

Original Article

TRAF2 activates the Akt signaling pathway via TRAF4-mediated ubiquitination of Akt and promotes migration and invasion of breast cancer cells

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Abstract: TRAF2, a classic member of the TRAF family, is highly expressed in a variety of tumors. In this study, we demonstrated that high TRAF2 levels correlated with the activity of the Akt signaling pathway and was closely related to pathological classification of breast cancers ($P < 0.05$). TRAF2 regulates the biology of breast cancer cells via the Akt signaling pathway. In MCF-7 and BT-549 cell lines, regulation of TRAF2 expression significantly affected the extent of Akt ubiquitination but did not affect the total Akt level. In addition, Akt phosphorylation changed with TRAF2 levels. Reports show that TRAF4 binds to Akt. Our previous studies showed that TRAF2 regulated TRAF4 localization. Here, we observed that TRAF4 knockdown in the presence of TRAF2 overexpression significantly inhibited phosphorylation and ubiquitination of Akt. Thus, TRAF2 affects the ubiquitination and activation of Akt via regulation of TRAF4 levels. In summary, we observed that TRAF2 affects the ubiquitination and activation of Akt through TRAF4-E3 ubiquitin ligase activity.

Keywords: TRAF2, Akt, TRAF4, breast cancer, ubiquitination

Introduction

Breast cancer is one of the most prevalent malignant tumors of this century. Despite rapid advances in breast cancer therapy in recent years, the high incidence of this cancer and the effect of mastectomy on the quality of human life is still a matter of concern [1]. An understanding of the molecular mechanisms of breast cancer would enable early diagnosis using breast cancer markers and development of targeted drugs, which would be critical for surmounting this challenge. Tumor necrosis factor (TNF) receptor associated factors (TRAFs) are important cytoplasmic adapter proteins that constitute a family of seven members. TRAFs form dimeric or trimeric polyprotein transduction complexes with the cytoplasmic portions of other TRAF family members, and are major signal transducers of the TNF receptor (TNFR) and interleukin-1/Toll-like receptor (TIR) family that mediate cell survival, proliferation, differentiation, and apoptosis via regulation of the NF- κ B, JNK, and other signal

transduction pathways. TRAFs play an important function in immune regulation reaction, inflammation, embryonic development, and bone metabolism [2-5]. TRAFs contain a C-terminal TRAF domain [6] and N-terminal ring finger and zinc finger domains. The ring finger domain is required for the E3 ubiquitin ligase activity of TRAF2, TRAF4, and TRAF6 [7].

TRAF2 is the most widely represented member of the TRAF family and can interact with other members of the family. TRAF2 was originally identified in CT6, a mouse cytotoxic T cell line [6]. It is mainly localized in the cytoplasm and is highly expressed in various cancers such as lung [8] and breast cancer [9]. Previously, we reported that TRAF2 activated the p70-S6 kinase signaling pathway [10]. Up-regulation of TRAF2 in the breast cancer cell line MCF-7 activated NF- κ B nuclear translocation and inhibited apoptosis of breast cancer cells [11].

Akt, also known as protein kinase B (PKB), is an evolutionarily conserved serine/threonine pro-

tein kinase, which acts at the intersection of the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signal transduction pathways [12-14]. The Akt signaling pathway plays a critical role in tumor cell biology and treatment of cancers as it transmits extracellular signals inside cells, regulating cellular survival, proliferation, migration, and invasion. Abnormal activation of Akt is closely related to the occurrence and development of tumors [13]. Akt levels are as high as 70% in human breast cancer [15, 16]. A key step in Akt activation is the growth factor-mediated membrane recruitment of Akt. In addition, PI3K activation is essential for Akt signaling, however recent studies have shown that Lys63-linked polyubiquitination can also promote membrane recruitment and activation of Akt, which plays an important role in Akt signaling [17, 18].

TRAF4, a TRAF family member that harbors a ring-finger domain with E3 ubiquitin ligase activity, can mediate Lys63-linked polyubiquitination of Akt and activate the Akt signaling pathway [17]. Similarly, the ring-finger domain of TRAF6 plays a key role in ubiquitination, membrane recruitment, and phosphorylation of Akt [18, 19]. Our preliminary study showed that TRAF2 binds to and regulates the intracellular distribution of TRAF4, thereby activating p70-S6 kinase and NF- κ B signaling pathways [10, 11]. Therefore, we speculated whether TRAF2 affected the activation of the Akt signaling pathway via TRAF4.

In this study, we demonstrate that high TRAF2 levels in breast cancer are positively correlated with activation of the Akt signaling pathway, and TRAF2 regulates the biology of breast cancer cells via Akt signaling. The TRAF2-regulated and TRAF4-mediated ubiquitination of Akt activates the Akt signaling pathway.

