tumor tissues than in adjacent breast tissues for both the mRNA and protein (**Figure 1**), indicating that high WIP1 levels tend to be strongly linked to the incidence of breast cancer. These results confirm previous findings demonstrating a potential link between WIP1 expression level and breast cancer [8, 14, 15].

WIP1 expression was correlated with survival rate of patients and P53 gene expression in breast cancer

The potential interrelationship was investigated between WIP1 expression level and breast cancer patients' clinicopathological characteristics by a follow up study of 3 years for 120 patients. Within the observation period, 94 patients survived with a total survival rate of 78.3%, 31 relapsed and the recurrence sites were as follows: 14 cases (45%) for the chest wall, 13 cases (41%; ribs in 5 cases, spine in 4 cases, skull in 1 case, limbs in 2 cases, including multiple bone metastases) for bone, 5 cases (16%) for lung, 2 cases (6%) for brain and 6 cases (19%) for liver. Some patients relapsed in multiple organs. The main cause of death was multiple organ failure. The patients were classified into groups depending on high (n = 77) or low (n = 43) WIP1 expression, and the overall survival (OS) is presented in **Figure 3**. The 3-year survival rate was slightly higher in patients with

Table 3. The associations between the clinical characteristics of breast cancer patients and WIP1 protein expression

Groups	Cases	WIP1 Protein Expression			
		-~+	++~+++	$\bar{x}$	P^*
Age					
≤ 50	48	13	35	2.664	0.103
> 50	72	30	42		
Lymph node metastasis					
N+	40	13	27	0.290	0.590
N0	80	30	50		
TNM stage					
I	59	18	41	1.431	0.232
II~III	61	25	36		
Estrogen receptor					
-	57	16	41	2.846	0.092
+	63	27	36		
Progesterone receptor					
-	63	20	43	0.964	0. 326
+	57	23	34		
HER2					
+	45	15	30	0.196	0.658
-	75	28	47		
P53					
-	80	22	58	38.496	0.000
+	40	5	35		

The Chi-square test was performed for comparisons of patient characteristics between two groups. $^*P < 0.05$.

low expression of WIP1 (83.7%) than that of patients with high expression of WIP1 (74.3%), but there was no significant difference.

The correlation of WIP1 protein expression with other clinicopathological characteristics of the breast cancer patients was also investigated and the result is summarized in **Table 3**. WIP1 expression was not obviously related to age, lymph node metastasis, estrogen/progesterone receptor levels, HER2, or TNM stage ($P > 0.05$), but was obviously related to p53 expression ($P < 0.001$).

Age, tumor size, TNM stage, lymph node metastasis, estrogen/progesterone receptor level, and progesterone receptor levels are not related to WIP1 expression which is compliance with the previous report of Bulavin [16]. However, WIP1 expression is negatively correlated with p53 expression, which might indicate an inhibitory effect of WIP1 on the p53 gene function of tumor suppressor and/or the induction of mutations [17-19]. Overexpression of WIP1 in breast cancer might induce tumor formation via the WIP1/p38MAPK/p53 signaling pathway. The present results also indicate that overexpression of WIP1 plays a vital part in the development of breast cancer by suppressing the function of the tumor suppressor gene p53.

Down-regulation of WIP1 expression is achieved by lentivirus infection

Lentivirus infection was used to pick out breast cancer cells with low WIP1 expression. Three WIP1-shRNA MCF-7 cell lines that showed the best effects of WIP1 knockdown were selected and named as MCF-7/shWIP1-1, -2, and -3. MCF-7/NC (control) and untreated MCF-7 (blank) cell lines were chosen for comparison. WIP1 mRNA and protein expression levels in the five groups are shown in **Figure 4**. MCF-7/shWIP1-1 showed the lowest expression and therefore appeared to be the most efficient knockdown line; therefore, MCF-7/shWIP1-1 was selected in the following research.

