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Original Article

p75NTR promotes survival of breast cancer resistant cells by regulating Bcl-2/Bax and MAPK pathway

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Abstract: In breast cancer, the neurotrophin receptor p75NTR is a critical factor that promotes resistance to the drug, however, its mechanism of action remains unclear. In our study, the 10 cases of positive expressions of p75NTR were detected in 86 cases of breast cancer tissues, accounting for 11.6% of cases detected. The immunohistochemistry detection of p75NTR was in the cytoplasm and cell membrane. The expression of p75NTR was significantly associated with histological grade (P<0.01), however, not with the menopause, tumor size and lymph node metastasis. Western blot result showed that p75NTR protein was induced by overexpression in the multidrug-resistant breast cancer cell lines. After transfection of pcDNA3.1-p75NTR, MDA-MB-231/ADR-p75NTR cell cycle was arrested in G0/G1 phase. However, the number of cells in G0/G1 phase increased and decreased in S phase cells (P<0.05). Additionally, apoptosis rate decreased (P<0.05). The p75NTR overexpression increased the expression of MDR related protein and activated MAPK signaling pathway.

Keywords: p75NTR, breast cancer, apoptosis, MAPK

Introduction

Breast cancer is one of the most common malignant tumors that threatens women's life and health. Yearly, more than one million women are diagnosed with breast cancer worldwide, and the alarming rates of breast cancer are high because approximately 1,7000,000 new cases are recorded each year making its preventive measures to be at a slow pace. The p75 neurotrophin receptor (p75NTR), which is also known as nerve growth factor receptor (nerve growth factor receptor, NGFR) or CD271, is a transmembrane receptor and a member of the tumor necrosis factor (TNF) receptor superfamily that exerts a variety of functions. Typically, p75NTR has no catalytic activity, but could recruit specific protein partners, which can bind intracellular chopper and death domains [1]. p75NTR is not only expressed in nervous tissues, it is also expressed in non-neuronal tissues and in several cancer cases, such as thyroid carcinoma [2], melanoma [3], bladder [4], prostate [5], stomach [6] and liver [7] cancers. However, p75NTR may serve opposite functions according to tumor types. Hence, it has been described to exert a tumor-promoting activity by favoring survival and metastasis in brain, prostate cancer and melanomas [5, 8, 9], and been identified as a potential tumor suppressor in bladder [4], stomach [6] and liver [7] cancers.

In breast cancer, a previous study showed that p75NTR was expressed in the majority of breast tumors [10], especially in basal-like breast carcinomas [11]. The stimulation of p75NTR leads to an increase in breast cancer cell's survival and resistance to apoptosis via promoting the activation of NF-κB [12] and p21waf1 [13]. Furthermore, NF-κB activation required the binding of TNF receptor-associated death domain protein (TRADD) on the death domain of p75NTR [14] and the brain-expressed X-2 protein (BEX2), which binds the death domain of p75NTR, and plays a key role in the downstream NF-κB activation [15]. Despite the recorded data on initial signaling activated by p75NTR in

Table 1. The relationship between the expression of p75NTR and the clinical pathology features of breast cancer

Clinical data	Category	p75NTR		n	*P* value
		+ (%)	-		
Menopause	Yes	5 (11.4)	39	44	0.42
	No	5 (11.9)	37	42	
Size (cm)	≤2	3 (7.8)	35	38	0.34
	2<, ≤5	7 (14.6)	41	48	
LNM	No	5 (11.1)	40	45	0.53
	Yes	5 (12.2)	36	41	
Histological grade	I	2 (12.5)	14	16	
	II	3 (7.5)	37	40	
	III	5 (16.7)	25	30	<0.01
Pathology	IDC	8 (11.6)	61	69	0.93
	Cephaloma	0 (00)	2	2	0.11
	ILC	1 (50)	1	2	0.42
	Carcinoma metaplastic	0 (0)	1	1	<0.01
	Carcinoma muciparum	1 (14.2)	6	7	0.13
	Others	0	5	5	

breast cancer cells, there is no information on potential changes induced by p75NTR at the proteome level.

In the present study, we demonstrated that p75NTR were overexpressed in breast cancer cell, especially in drug-resistance cells. Overexpression of p75NTR could arrest cell in G0/G1 phase, block in S phase and promote P-gp expression. The expression levels of the MDR related proteins MDR1, MRP1, BCRP, Bcl-2 and MAPK pathway-related proteins, such asJNK1, ERK1, ERK2, p38 in p75NTR were induced by overexpression.

Materials and methods

Cell culture and breast cancer tissues

MCF-7 and MDA-MB-231/ADR cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% of fetal bovine serum (FBS) at 37°C and 5% CO_2. Moreover, 86 samples of patients with breast cancer were collected from January 2013 to December 2013 in our hospital.