Materials and methods

Patients and specimens

Surgically resected, paraffin-embedded breast tissue specimens from 2009 to 2012 were obtained from the Department of Pathology, the First Affiliated Hospital of China Medical University. The use of specimens was approved by the Ethics Committee of the China Medical University First Affiliated Hospital and signed informed consent was provided by the patients.

The patients had not undergone radiotherapy or chemotherapy prior to the surgery, and received standard treatments after the surgery. Data was evaluated based on the 2012 World Health Organization's (WHO) classification of breast tumors.

Cell culture

The normal mammary epithelial cell line MCF-10A was cultured in Dulbecco's modified Eagle medium (DMEM)/F12 (Gibco, USA) containing 5% horse serum, 10 μ g/ml insulin and 20 ng/ml epidermal growth factor (EGF). The breast cancer cell lines MDA-MB-231 and MDA-MB-468 were cultured in L15 medium (Gibco, USA) containing 10% FBS, MCF-7 was cultured in DMEM (Gibco, USA) plus 10% FBS, and BT-549 was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, USA) with 10% FBS. Except for MDA-MB-231, which does not require CO₂ for growth, all other cell lines were cultured at 37°C in the presence of 5% CO₂.

Immunohistochemistry

Paraffin-embedded breast tissue specimens were excised into 4- μ m thick sections, and placed on slides. Then, they were subjected to dewaxing by xylene and treatment with a gradient of alcohol to benzene, followed by hydration. The antigen was repaired with citrate buffer at high temperature and pressure and washed with phosphate buffered saline (PBS) three times for 5 min each. The slides were treated with a peroxidase inhibitor, placed in a wet box, and incubated at 37°C for 15 min. This was followed by washing three times with PBS for 5 min, addition of 10% non-immune animal serum, and incubation in a 37°C incubator for 60 min in a wet box. Primary antibodies (50-100 μ l) (1:100 dilution of TRAF2 antibody, 1:200 dilution ratio of Akt antibody in PBS) were added to each slide, and PBS was used instead of antibodies as a control. The slides were placed in a wet box and placed in a 4°C refrigerator overnight. The next day, the slides were washed three times with PBS for 5 min, followed by addition of biotin-labeled secondary antibody, and incubated at 37°C for 30 min in a wet box. Then, the slides were washed three times with PBS for 5 min, followed by addition of streptavidin-avidin peroxidase solution, and incubated at 37°C for 30 min in a wet

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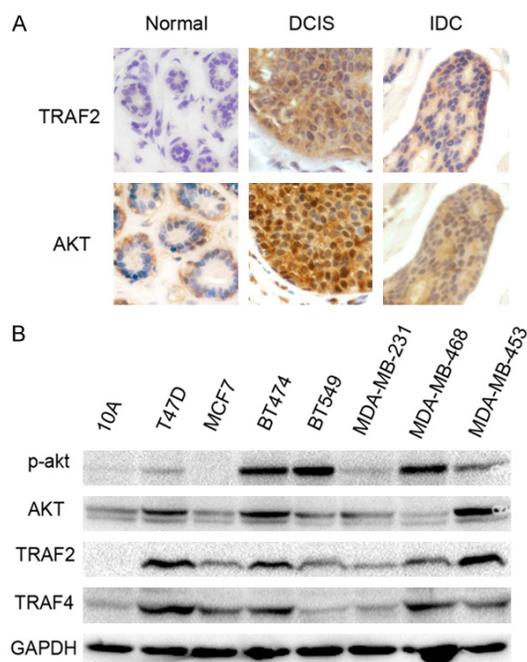


Figure 1. TRAF2 and Akt are highly expressed in breast cancer tissues and cell lines. A. Immunohistochemistry was used to analyze the expression of TRAF2 and Akt in normal breast and breast cancer tissues. B. Western blot results showed that the expression of Akt and TRAF2 in breast cancer cell lines was significantly higher than that in normal breast epithelial cells.

Table 1. Expression of TRAF2 in different types of human breast cancers

Histological grade	TRAF2 expression		
	n	+	PR %
Normal	17	5	29.4%
DCIS	37	23	62.2%
IDC	77	58	75.3%

box. Next, the slides were washed three times with PBS for 5 min and stained with 3,3'-diaminobenzidine (DAB) and hematoxylin (for staining nuclei). The slides were then treated with hydrochloric acid and alcohol for rapid differentiation and washed with tap water for "blueing". Finally, the slides were treated with an ethanol gradient for dehydration, dewaxed using xylene for transparency, and sealed with a neutral resin. The final staining score of all samples depended on the percentage of positive cells, which included the percentage of tumor cells, and the staining intensity. The samples were categorized into three grades based on staining percentage: grade 1 (1-50%), grade 2 (51-

75%), and grade 3 (> 75%); they were divided into three grades according to the staining intensity: 0 (no color), 1 (light yellow), 2 (brownish yellow), and 3 (brown). Final score in the range of 0-9 was obtained by multiplying the staining intensities and percentages. Scores ≥ 3 were considered as cases of positive expression.