After infection, the MCF-7/shWIP-1 group showed strong fluorescence intensity 48 hr whereas the infection efficiency in MCF-7/NC was only ~20% and showed weak fluorescence intensity (**Figure 5**). The q-PCR and western blot results indicated that the expression of WIP1 was obviously lower in the MCF-7/shWIP-1 group than in the MCF-7/NC group for both mRNA and protein expression.

Down-regulation of WIP1 inhibits tumor malignancy and metastasis potential in vitro

To investigate whether down-regulation of WIP1 in MCF-7 cell affects the proliferation ability of tumor cells, MCF-7/shWIP-1 and MCF-7/NC cells were seeded and cultured for 24, 48, 72 and 96 hr and MTT assay was adopted to evaluate cell viability. The results showed that cell viability in the MCF-7/shWIP-1 group was obvi-

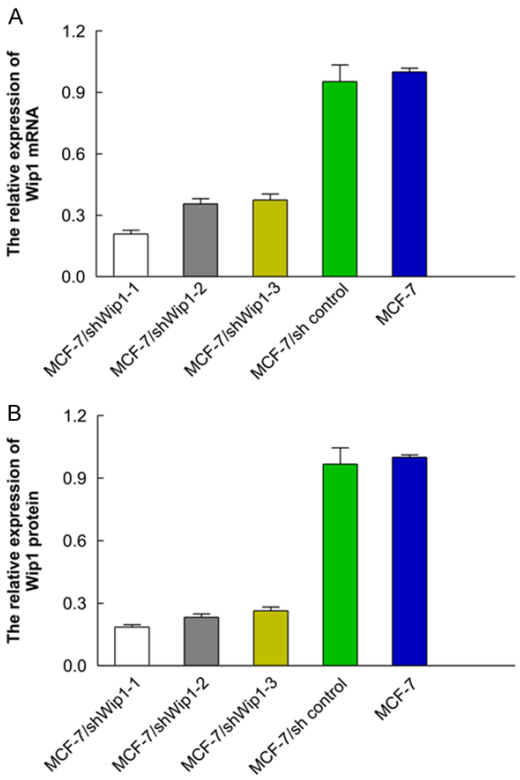

Figure 4. WIP1 mRNA and protein expression levels in the MCF-7/shWIP1-1, 2, 3, MCF-7/NC and MCF-7 groups. One-way ANOVA was used to analyze the differences among the WIP1-shRNA and NC-shRNA groups. Data are shown means ± SD (*n* = *3*).

ously lower than MCF-7/NC group at each time point ($P < 0.05$) (**Figure 6A**). Apoptosis rate of the MCF-7/shWIP-1 group was also significantly improved compared to the MCF-7/NC group (17.6 ± 0.9% *vs*. 5.4 ± 0.06%, $P < 0.05$; **Figure 6B**). Furthermore, there was a significantly higher proportion of MCF-7/shWip1 cells in the G_0/G_1 phase (72.3 ± 5.2% vs. 53.5 ± 3.6%) and a lower proportion in the S phase (14.6 ± 0.8% vs. 27.3 ± 1.5%) than MCF-7/NC cells (**Figure 6C**).

To corroborate the effect of WIP1 expression on tumor metastasis and invasion ability, transwell invasion assays were carried out. The result showed that the number of migrating cells in the MCF-7/shWIP-1 group was obviously decreased compared to the MCF-7/NC group (49.0 ± 6.0 vs. 106.0 ± 11.0, $P < 0.05$; **Figure 7**). Besides, the number of invading cells in the MCF-7/shWIP-1 group was also reduced as compared to the MCF-7/NC group (42.0 ± 4.0 vs. 96.0 ± 9.0, $P < 0.05$; **Figure 7**). The above findings demonstrate that inhibiting the expression of WIP1 in MCF-7 cells weakened the cell ability of transwell invasion. This evidence implied that WIP1 might have endowed MCF-7 cells with higher invasion ability.