Immunohistochemical analysis

Immunohistochemical staining was performed onformalin-fixed, and paraffin-embedded sections. Moreover, 5 μm sections of cancer samples were obtained and immunostaining was performed using p75NTR monoclonal antibody (1:800, Abcam) by DAB Kit (Vector, USA) according to the manufacturer's protocol.

CCK-8 assay

The Cell Counting Kit (CCK)-8 was used to assess the effect of cell proliferation according to the standard protocol. Typically, 6×10^4 MDA-MB-231 and MDA-MB-231/ADR cells were pipetted into wells of a 96-well microplate and subjected to various treatments (GEM, OXA, ADM, PTX and DDP) before carrying an analysis with the CCK-8 assay. The absorbances were measured at a wavelength of 450 nm.

Western-blot

In this study, cells were collected and lysed in ice-cold RIPA lysis buffer (), then, 40 μg of cell lysate from each sample was separated on 10% SDS polyacrylamide gel electrophoresis (PAGE). Electrophoretic transfer of proteins from gels onto nitrocellulose membranes was carried out in a transblotting chamber. Membranes were blocked by immersing them in 5% PBS nonfat milk (w/v) for 30 min before being incubated with primary antibodies at 4°C for 12 hours. After rinsing with PBS/0.1% Tween-20, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Zhongshan Boil Tech Co, Beijing, China). Immunocomplexeswere visualized by incubation using an enhanced chemiluminescence system (Thermo Fisher Scientific, MA, USA).

Cell cycle analyses

Before embarking on treatments, cells were cultured inserum-free medium for 12 h to arrest the cell cycle, then the serum-free supernatant was replaced by fresh medium containing 10% FBS. After transfection of pcDNA3.1-p75NTR and siRNA-p75NTR, MDA-MB-231/ADR cells were stained with propidium iodide (PI) at a final concentration of 50 mg/l. Mo-

Table 2. The relationship between the expression rate of p75NTR and the number of breast cancer lymph node metastasis

Numbers of LNM	p75NTR		
	+	-	n
<4	2	23	25
≥4	3	13	16
Total	5	36	41

P<0.05.

Table 3. The relationship between expression of p75NTR and subtypes of breast cancer

	p75NTR			
Subtypes	+ (%)	-	n	*p* value
Luminal A	3 (7.1)	39	42	0.078
Luminal B	5 (16.1)	26	31	0.037
Her2-OE	1 (14.3)	5	7	0.258
TNBL	1 (16.7)	5	6	0.021

reover, DNA content was analyzed with a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), and the distribution of cells in different cell cycle stages was determined according to the DNA content.

Annexin V-FITC/PI staining

Cell apoptosis was determined by Annexin V-FITC assay. After transfection of pcDNA3.1-p75NTR and siRNA-p75NTR, MDA-MB-231/ADR cells, MDA-MB-231/ADR cells were collected and subjected to Annexin V-FITC/PI apoptosis detection kit. Approximate 1×10^5 cells were analyzed by utilizing flow cytometry (BD Biosciences). The cell apoptosis was expressed as a percentage of apoptotic cells.

Real time PCR

Total RNA was extracted from MDA-MB-231/ADR-p75NTR, MDA-MB-231/ADR-pcDNA, MDA-MB-231/ADR-pSilencer, MDA-MB-231/ADR-p75-NTRsi1 according to manufacturer's instructions (Takara, Dalian). Moreover, 1 μg of total RNA was reverse transcribed (RT). The real time RT-PCR was performed in a 20 μl reaction volume containing 0.5 μl (10 pM) forward and reverse specific primers. The 10 μl of SYBR Green master mix (Applied Bio systems, Carlsbad, Califonia), 2 μl of cDNA and 7 μl of nuclease-free water was carried out.

Gene	Primer sequence (5'-3')	Product length/bp
β-actin	5'-TGTCCACCTTCCAGCAGATGT-3' 5'-AAGTCATAGTCCGCCTAGAAGCA-3'	97
MDR1	5'-CCTGTGAAGAGTAGAACATGAAG-3' 5'-GCTTCCGTTGCACCTCTCTTT-3'	138
MRP1	5'-CATTGGTGTGGTGAGTCAGGAA-3' 5'-TCTCAATCTCATCCATGGTGACA-3'	98
BCRP	5'-AAGCAGGGACGAACAATCATCT-3' 5'-CCAATAAGGTGAGGCTATCAAACA-3'	82
Bcl-2	5'-TTCTTTGAGTTCGGTGGGGTC-3' 5'-TGCATATTTGTTTGGGGCAGG-3'	304
Bax	5'-TCCACCAAGAAGCTGAGCGAG-3' 5'-GTCCAGCCCATGATGGTTCT-3'	257
p38α	5'-AGATGAGTGGAAAAGCCTGACCT-3' 5'-A AGGACTCCATCTCTTCTTGGTCAA-3'	80
JNK1	5'-GCAGGAACGAGTTTTATGATGAC-3' 5'-GTAGCCCATGCCAAGGATGA-3'	81
ERK1	5'-GCTGGACCGGATGTTAACCTT-3' 5'-TCATCCGTCGGGTCATAGTACTG-3'	99
ERK2	5'-TATTTGTTCCTGCCACTGTGTACT-3' 5'-AGGCAAATTTCTCACCACAGA-3'	105