Plasmids and transfection

hTRAF2pLPCX-HA-FLAG/P874 (full-length plasmid with TRAF2) was purchased from Addgene Corporation (USA). siRNAs against TRAF4 and Akt, and scrambled small interfering RNA (siRNA) sequences were designed by Guangzhou RiboBio. The Attractene transfection reagent and HiPerFect transfection reagents were purchased from Qiagen (Germany) and used according to the manufacturer's instructions for transient transfection and interference experiments. The empty plasmid was used as the control.

Western blotting

MCF-7 and BT-549 cells were collected and the supernatant was extracted post-lysis after transfection and plasmid interference for 48 h. The Bradford method was used to estimate protein concentration, and equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred (60 V, 2 h) to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 2 h at room temperature with 5% non-fat milk or 5% bovine serum albumin (BSA), and incubated overnight with primary antibodies at 4°C. Antibodies against Akt, phospho-Akt (p-Akt) (1:1,000 for each antibody; Cell Signaling Technology), TRAF4, TRAF2, β -actin (1:100 for each antibody; Santa Cruz Biotechnology), TRAF2 (1:200; BD Biosciences, USA), GAPDH (1:2,000; Beijing Zhongshan Jinqiao Biotechnology, China), HA (1:500; Beijing Quanshijin Biological Technology, China), and tubulin (1:300; Beyotime Biotechnology) were used as primary antibodies. Peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:1,000; Beijing Zhongshan Jinqiao Biotechnology, China) were used as secondary antibodies for 2 h at room temperature, followed by enhanced chemiluminescent (ECL) color rendering, image acquisition, and gray value measurement.

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Table 2. Relationship between TRAF2 expression and clinicopathological factors in human breast tissues

Clinico-pathological factors	n	TRAF2 expression		p-value
		Negative	Positive	
Histological grade				
Normal	17	12	5	0.001**
DCIS	37	14	23	
IDC	77	19	58	
Age				
≤ 50 years	70	26	44	0.513
> 50 years	60	19	41	
Stage				
I+II	94	38	56	0.771
III	19	7	12	
Tumor size				
≤ 2.0 cm	13	6	7	0.420
> 2.0 cm	112	39	73	
LNM				
-	94	33	61	0.772
+	37	12	25	
ER				
-	37	14	23	0.689
+	88	30	58	
PR				
-	29	9	20	0.592
+	96	35	61	
HER2				
-	18	8	10	0.375
+	107	36	71	
BRCA1				
-	13	6	7	0.159
+	59	17	42	
BRCA2				
-	24	9	15	0.352
+	38	10	28	

**IDC vs normal breast tissues/DCIS vs normal breast tissues/IDC vs DCIS.

Table 3. Correlation between TRAF2 and Akt expression in human breast cancers

	TRAF2 expression		n	p-value
	Negative	Positive		
Akt Negative	24	5	29	0.001
Positive	21	81	102	

Wound healing assay

MCF-7 cells were transfected with TRAF2 and/or si-Akt and incubated for 48 h in 6-well plates where vertical lines (wound) were pre-drawn

using a 200 µl pipette tip. Cell migration into the wound was observed at 0 h and 12 h. Image J software was used to analyze migration ability after imaging.

Matrigel invasion

MCF-7 cells were transfected with TRAF2 and/or si-Akt for 48 h and resuspended uniformly in the upper chamber (containing Matrigel) of a Transwell chamber. Six hundred microliters complete medium was added into the lower chamber, and the cells were incubated for 24 h. Cotton swabs were used to wipe the upper chamber. The cells were fixed with 4% paraformaldehyde and stained with hematoxylin. After microscopic observation, five high magnification images were randomly selected and counted, followed by statistical analysis.

Ubiquitination assay

The plasmids expressing TRAF2, TRAF4, and HA-tagged ubiquitin were transfected in the MCF-7 cells. After 24 h, 20 µM proteasome inhibitor MG-132 was added, and the cells were harvested after another 4 h. Ubiquitination level was detected after immunoprecipitation using Western blotting.

Immunoprecipitation

One microgram primary antibody or mouse/rabbit IgG were added to a certain amount of whole cell lysates and incubated overnight at 4°C. Protein A and G beads were added to the lysate the following day and incubated at 4°C for 4 h. The immunoprecipitation complex was eluted and subjected to Western blot analysis.

Detection of apoptosis by flow cytometry

MCF-7 cells were harvested after 48 h of transfection with TRAF2 and/or si-Akt. The cells were stained and analyzed by flow cytometry according to the manufacturer's protocol for the Annexin V-FITC apoptosis detection kit (KeyGen Biotech. Co. Ltd., China).