Down-regulation of WIP1 promoted the p53 gene expression

Our statistical results of 120 patients proved that WIPl expression is negatively correlated with p53 expression. Previous publications have proved that tumor genesis and metastasis often accompanied with the p53 tumor suppressor inactivation and p53 was an important regulator in breast cancer. Therefore, we studied the possible mechanism for WIP1-related breast cancer cell invasion and migration by investigating the expression difference of p53 in MCF-7/shWIP-1 group and MCF-7/NC group. Quantitive western blot for p53 indicated that, after WIP1-shRNA infection, the relative expression level of p53 protein was obviously improved compared to the MCF-7/NC group (0.765 ± 0.067 vs. 0.315 ± 0.033, $P < 0.05$; **Figure 8**).

Discussion

WIP1 is an oncogene that has been confirmed in many human cancers. Some previous studies reported that WIP1 hamper the DNA damage repair response by inactivating the phosphorylation removing process of p53 and other tumor suppress gene [20]. Abnormal expression of WIP1 was correlated to cervical cancer, colorectal cancer, salivary adenoid cystic carcinoma, renal cell carcinoma, non-small cell lung cancer [20-22]. Therefore, the above research suggesting a close association between WIP1 and its prognosis value. However, little research has been done on the correlation between WIP1 and breast cancer metastasis and tumorigenicity. In the research, the results indicated that WIP1 expression was increased in breast tumor tissues than normal tissues, and the overall 3-year survival rate was related to WIP1 expression level. Our research proved that WIP1 may involve in the malignancy and metastasis of breast cancer.

Breast cancer is a serious hazard that caused one of the most mortality in female cancer. Previous studies proved that down-regulation of WIP1 inhibiting tumor cell proliferation and inducing apoptosis [21]. However, the relation-