Statistical analysis

SPSS 13.0 software was used for data analysis. Chi-square test or Fisher's exact test was used to analyze the relationship between the expression of p75NTR and the clinical pathology features of breast cancer, the number of breast cancer lymph node metastasis and subtypes of breast cancer. Spearman correlation analyses were performed for the relationship between expression of p75NTR and ER, PR, Her2 and Ki-67. The t-test was conducted to compare the biochemistry index of membrane glycolipids between two groups. The statistical analyses of more than two groups for IC50, cell cycle and apoptosis were performed by using one-way ANOVA with the S-N-K post hoc test. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Expression of p75NTR in breast cancer and clinical pathological features of breast cancer

There was no significant difference between the expression of p75NTR and the menopausal status, tumor size and lymph node metastasis. The expression of p75NTR was correlated with histological grade. The higher the histological grade, the higher the positive expression rate of p75NTR. In the 86 cases of breast cancer,

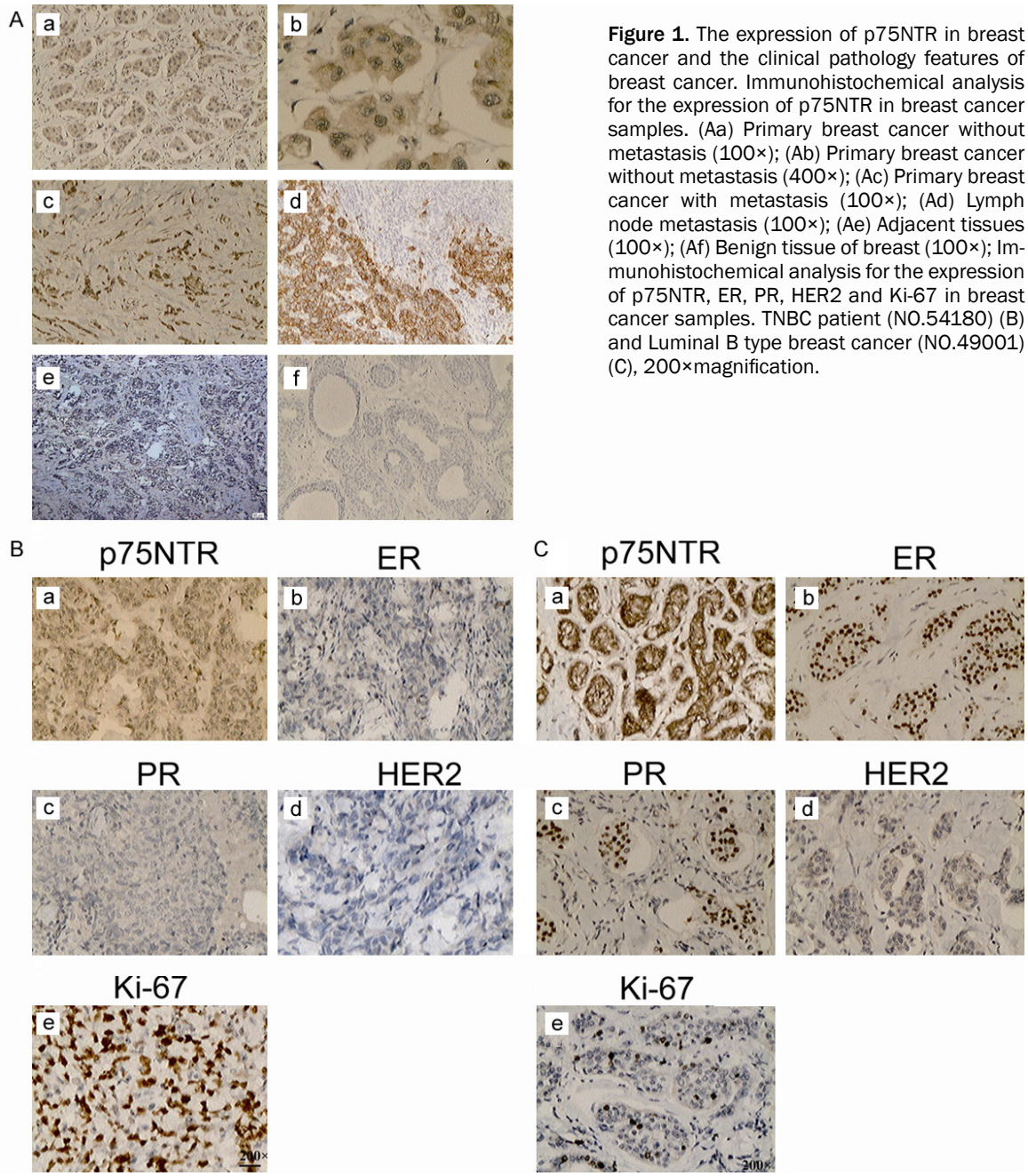

Figure 1. The expression of p75NTR in breast cancer and the clinical pathology features of breast cancer. Immunohistochemical analysis for the expression of p75NTR in breast cancer samples. (Aa) Primary breast cancer without metastasis (100×); (Ab) Primary breast cancer without metastasis (400×); (Ac) Primary breast cancer with metastasis (100×); (Ad) Lymph node metastasis (100×); (Ae) Adjacent tissues (100×); (Af) Benign tissue of breast (100×); Immunohistochemical analysis for the expression of p75NTR, ER, PR, HER2 and Ki-67 in breast cancer samples. TNBC patient (N0.54180) (B) and Luminal B type breast cancer (N0.49001) (C), 200×magnification.