Statistical analysis

The SPSS 22.0 statistical software was used to analyze experimental data. Fisher's exact test and Pearson's Chi-square test were used to

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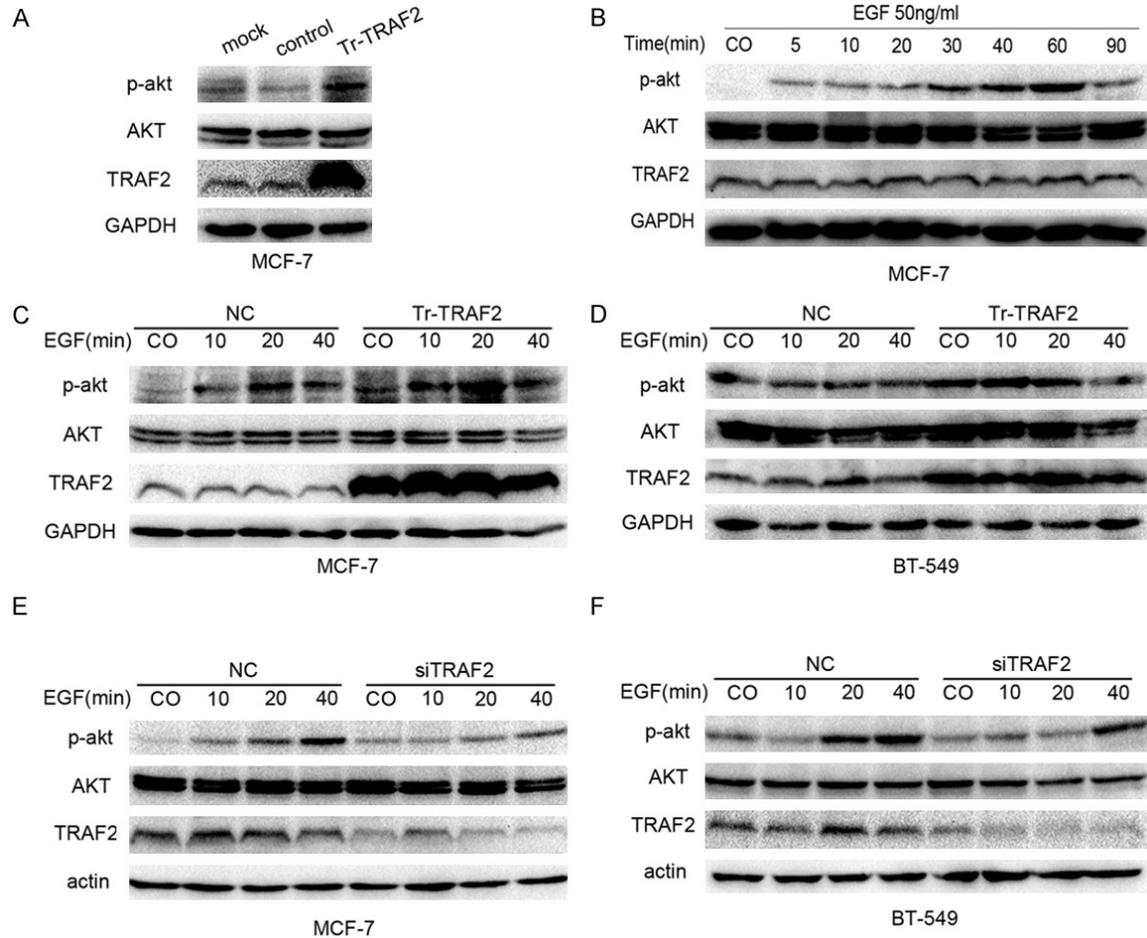


Figure 2. TRAF2 activates the Akt signaling pathway. A. Western blot results showed that up-regulation of TRAF2 significantly increased Akt phosphorylation levels in MCF-7 cells. B. EGF induced phosphorylation of Akt. Western blot showed that when MCF-7 cells were treated with EGF (50 ng/ml), the phosphorylation level of Akt increased gradually at different time points. C, D. Western blot showed that up-regulation of TRAF2 in MCF-7 and BT-549 cells increased EGF-induced Akt phosphorylation level at different time points. E, F. Western blot showed that down-regulation of TRAF2 in MCF-7 and BT-549 cells decreased Akt phosphorylation level induced by EGF (50 ng/ml) at different time points.

analyze the correlation between TRAF2 and Akt expression and the relationship with clinicopathological factors in human breast tissues. The Student's t-test was used to analyze statistical significance. All experiments were performed at least three times and data are expressed as mean \pm standard deviation (SD). $P < 0.05$ was considered statistically significant.