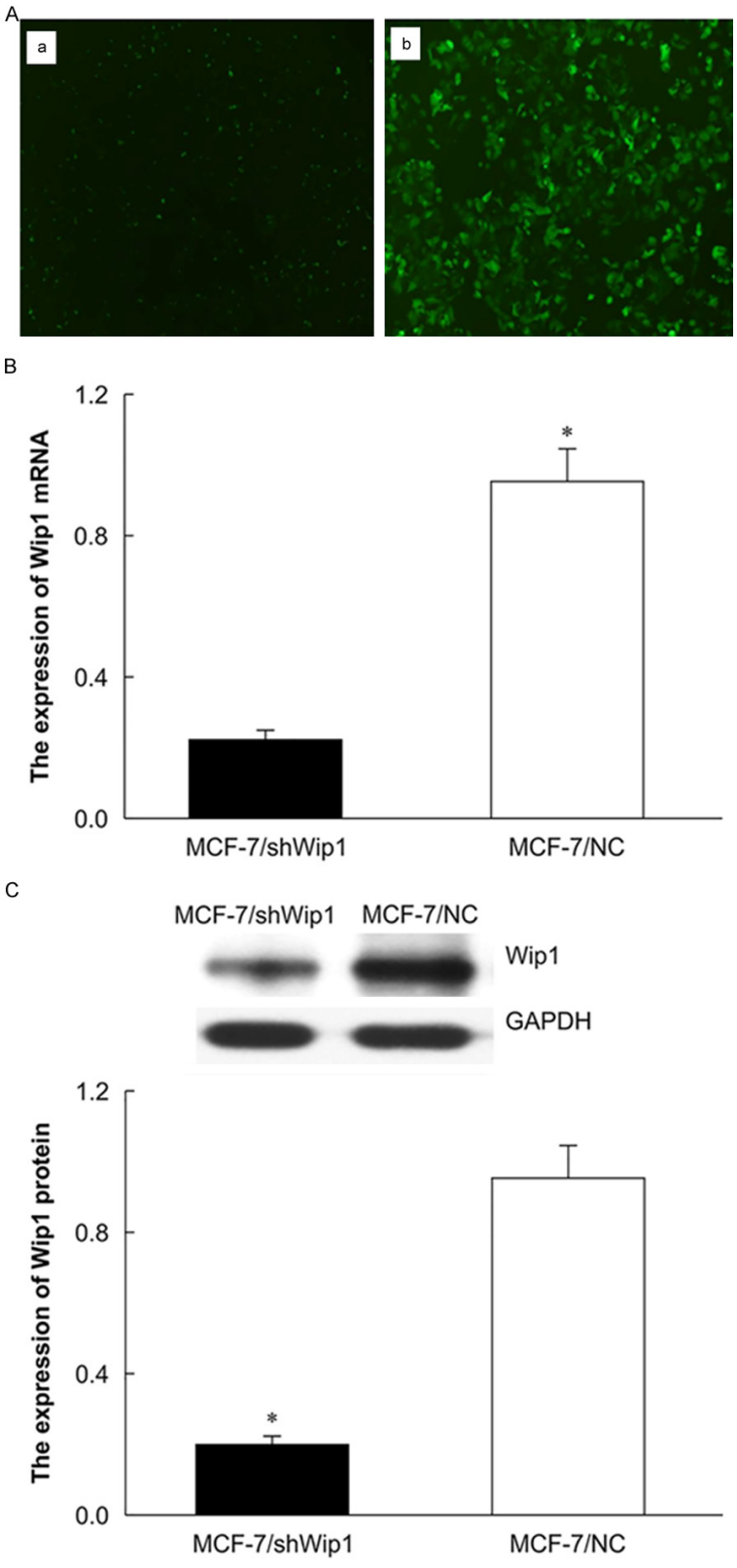

Figure 5. (A) Lentivirus infection of (a) MCF-7/NC cells and (b) MCF-7/shWip1 cells. (B) mRNA and (C) protein expression levels of WIP1 in (A). Independent samples t-test was used to analyze the differences between the MCF-7/shWip1 and MCF-7/NC groups. Data are shown means ± SD (*n* = 3).

ship between WIP1 expression level as well as its clinical significance in breast cancer remain unclear. The present results show that WIP1 mRNA and protein expression in breast cancer tissues was significantly higher than the adjacent tissues. However, WIP1 expression was not obviously correlated with clinicopathological characteristics such as age, estrogen/progesterone receptor levels, tumor size, HER2, TNM stage or lymph node metastasis. In the future study, it is necessary to further investigate the roles of WIP1 protein expression in breast cancer development.

Anyway, another interesting finding was that WIP1 expression was significantly correlated with p53 expression. The expression of WIP1 was proved to be related to the wild-type p53 gene, and exerts a significant effect on DNA repair processes [3, 14, 16]. Shreeram reported that WIP1 could suppress the activity of other tumor suppressor genes (ATM e.g.) [14]. Baxter silenced WIP1 expression in medulloblastoma D283 cells using RNA interference, which enhanced p53 expression and induced tumor cell apoptosis [23]. Besides, WIP1 over-expression in a variety of tumors acts as a negative feedback regulator of p53 expression via the p38/MAPK/p53 signaling pathway and consequently induces p53 mutations [24, 25]. Therefore, in this study the high expression of WIP1 in breast cancer patients may decrease p53 expression, which in turn inhibit the function of p53 tumor suppresser and lead to the development of breast cancer. It is reported that, inhibition of WIP1 function might enhance

