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Down-regulation of tripartite motif protein 59 inhibits proliferation, migration and invasion in breast cancer cells

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ABSTRACT

Tripartite motif protein 59 (TRIM59), also known as mouse ring finger protein 1 (MRF1), is a surface molecule and belongs to the TRIM family. Recently, TRIM59 has been described in multiple cancers such as gastric, prostatic and lung cancer. However, there have been no reports on its functions in breast cancer. In this study, we elucidated the biological roles of TRIM59 in breast cancer. We found that TRIM59 was up-regulated in breast cancer cells. Down-regulation of TRIM59 inhibited breast cancer cell proliferation, migration and invasion *in vitro* as well as tumor growth *in vivo*. In addition, TRIM59 down-regulation reduced the protein expression level of *p*-Smad2 and thus inhibited the activity of transforming growth factor-β (TGF-β) signaling. Taken together, our study results provided new evidence showing that TRIM59 may be considered as a promising therapeutic target for breast cancer.

1. Introduction

Breast cancer, a kind of malignant tumor originated from the breast ductal epithelial cells, is a great threat to women [1]. This disease has a high frequency of occurrence and its incidence rate has been increasing every year [2,3]. According to the statistics, breast cancer is ranked the second leading cause of cancer-related death in women [4]. The high mortality in breast cancer patients is mainly due to recurrence and metastasis [5]. Their rate can reach as high as 70% [6]. All current therapeutic approaches including radiation, chemotherapy and hormone therapy are not effective enough to control them [7]. Thus, identifying new biomarkers and understanding the underlying mechanisms will be of great importance for improvement in the management of breast cancer patients.

The tripartite motif (TRIM) protein family is composed of more than 70 members [8]. All members are evolutionarily conserved proteins [9]. The most striking feature of this protein family is their unique structure: zinc-binding domains and coiled-coil regions [10,11]. Normally, these proteins function as scaffolds to assemble multi-protein complexes *via* multimerization [12]. Over the past decade, a great attention has been given to them because of their involvement in diverse cellular processes such as membrane repair, transcriptional regulation and cytoskeleton remodeling [13–15]. Currently, the protein family is again put in the center of focus because of their implication in cancer progression. For example, TRIM28 has been reported to decrease lung cancer cell proliferation through bridging HDAC1/E2F interactions; TRIM16 has been found exerting an inhibitory effect on the proliferation and migration of neuroblastoma cells [16,17]. These findings indicated the essential roles of the TRIM protein family in tumorigenesis. TRIM59, another member of the TRIM family, has been described in multiple cancers such as gastric, prostatic and lung cancer [18–20]. However, there have been no reports on its functions in breast cancer.

The aim of this study was to elucidate biological functions of TRIM59 in breast cancer. We found that TRIM59 was up-regulated in breast cancer cells. Down-regulation of TRIM59 inhibited breast cancer cell proliferation, migration and invasion *in vitro* as well as tumor growth *in vivo*. In addition, TRIM59 down-regulation inactivated the TGF-β signaling pathway by reducing the protein expression level of p-Smad2.

2. Materials and methods

2.1. Cell lines

Human breast cancer cell lines (MCF-7 and MDA-MB-231) and human mammary epithelial cell line (HMEC) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were maintained in RPMI-1640 medium containing 10% fetal

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bovine serum (FBS; Sigma, St. Louis, MO, USA), 100 U/mL penicillin and 100 μg/mL streptomycin, followed by incubation at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription for cDNA synthesis was performed with ThermoScript RT-PCR system (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. RT-PCR was conducted under the following conditions: 95 °C for 5 min, 40 cycles of 95 °C for 30 s, 60 °C for 45 s and 72 °C for 30 s. The following primers were used: TRIM59, 5′-ATGATCCAAGGCGATAAGGAAGC-3′ (forward) and 5′-ATCACAGAGAGCCGTTAGGAA-3′ (reverse); β-actin, 5′-AGAAAATCTGGCACCACACC-3′ (forward) and 5′-TAGCACAGCCTGGATAGCAA-3′ (reverse). β-actin was employed for normalization. The relative mRNA expression level was determined by the $2^{-\Delta\Delta CT}$ method [21].

2.3. Western blot

Cells were lysed in lysis buffer on ice. Proteins were electrophoresed with 12% SDS-PAGE and then transferred to nitrocellulose membranes (Invitrogen, Carlsbad, CA, USA). After blocking in 5% non-fat milk for 2 h at room temperature, the membranes were incubated with primary antibodies against TRIM59 (1:400), *p*-Smad2 (1:400), Smad2 (1:400) and β-actin (1:2000). Subsequently, the membranes were washed three times with TBST for 15 min and then incubated with corresponding secondary antibodies. All antibodies used in the study were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). β-actin was used as an internal control. An enhanced chemiluminescence kit (Thermo Fisher Scientific, Waltham, MA, USA) was employed for analysis of the protein levels and an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA) for visualization of fluorescent signals.