Table 4. The relationship between expression of p75NTR and ER, PR, Her2 and Ki-67

	ER	PR	Her2	Ki-67
Spearman	-0.131	-0.090	0.001	0.201
P value	0.003	0.073	0.897	<0.01
n	85	86	85	84

invasive ductal carcinoma (IDC) was 69 cases, cephaloma was 2 cases, infiltrating lobular carcinoma (ILC) was 2 cases, metaplastic carcinoma was 1 cases, carcinoma muciparum was 7 cases, others were 5 cases (**Table 1**). In **Table 2**, a comparison of patients with lymph node metastasis (LNC), of more than 4 and less than 4, was significantly different in the expression of p75NTR. In **Table 3**, the expression of p75NTR was positively correlated with Luminal B and triple-negative breast cancer (TNBC) (P<0.05).

Immunohistochemical results of p75NTR and ER, PR, HER2, Ki-67 were shown in **Figure 1**. The expression of p75NTR was negatively cor-

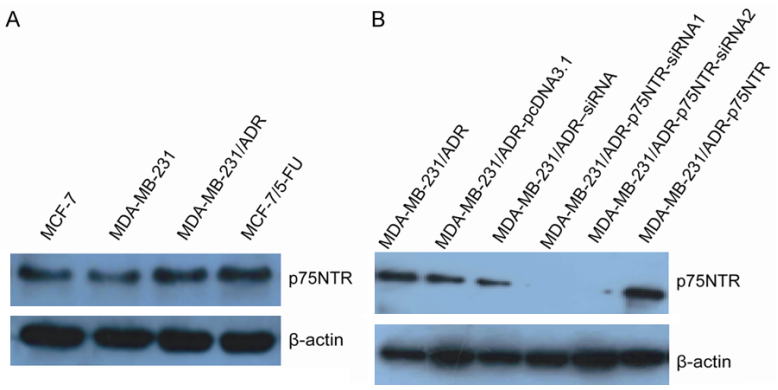

Figure 2. The effects of p75NTR on the cell cycle of MDA-MB-231/ADR cells. Western blot analysis of p75NTR expression in breast cells. A. The expression of p75NTR in MCF-7, MDA-MB-231, MDA-MB-231/ADR and MCF-7/5-FU. B. After transfection with p75NTR-siRNA and p75NTR, the expression of p75NTR was determined by Western blot.

Table 5. Sensitivity to different chemotherapeutic drugs on MDA-MB-231 and MDA-MB-231/ADR cell by CCK-8 (x±s, n=3)

	IC50 (ug/ml)		
Drug	MDA-MB-231	MDA-MB-231/ADR	RF
GEM	0.28±0.05	11.10±1.23*	39.6
ADM	8.58±0.83	126.7±3.54*	14.7
OXA	4.24±0.71	39.12±2.12*	9.2
PTX	0.49±0.06	2.67±0.41	5.5
DDP	1.57±0.24	6.75±1.23	4.3

*P<0.05. vs MDA-MB-231.

related with ER (P<0.01), and positively correlated with Ki-67 (P<0.01). However, the expression of p75NTR was not statistically significantly correlated with PR and HER2 (**Table 4**).