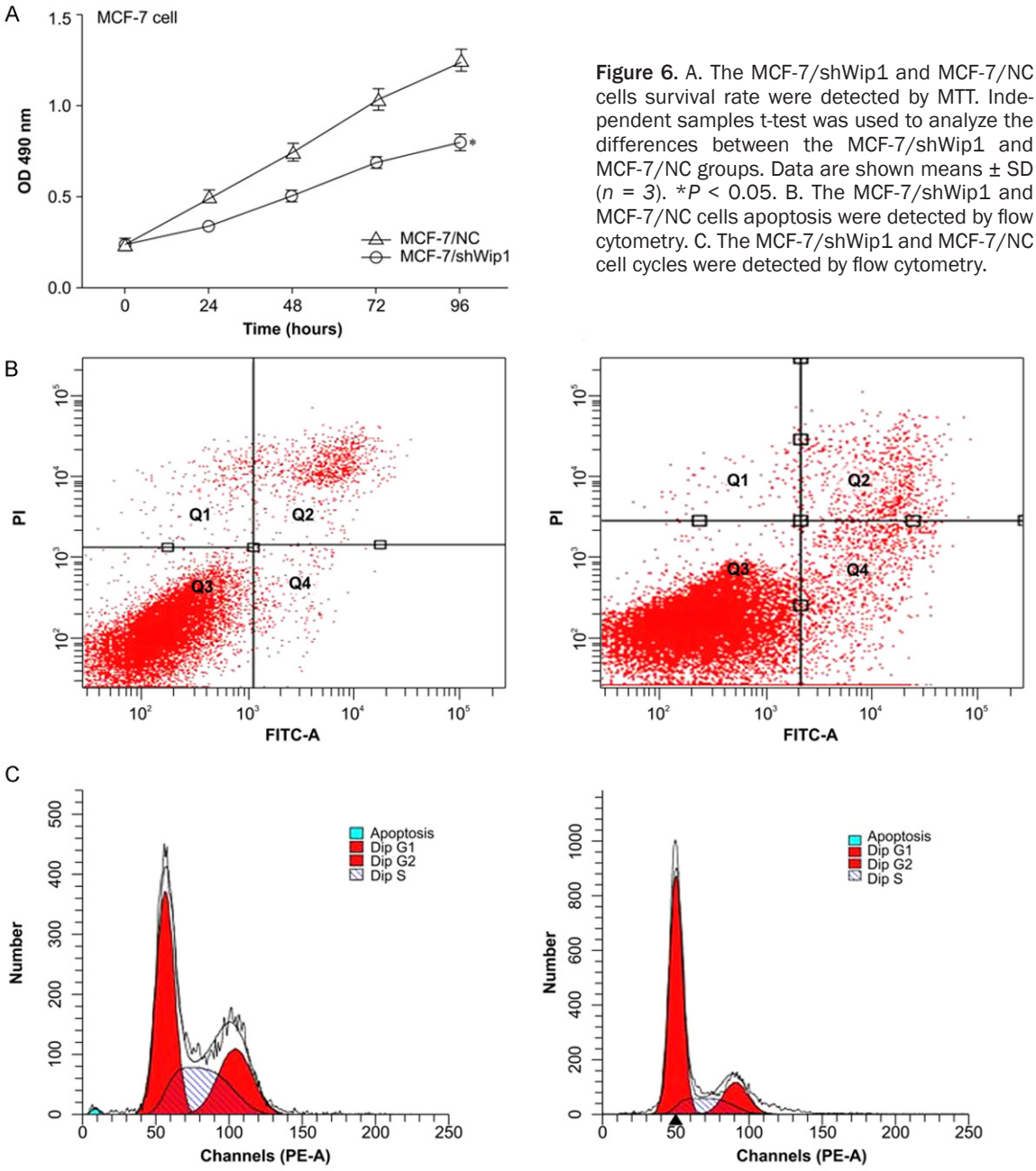

Figure 6. A. The MCF-7/shWip1 and MCF-7/NC cells survival rate were detected by MTT. Independent samples t-test was used to analyze the differences between the MCF-7/shWip1 and MCF-7/NC groups. Data are shown means ± SD ($n = 3$). *$P < 0.05$. B. The MCF-7/shWip1 and MCF-7/NC cells apoptosis were detected by flow cytometry. C. The MCF-7/shWip1 and MCF-7/NC cell cycles were detected by flow cytometry.

the activity of tumor suppressor genes to prevent tumor formation which further support our speculation [26].