Results

Correlation between TRAF2 and Akt expression in breast cancer

TRAF2 and Akt levels in normal breast tissues and breast cancer tissues were detected using

immunohistochemistry. Results showed that TRAF2 was negligibly expressed in normal breast tissues, whereas its expression was high in breast carcinoma (62.2%) and invasive ductal carcinoma (75.3%) (Figure 1A, Table 1), which were significantly higher than that in normal breast tissue ($P < 0.05$) (Table 1). The correlation between TRAF2 expression and clinicopathological factors is summarized in Table 2. There was no significant correlation between TRAF2 levels and patient age, tumor size, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) ($P > 0.05$), whereas there was significant correlation with the pathological type of the tumor ($P < 0.05$). Next, we analyzed wheth-

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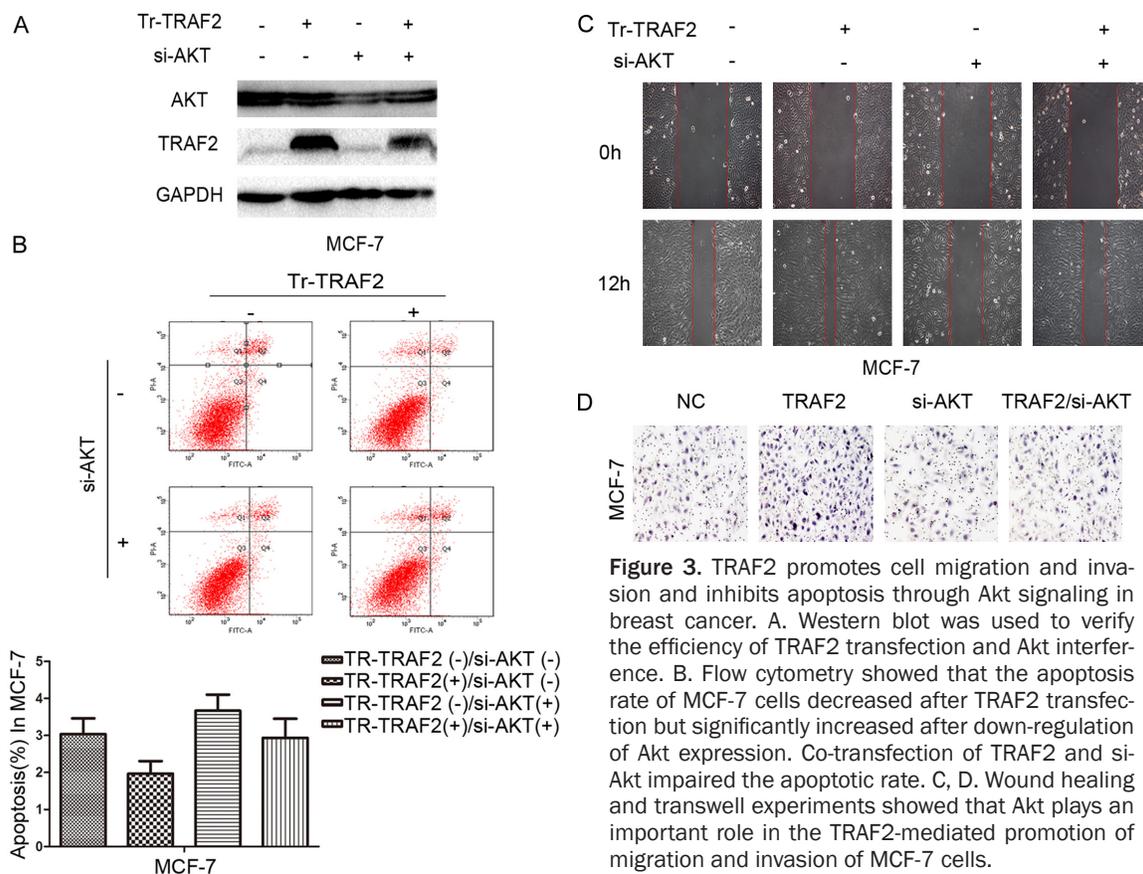


Figure 3. TRAF2 promotes cell migration and invasion and inhibits apoptosis through Akt signaling in breast cancer. **A.** Western blot was used to verify the efficiency of TRAF2 transfection and Akt interference. **B.** Flow cytometry showed that the apoptosis rate of MCF-7 cells decreased after TRAF2 transfection but significantly increased after down-regulation of Akt expression. Co-transfection of TRAF2 and si-Akt impaired the apoptotic rate. **C, D.** Wound healing and transwell experiments showed that Akt plays an important role in the TRAF2-mediated promotion of migration and invasion of MCF-7 cells.

er TRAF2 positivity was associated with Akt expression. Results showed that tumors with high TRAF2 expression had higher Akt-positive rate than the TRAF2-low group (**Table 3**). Therefore, we conclude that TRAF2 expression correlates positively with Akt expression.

Western blot was used to detect Akt and TRAF2 in the normal breast cell line MCF-10A and breast cancer cell lines T47D, MCF-7, BT474, BT549, MDA-MB-231, MDA-MB-468, and MDA-MB-453 (**Figure 1B**). Akt and TRAF2 levels were both significantly higher in breast cancer cell lines than in the normal breast cell line. This suggests that TRAF2 may be involved in the activation of the Akt signaling pathway in breast cancer.