Content of p75NTR in breast cancer cell and drug-resistance of breast cancer cell

A difference of expression of p75NTR between breast cancer cell and breast cancer cell line with drug resistance showed that western blot results of an expression of p75NTR in breast cancer cell lines with drug resistance (MDA-MB-231/ADR, MCF-7/5-FU) were higher than the breast cancer cells (MCF-7, MDA-MB-231) (**Figure 2A**). To analyze whether breast cancer cell line with drug resistance had different sensitivity to different chemotherapeutic drugs, we used GEM, ADM, OXA, PTX and DDP to test the sensitivity of cells. The results demonstrated that MDA-MB-231 and MDA-MB-231/ADR cells had a great difference in sensitivity to anticancer drugs, such as GEM, ADM and OXA. However, they had little difference in sensitivity to PDX and DDP (**Table 5**).

The previous study had shown that p75NTR was expressed in breast cancer tissues, especially in TNBC, and it was highly expressed in drug-resistant cells, such as MDA-MB-231/ADR and MCF-7/5-FU. To reduce the expression of p75NTR in breast cancer cells, a specific siRNA was designed according to CDS region of p75NTR. p75NTRsi1 and p75NTRsi2, which was successfully constructed by gene recombination. The recombinant plasmid p75NTRsi1 and p75NTRsi2 were transfected into breast cancer MDA-MB-231/ADR and MDA-MB-231 cells respectively. The expression of p75NTR in transfected cells were determined by Western Blot. The results demonstrated that the expression level of p75NTR in MDA-MB-231/ADR-p75NTR cells were higher than the expression level of MDA-MB-231/ADR-pcDNA. Moreover, its expression in MDA-MB-231/ADR-p75NTRsi1 cells and MDA-MB-231/ADR-p75NTRsi2 cells were significantly lower than that of MDA-MB-231/ADR-siRNA. The expression level of p75NTR in MDA-MB-231/ADR-p75NTRsi1 was lower than the MDA-MB-231/ADR-p75NTRsi2, however, the difference was not significant. In this study, we selected p75NTRsi1 transfected cells.

To study the role of p75NTR in drug-resistant of MDA-MB-231 cells, the CCK-8 method was used to detect chemotherapeutic drugs resistance of MDA-MB-231/ADR-p75NTR, MDA-MB-231/ADR-pcDNA, MDA-MB-231/ADR-p75NTRsi1, MDA-MB-231/ADR-pSilencer, MDA-MB-231-p75NTR, MDA-MB-231-pcDNA, MDA-MB-231-p75NTRsi1, and MDA-MB-231-pSilencer. The results showed that MDA-MB-231/ADR was resistance to ADM, GEM and OXA. After transfection with pcDNA3.1-p75NTR, the drug resistance of MDA-MB-231/ADR-p75NTR and MDA-MB-231-p75NTR cells were enhanced (P<0.05). The results indicated that overexpression of p75NTR can enhance the drug resistance of MDA-MB-231/ADR cells to ADM, GEM and OX. In this wise, inhibition of p75NTR

Table 6. The drug resistance effect of p75NTR to MDA-MB-231/ADR cells was detected by CCK-8 method (x±s, n=3)

Group	IC50 (μg/ml)			*P* value
	ADM	GEM	OXA	
MDA-MB-231/ADR-p75NTR	183.37±4.45	25.43±2.15	65.72±1.85	<0.05
MDA-MB-231/ADR-pcDNA	124.28±4.94	12.13±2.02	38.45±1.56	
MDA-MB-231/ADR-pSilencer	124.48±4.35	13.24±1.86	40.15±1.73	
MDA-MB-231/ADR-p75NTRsi1	66.9±3.17	7.36±1.43	28.17±1.42	<0.05

expression can reduce MDA-MB-231/ADR cells resistance (**Table 6**).

Effects of p75NTR on the cell cycle of MDA-MB-231/ADR cells

Furthermore, we studied the impact of cell cycle on MDA-MB-231/ADR-p75NTR, MDA-MB-231/ADR-pcDNA, MDA-MB-231/ADR-p75NTRsi1, MDA-MB-231/ADR-pSilencer. The results showed that cell cycle of MDA-MB-231/ADR-p75NTR was arrested in G0/G1 phase and blocked in S phase. Moreover, MDA-MB-231/ADR-p75NTRsi1 cells blocked in G0/G1phase could promote their synthesis of DNA in S phase. The two groups were statistically significant (P<0.05).