2.4. Cell transfection

TRIM59 siRNA (si-TRIM59: 5′-GCCUCUCUAUCUGUUUACCAAAGUU-3′) and a negative control siRNA (si-NC) were purchased from Life Technologies (Carlsbad, CA, USA). Cells were seeded on 6-well plates at a density of 2×10^5 cells/well. After 80% confluence was reached, si-TRIM59 or si-NC was transfected into cells using SuperFectin siRNA Transfection Reagent (Pufei, Shanghai, China). 48 h later, western blot analysis was performed to determine the transfection efficiency.

2.5. MTT assay

The MTT assay was performed to measure cell proliferation. In brief, cells were plated in 96-well plates at a density of 5×10^3 cells/well and cultured for different time. 50 μL of MTT reagent (Sigma, St. Louis, MO, USA) was added to each well and cells were further incubated for 4 h at 37 °C. Following removal of the MTT reagent, DMSO (Sigma, St. Louis, MO, USA) was added to each well. The absorbance was measured at a wavelength of 490 nm using a microplate reader.

2.6. Colony formation assay

Cells were seeded in a 6-well plate at a density of 1×10^3 cells/well and cultured in DMEM containing 10% FBS at 37 °C with 5% CO_2. After two weeks, the cells were washed twice with PBS, fixed with 100% methanol and stained with 0.1% crystal violet (Sigma, St. Louis, MO, USA). Subsequently, the plate was washed gently with PBS and then air dried. The number of colonies (≥50 cells) was counted under a light microscope.

2.7. Transwell assay

For the cell migration assay, transwell chambers with 8-μm-pore polycarbonate membranes were used. Cells in serum-free RPMI-1640 medium were added to the upper chamber at a density of 2×10^5 cells/well. RPMI-1640 medium supplemented with 10% FBS was added to the lower chamber. After incubation for 24 h at 37 °C, cells failing to migrate to the lower membrane surface were removed with cotton swabs. Migrating cells were fixed with methanol and stained with crystal violet and their number was counted using a microscope. The cell invasion assay was performed according to the same procedure as described above, except that Matrigel-coated membranes were used.

2.8. Tumorigenesis assay in nude mice

Male BALB/c nude mice (4–6 weeks old) were obtained from Shanghai Laboratory Animal Center (Shanghai, China) and housed under specific-pathogen-free conditions. All animal experiments were conducted with approval of the Institutional Animal Care and Use Committee of Xi'an Jiaotong University. Mice were divided into two groups and each group contained eight mice. 1×10^6 transfected cells in 0.1 mL of culture medium were subcutaneously injected into flanks of nude mice (n = 8). Tumors were measured every five days since their appearance. 35 days later, mice were euthanized and tumors were weighed.

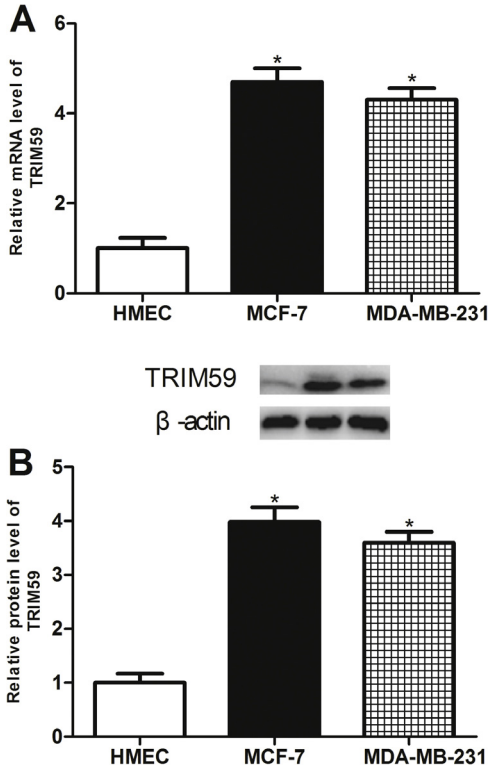

Fig. 1. Expression of TRIM59 in breast cancer cell lines. (A) The mRNA expression of TRIM59 in breast cancer cell lines was measured by RT-PCR. (B) The protein expression of TRIM59 in breast cancer cell lines was detected by western blot. $^*P < 0.05$.