TRAF2 activates the Akt signaling pathway

Western blot showed that the total Akt level did not change significantly, but the level of p-Akt increased significantly after overexpressing TRAF2 in MCF-7 (**Figure 2A**). Since p-Akt levels were low, EGF which is the activation effector of the Akt signaling pathway, was introduced to

further detect TRAF2-mediated p-Akt regulation in subsequent experiments (**Figure 2B**). First, we examined p-Akt levels at different time points in the presence of EGF, and selected the appropriate time points for subsequent experiments. In the EGF-induced MCF-7 and BT-549 cell lines, the Akt phosphorylation level increased significantly after TRAF2 up-regulation (**Figure 2C, 2D**) and decreased with TRAF2 down-regulation (**Figure 2E, 2F**). This suggested to us that TRAF2 promotes activity of the Akt signaling pathway.

TRAF2 promotes migration and invasion and inhibits apoptosis of breast cancer cells through the Akt signaling pathway

TRAF2 plays an important role in the NF- κ B and other signaling pathways owing to their common involvement in the regulation of apoptosis. Therefore, we regulated the expression of TRAF2 and Akt in the breast cancer cell line MCF-7 and determined apoptotic rate and migration ability. Western blot was used to verify the efficiency of transfection (**Figure 3A**). Flow cytometry showed that TRAF2 transfection

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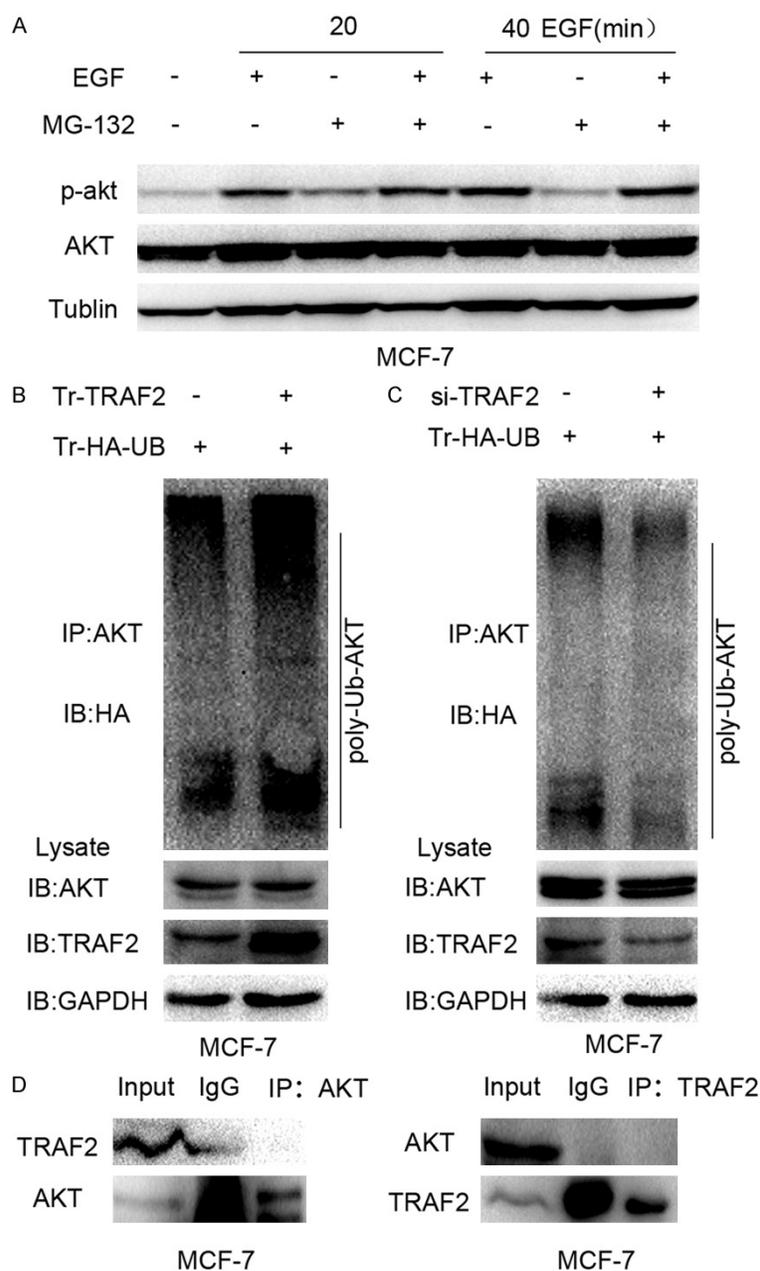