Effects of p75NTR on drug resistance and cell cycle of MDA-MB-231/ADR cells

Moreover, we studied the impact of cell cycle on MDA-MB-231/ADR-p75NTR, MDA-MB-231/ADR-pcDNA, MDA-MB-231/ADR-p75NTRsi1, MDA-MB-231/ADR-pSilencer. The results showed that cell cycle of MDA-MB-231/ADR-p75NTR was arrested in G0/G1 phase and blocked in S phase. In MDA-MB-231/ADR-p75NTRsi1 cells, it blocked in G0/G1phase, and promoted its synthesis of DNA in S phase. The two groups were statistically significant (P< 0.05) (**Figure 3A**). We also found that p75NTR could inhibit apoptosis. After transfection of pcDNA3.1-p75NTR, the apoptosis rate of MDA-MB-231/ADR-p75NTR cells decreased, however, the apoptosis rate of MDA-MB-231/ADR-p75NTRsi1 cells increased after transfection of p75NTR-siRNA. The difference was statistically significant (P<0.05) (**Figure 3B**; **Table 7**).

Effects of p75NTR on the MDR related genes expression inMDA-MB-231/ADR cells

After transfection of p75NTR, P-gp, MRP1, BCRP and Bcl-2 were over expressed in MDA-MB-231/ADR-p75NTR cells (**Figure 3C**). However, MDA-MB-231/ADR-p75NTRsi1 cells promoted Bax expression and inhibited P-gp, MRP1, BCRP and Bcl-2 expression (**Figure 3D**). There was a significant difference between the two groups. After transfection with p75NTR, MDA-MB-231/ADR-p75NTR cells were over expressed ERK1, ERK2, JNK1 and p38α. However, after transfection with p75NTR-siRNA, expressions of ERK1, ERK2, JNK1 and p38α were inhibited. There was a significant difference between the two groups (P<0.05).

To analyze expression of the resistant gene in drug-resistant cells after transfection with p75NTR and p75NTR-siRNA, MDR1, MRP1, BCRP, Bcl-2 and Bax were detected by real time quantitative PCR. The results showed that the expressions of MDR1, MRP1, BCRP, Bcl-2 were upregulated and Bax was down-regulated after transfection with p75NTR. On the other hand, the expressions of MDR1, MRP1, BCRP, Bcl-2 were down-regulated and Bax was upregulated after transfection with p75NTR-siRNA. It suggested that overexpression of p75NTR can increase MDR1, MRP1, BCRP and Bcl-2 levels and decrease Bax level in MDA-MB-231/ADR cells (**Figure 3E** and **3F**). There was a significant difference between the two groups.

Discussion

The present study demonstrated that P75NTR, the molecular weight of 75 kD, was a transmembrane protein receptor of NTs located in 17q12-17q22 and contained 6 exons. NTs has two different types of receptors: high-affinity nerve growth factor tyrosine kinase receptor (Trk A, Trk B, Trk C) and low-affinity nerve growth factor receptor. In addition to the expression in the nervous system, p75NTR expressed in a variety of malignant tumors, such as prostate cancer, colorectal cancer, pancreatic cancer and leukemia cells. The present study showed that p75NTR expressed in basal-like breast carcinoma, which could inhibit the apoptosis of tumor cells, mediated breast cancer stem cell self-renewal and promoted tumor proliferation by regulation of gene expression.