We next studied the possible mechanism of WIP1 in the malignancy and metastasis of breast cancer. Our *in vitro* experiments showed that the constructed lentiviral vector effectively decreased WIP1 protein expression in MCF-7 cells which in turn significantly changed the MCF-7 cell cycle and inhibited MCF-7 cells growth. Our results demonstrated that WIP1 down-expression dramatically inhibited the invasion and migration of breast cancer cell. This might be the cause of higher mortality in WIP1 high expression patients.

Furthermore, we demonstrated in this study that WIP1 gene silencing significantly inhibited the migration and invasion of breast cancer cells, indicating that WIP1 has a significant influence on the metastasis of breast cancer. In this study, down-regulation of WIP1 promoted the p53 gene expression in breast cancer cell.

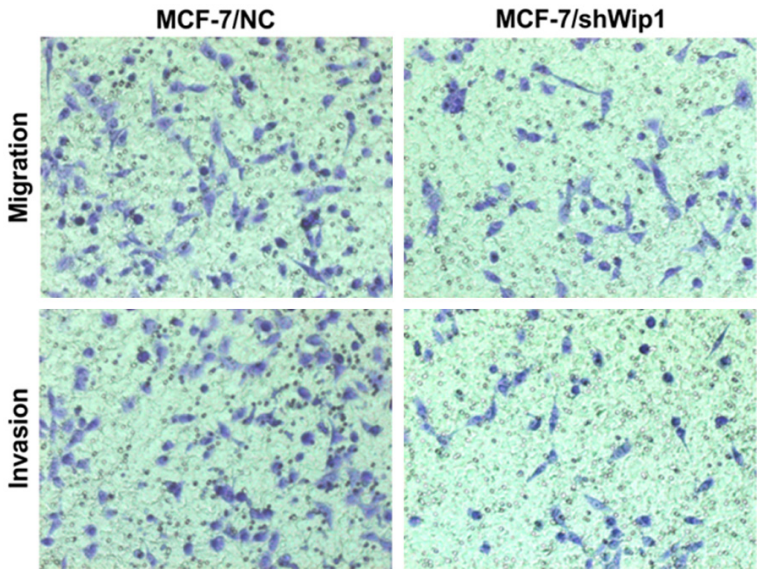

Figure 7. The invasion and metastasis of MCF-7/shWip1 and MCF-7/NC cells were detected by transwell.

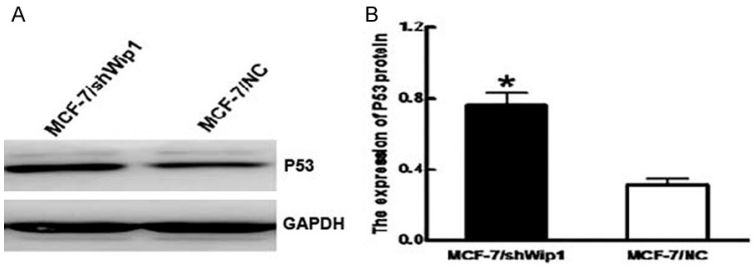

Figure 8. A. Western blot analysis for P53 in MCF-7/shWIP-1 group and MCF-7/NC group. B. Quantitative analysis of P53 levels (GAPDH internal control). Independent samples t-test was used to analyze the differences between the MCF-7/shWip1 and MCF-7/NC groups. Data are shown means ± SD ($n = 3$). ($^{*}P < 0.05$ MCF-7/shWip1 versus MCF-7/NC).

According to the results above, we suggest that the up-expression of WIP1 may induce breast cancer cells metastasis and invasion by inhibiting the p53 expression.

In summary, our study indicates that the expression of WIP1 is significantly increased in breast cancer tissues suggesting its clinical significance in breast cancer diagnosis. The overexpression of WIP1 is closely related to cancer cell viability and invasion in breast cancer cell. Down-regulation of WIP1 significantly ameliorates the malignancy and metastasis of breast cancer cells via regulation of p53 expression. Given that WIP1 expression is strongly associated with the malignancy and metastasis of breast cancer cell, WIP1 appears to be a worthy target for further exploration in breast cancer treatment.

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Disclosure of conflict of interest

None.

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