Figure 4. TRAF2 enhances Akt ubiquitination levels. **A.** MCF-7 cells were pre-treated with the proteasome inhibitor MG-132 for 4 h and treated with EGF (50 ng/ml) for 20 and 40 min. Pre-treatment with MG-132 significantly increased the level of EGF-induced Akt phosphorylation as observed by Western blotting. **B.** TRAF2 and HA-Ub plasmids were co-transfected in MCF-7 cells. After 24 h, the proteasome inhibitor MG-132 was added and incubated for 4 h. The cells were collected for immunoprecipitation and Western blot. **C.** si-TRAF2 and HA-Ub plasmids were co-transfected in MCF-7 cells. After 24 h, the proteasome inhibitor MG-132 was added and incubated for 4 h. The cells were collected for immunoprecipitation and Western blot. **D.** Immunoprecipitation showing that TRAF2 and Akt do not interact physically in MCF-7.

tion in MCF-7 inhibited apoptosis, whereas transfection of si-Akt enhanced the apoptotic

rate. Co-transfection with TRAF2 and si-Akt significantly impaired the apoptotic rate triggered by TRAF2 overexpression ($P < 0.05$) (**Figure 3B**). Wound healing and Matrigel invasion assays showed that TRAF2 transfection promoted the invasiveness of breast cancer cells, whereas si-Akt transfection inhibited the invasive ability. Co-transfection with TRAF2 and si-Akt weakened the invasiveness of breast cancer cells (**Figure 3C, 3D**). This suggested that TRAF2 regulates the biology of breast cancer cells through Akt.

TRAF2 up-regulates the level of Akt ubiquitination in breast cancer cells

The ring-finger domain of TRAF2 possesses characteristic E3 ubiquitin ligase activity, which promotes the activation of the NF- κ B signaling pathway. Reports show that Akt can also be polyubiquitinated at Lys63, which promotes Akt membrane translocation and activation. Therefore, we further examined whether TRAF2 affected the extent of Akt ubiquitination via its E3 ubiquitin ligase domain. First, we determined whether Akt phosphorylation was maintained after treatment with the proteasome inhibitor, MG-132. The amplification effect of EGF on Akt activation and the synergistic enhancement of the EGF-induced increase in Akt phosphorylation were not affected in the MG-132-treated group (**Figure 4A**).

Next, we assessed whether TRAF2 regulates ubiquitination of Akt. We observed that the extent of Akt ubiquitination increased in MCF-7 overexpressing TRAF2 (**Figure 4B**),

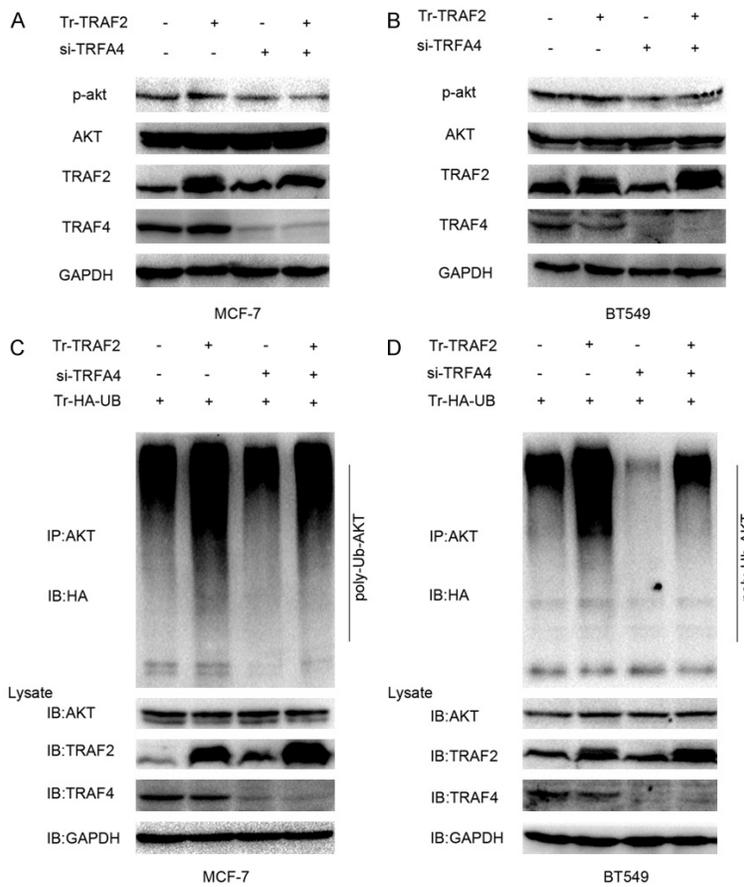


Figure 5. TRAF2 increases Akt ubiquitination levels and affects TRAF4-mediated activation. A, B. TRAF2 increased phosphorylation of Akt through TRAF4. Knocking down TRAF4 significantly impaired TRAF2-induced p-Akt up-regulation. C, D. TRAF2 promotes the ubiquitination of Akt through TRAF4. Knocking down TRAF4 significantly impaired TRAF2-induced ubiquitination of Akt.

whereas Akt ubiquitination decreased with down-regulation of TRAF2 (Figure 4C). We performed immunoprecipitation experiments to detect physical interactions between TRAF2 and Akt, however the results showed that TRAF2 was not able to bind to Akt in MCF-7 (Figure 4D). Therefore, we speculate that TRAF2 indirectly regulates Akt.