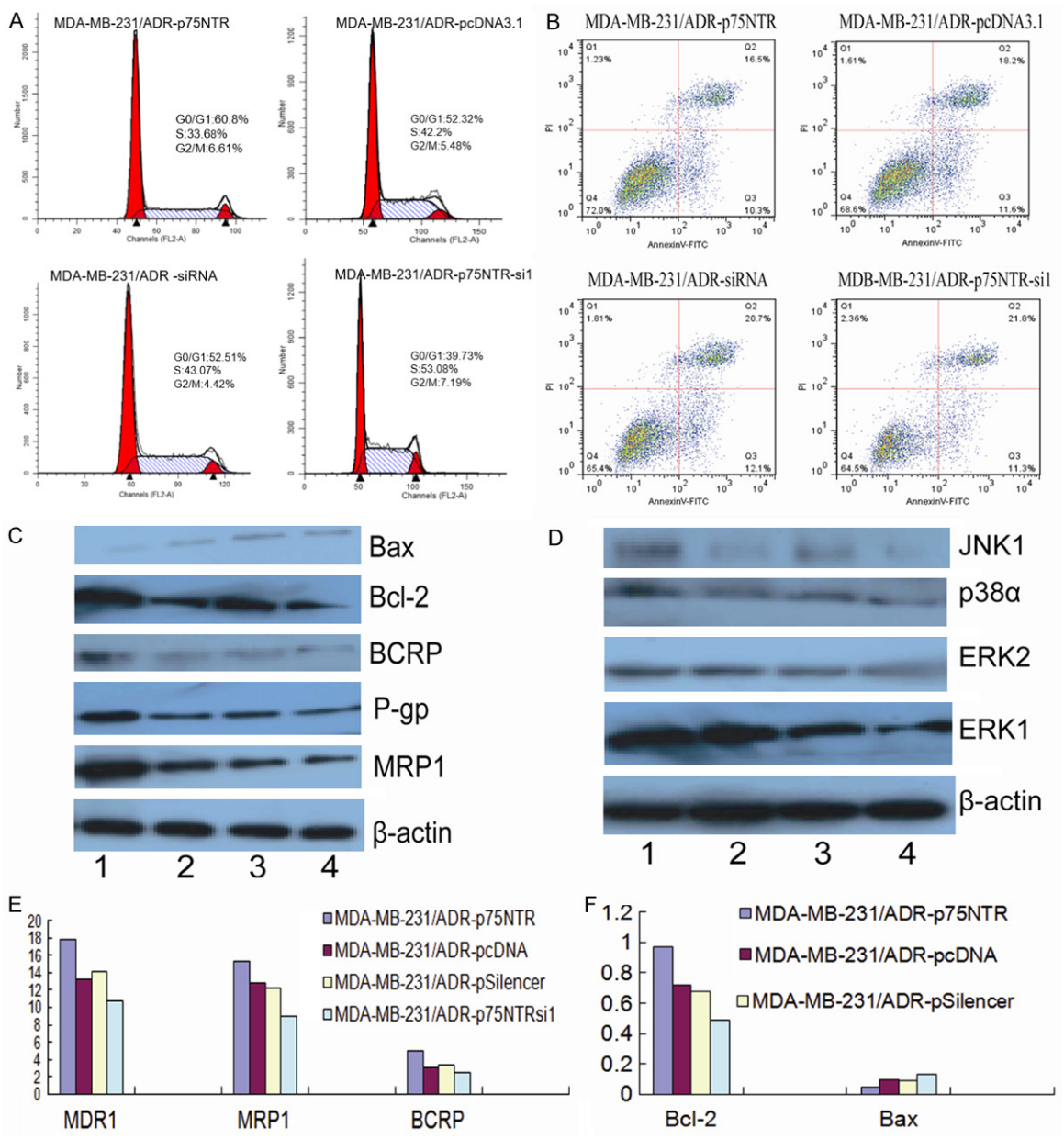

Figure 3. The effects of p75NTR on the MDR related genes expression in MDA-MB-231/ADR cells. A. FCM analyzed the cell cycle; B. FCM analyzed cell apoptosis; C. Western blot analyzed the expression of Bax, Bcl-2, BCRP, P-gp and MRP1. 1: MDA-MB-231/ADR-p75NTR; 2: MDA-MB-231/ADR-pcDNA; 3: MDA-MB-231/ADR-SiRNA; 4: MDA-MB-231/ADR-p75NTRsi1; D. Western blot analyzed the expression of JNK1, p38α, ERK1 and ERK2. 1: MDA-MB-231/ADR-p75NTR; 2: MDA-MB-231/ADR-pcDNA; 3: MDA-MB-231/ADR-SiRNA; 4: MDA-MB-231/ADR-p75NTRsi1. E and F. Real-time PCR analyzed mRNA of MDR1, MRP1, BCRP, Bcl-2 and Bax in MDA-MB-231/ADR-p75NTR, MDA-MB-231/ADR-pcDNA MDA-MB-231/ADR-pSliencer and MDA-MB-231/ADR-p75NTRsi1.

Tsang showed that the expression of p75NTR was not associated with age, tumor size and lymph node metastasis in 602 patients with breast cancer, which was closely related to histological grade and prognosis [16]. Our results also demonstrated that there was no significant difference between the expression of p75NTR and menopause and tumor size. Other studies found that the more lymph node metastasis, the higher expression of p75NTR, suggesting that low expression of p75NTR was in the early stage of breast cancer. Typically, p75NTR expressed in a variety of malignant tumors, such as oral squamous cell carcinoma,

Table 7. The effects of p75NTR on the cell cycle and apoptosis distribution (x±s, n=3%)

Group	Cell cycle			Apoptosis
	G0/G1	S	G2/M	
MDA-MB-231/ADR-p75NTR	61.12±1.89*	32.76±0.23*	6.12±0.48*	16.83±1.29*
MDA-MB-231/ADR-pcDNA	51.57±1.28	42.68±1.37	5.75±0.88	18.59±1.53
MDA-MB-231/ADR-pSilencer	52.43±1.74	43.19±1.16	4.38±0.73	20.57±1.65
MDA-MB-231/ADR-p75NTRsi1	39.26±1.31*	52.88±1.74*	7.86±1.02*	21.49±1.41*

*P<0.05.

esophageal cancer, gastric cancer, prostate cancer. The expression rate was from 4.2% to 64%.

Moreover, P75NTR is a member of the tumor necrosis factor receptor superfamily. Recent studies demonstrated that p75NTR can promote cells growth of esophageal cancer and breast cancer to resist the anti-tumor therapy [17]. More importantly, p75NTR can promote the brain metastasis of melanoma cells serving as a survival receptor. With further study of p75NTR, it has been confirmed that a variety of drugs can regulate the tumor cells resistance by acting on p75NTR [18, 19]. Our study showed that the expression of p75NTR in the drug resistant cell, such as MDA-MB-231/ADR and MCF-7/5-FU, was higher than their parental cell lines, suggesting that p75NTR might be involved in the drug resistance of breast cancer cell lines.

Tumorigenesis and its development are usually characterized by abnormal cell cycle, misregulation of molecular mechanism of cell cycle and unlimited proliferation potential. Typically, G1/S and G2/M phase is the key checkpoint of the cell cycle. Vrbeke confirmed that overexpression of p75NTR could upregulate p21, which was a cell cycle inhibitor of cyclin/CDK and arrested in G0/G1 phase, decreased sensitivity to chemotherapy and increased cell resistance. We confirmed that p75NTR had a major impact on cell cycle. After overexpression of p75NTR, cells were arrested in G0/G1 phase. Our study proved that regulation of p75NTR expression was related with cell cycle and played a critical role in the pathogenesis of the disease.

Drug resistance is the main reason of chemotherapy failure, recurrence and metastasis, which seriously affects the survival and prognosis of patients with breast cancer. Multiple drug resistance (MDR) describes a phenomenon, whereby resistance to one drug is accompanied by resistance to drugs whose structures and mechanisms of action may be completely different [20]. ABC family is main drug transporter, and P-gp protein is recognized as the most classic mechanism of MDR. Typically, the overexpression of P-gp is biology foundation of MDR [21], which cannot only remove the toxic substances to protect cells from them, but it can also be involved in cell proliferation, differentiation and MDR occurrence. Long-term stimulation of anti-tumor drugs can induce MDR1 gene amplification and secrete P-gp protein. By exception, P-gp, MRP1/ABCC1 can deliver drugs to intracellular vesicles and function in the nucleus making tumor cells to be drug resistance. Typically, BCRP is a semi transporter, and the present study shows that breast cancer resistance may be associated with high expression of BCRP, however, the mechanism requires further study. It has been reported that BCRP can induce resistance to adriamycin in breast cancer through C-MET/PI3-K pathway [22]. Our work showed that P-gp, MRP1 and BCRP were a high expression in MDA-MB-231/ADR-p75NTR cells, but not in MDA-MB-231/ADR-p75NTR-si1 cells, suggesting that p75NTR might involve with these transporters distribution.

The resistance of tumor is associated with a variety of mechanisms, such as increased drug efflux, changed drug target, blocked apoptosis pathway, and DNA damage repair enhancement, suggesting that MDR is closely related to cell metabolism and signal transduction. MAPK signaling pathway is an important pathway for regulating cell proliferation, differentiation, apoptosis, adhesion and migration. Typically, chemotherapeutic drugs can activate ERK, p38 and other signaling molecules in MAPK signaling pathway [23]. The activation of MAPK pathway is associated with the drug resistance of ovarian cancer, oral squamous cell carcinoma, gastric cancer and leukemia [24]. Kommaddi

found that nerve growth factor could activate Trk A and p75NTR, which could lead to ADAM17 phosphorylation and p75NTR hydrolysis, thereby activating ERK and AKT signaling pathways [25]. Our study also proved that p75NTR could promote the expression of MAPK signaling protein in breast cancer cells. P75NTR is a cell membrane glycoprotein receptor, which can bind and phosphorylate ligand on target cells to activate multiple signaling pathways, including PI3K/AKT, JNK/AP-1, NF-κB, p38, MAPK and so on These pathways can phosphorylate P-gp protein [26-28]. MAPK inhibitors can down-regulate MDR of drug resistant cells, suggesting that drug resistance of tumor cells is related to the activation of MAPK kinase system [29, 30]. The ERKs pathway most promoted cell proliferation and survival, p38 and JNKs induced cell apoptosis [31, 32]. We observed that expression levels of ERK, JNK and p38α3 were increased in MDA-MB-231/ADR-p75NTR cells, suggesting that p75NTR played a critical role in resistance mechanism. In our study, p75NTR can improve drug-resistant cells survival, which implies that p75NTR can increase the expression of ERK1. Moreover, a compensatory increase of JNK and p38 can serve as a negative feedback mechanism, which indicates that there may be a dynamic balance in the MAPK pathways and the threshold can be changed after sustained stimulation [33, 34]. Based on upregulation of P-gp and down-regulation of BCRP after MAPK/ERK activation, it was suggested that MAPK pathway played an important role in resistance of different membrane transport channel proteins.

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Disclosure of conflict of interest

None.

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