TRAF2 regulates the Akt signaling pathway through TRAF4

Our previous studies demonstrated that TRAF2 binds to TRAF4 and affects its nuclear-cytoplasmic distribution [10, 11]. Other studies showed that TRAF4 regulates the Akt signaling pathway via ubiquitination [17]. Therefore, we speculated that TRAF2 may regulate the Akt signaling pathway via TRAF4. p-Akt levels

increased in TRAF2-transfectants of the breast cancer cell lines MCF-7 and BT-549. However, p-Akt level decreased after transfection with si-TRAF4. Simultaneous transfection with TRAF2 and si-TRAF4 significantly reduced p-Akt level (Figure 5A, 5B).

Next, we examined whether TRAF4 was involved in TRAF2-mediated activation of Akt ubiquitination. We transfected the TRAF2 plasmid and/or si-TRAF4 in MCF-7 and BT-549 cells. Results showed that TRAF2 up-regulated and si-TRAF4 down-regulated Akt ubiquitination. Co-transfection of TRAF2 and si-TRAF4 displayed that TRAF4 knock down strongly inhibited the increase in Akt ubiquitination induced by TRAF2 up-regulation (Figure 5C, 5D). Therefore, we speculate that TRAF2 affects ubiquitination of Akt through TRAF4.

Discussion

TRAF2 is the most widely expressed TRAF family member that participates in a variety of protein modifying processes such as phosphorylation, ubiquitination, and self-oligomerization, and plays a key role in NF-κB and JNK signaling pathways [20-22]. First, we used immunohistochemistry to determine TRAF2 levels in breast cancer specimens and observed that TRAF2 was highly expressed in breast cancer samples and was closely related to the pathological classification of breast cancers.

The Akt pathway is a crucial signaling pathway that operates at the intersection of several key signaling cascades and is involved in tumor development and cancer progression [13]. Reports show that TRAF2 can promote the development of cardiac hypertrophy by activating the Akt/GSK-3β signaling pathway [23]. Therefore, we further investigated the correlation between TRAF2 and Akt signaling in this

study. Immunohistochemistry showed that TRAF2 and Akt levels correlated positively in breast cancer tissues. Western blot showed that the levels of TRAF2 and Akt in breast cancer cell lines were significantly higher than in normal breast cell line. Further analysis of the biological function of TRAF2 and the Akt signaling pathway in breast cancer cells showed that TRAF2 inhibited apoptosis and promoted cell migration and invasion through Akt signaling.

Next, we examined the effect of TRAF2 on the Akt signaling pathway. TRAF2 significantly affected the level of Akt ubiquitination in breast cancer cells, but did not affect the amount of total Akt. In addition, the phosphorylation level of Akt changed with TRAF2 levels, indicating that TRAF2 activated the Akt signaling pathway via Akt ubiquitination. However, immunoprecipitation showed that TRAF2 did not physically bind to Akt in the breast cancer cell line, MCF-7. Hence, we hypothesized that TRAF2 indirectly regulated Akt.

Studies show that TRAF4 binds to Akt and regulates activation of the Akt signaling pathway via its E3 ubiquitin ligase activity [17]. Our preliminary results demonstrate that TRAF2 can affect the biological behavior of breast cancer cells by regulating the localization of TRAF4 [10, 11]. Therefore, we speculate that TRAF2 can affect the activation of the Akt signaling pathway through TRAF4. Our results show that TRAF4 down-regulation in the presence of TRAF2 overexpression significantly inhibits Akt phosphorylation and ubiquitination. Thus, TRAF4 is involved in TRAF2-mediated activation of Akt signaling.

In summary, we observed that TRAF2 was overexpressed in breast cancers and was closely related to the pathological classification of breast cancers. TRAF2 inhibited apoptosis and promoted cell migration and invasion via the Akt signaling pathway. TRAF2 regulated the ubiquitination and activation level of Akt through TRAF4 E3 ubiquitin ligase activity. Most of the current TRAF2-related studies have focused on the transduction of anti-apoptotic signaling pathways, particularly the NF- κ B signaling pathway. Reports suggest that the N-terminal ring finger domain of TRAF2 also possesses E3 ubiquitin ligase activity [24, 25], which could regulate NF- κ B signaling and affect the biological behavior of cells. Therefore,

whether TRAF2 regulates Akt ubiquitination or activates the Akt pathway through alternative pathways requires further investigation. Our findings broaden the understanding of the role of TRAF2 in breast cancer progression and provide new prospects for future cancer research and therapy.

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Disclosure of conflict of interest

None.

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