

Original Article

TRIM68 regulates ubiquitinated degradation of Rabex-5 and plays a role in progression of breast cancer through Rab5-Rac1

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Abstract: TRIM family proteins are widely involved in tumorigenesis and progression, while TRIM68 is less explored in breast cancer. The expression of TRIM68 in breast cancer was explored in two public databases (GEPIA and UALCAN), and the survival was tested on Kaplan-Meier plotter database. The expression of TRIM68 in local breast cancer and corresponding para-carcinoma tissues were determined using qRT-PCR and immunohistochemistry. The gain- and loss-of function of TRIM68 were carried in breast cancer cells by knockdown or overexpression, the migration and invasion of breast cancer cells were examined by transwell, epithelial-mesenchymal transition (EMT) was determined by western blot, cell morphology was observed under light microscope. The activation of Rab5-Rac1 was determined using GST-pull down assay. The ubiquitination of Rabex-5 was determined using in vitro ubiquitination assay. The lower expression of TRIM68 in breast cancer tissues compared to the non-tumor tissues, both in public data and our local clinic samples. Also, lower TRIM68 expression was in breast cancer cells. TRIM68 overexpression impaired, but TRIM68 knockdown facilitated the migration, invasion and EMT in breast cancer cells. TRIM68 regulates the activation of Rab5-Rac1 signaling, and Rabex-5 was identified to be mediated by TRIM68 in a ubiquitination-dependent degradation manner. Furthermore, Rabex-5 knockdown completely abrogated the pro-tumor effects of TRIM68 knockdown in breast cancer cells. Our study first uncovered the crucial role of TRIM68 in malignant behaviors of breast cancer cells, through Rabex-5-mediated Rac5 activation, and shed new light on the development of the diagnostic and therapeutic strategies of breast cancer.

Keywords: Tripartite motif 68 (TRIM68), Rab5, Rabex-5, breast cancer, ubiquitination-dependent degradation

Introduction

Breast cancer is the most prevalent female malignancy worldwide with 2.3 million new cases each year, accounting for approximately one forth new newly diagnosed cancer cases, and also the leading cause of cancer death in women [1, 2]. By the developing of therapeutic strategies, ~70-80% of patients could be cured, usually in early diagnosis, for those advanced breast cancers, however, no strategy available [3]. Distant metastasis is the main reason for the breast cancer death [4], and data indicates that the five-year survival of patients with metastatic breast cancer is 27%-33% [5, 6]. Since the poor prognosis of patients with metastatic breast cancer, a better understanding of the molecular basis of pathogenesis and progres-

sion, as well as the key oncogenes involved in invasion and metastasis in breast cancer, are urgently needed for the development of new therapeutic strategies.

Rab proteins are important members of the small GTPase family which locate in intracellular structures and play crucial roles in vesicle transport, including translocation, adhesion, anchoring and fusion [7]. Rab5 GTPase is located in early endosomes, membrane and clathrin-covered vesicles and is involved in intracellular transport [7]. Similar to other small GTPases, Rab5 constantly cycles between the GTP-bound active form and the GDP-bound inactive form [8]. In addition to its role in endocytosis, Rab5 is also involved in a variety of other cellular processes, including cell adhesion and migration,

mitosis, autophagy and etc. [9-12]. Rab5a, an important isoform of Rab5, plays an important role in regulating the internalization of membrane proteins, as well as their return to cell surface. Rab5a not only regulates the transport of G protein-coupled receptors, but also participates in regulating the localization of junctional adhesion proteins [13]. It has been reported that increased active Rab5 is positively associated with migratory behavior in tumor cells, and overexpression of wild-type Rab5 promotes dephosphorylation of the focal adhesion kinase (FAK) and depolymerization of FAK, thereby promoting cell migration [14]. The regulatory role of Rab5 in cell migration, to some extent, also could be achieved through its effector APPL1. When stimulated by EGF or oxidative stress, APPL1 translocates from the membrane to the nucleus, thereby interacting with the NuRD/MeCP1 complex to regulate chromatin structure and gene expression [15]. Rab5 is highly expressed in a variety of tumor cells including breast, cervical, and thyroid cancers [16]. Down-regulation of Rab5 expression in mouse melanoma cells B16-F10 as well as human lung cancer cells A549 inhibited tumor cell proliferation, promoted their apoptosis, and hindered non-adherent growth of tumor cells [17]. Frittoli et al. [18] found that the increased Rab5 expression could promote HGF/MET signaling by stimulating the transport of MT1-MMP and integrin $\beta 3$, thus facilitating the transformation of ductal carcinoma in-situ to invasive ductal carcinoma. Therefore, the high expression of Rab5 could predict the invasive behavior and metastatic ability of human breast cancer.

The TRIM family proteins, characterized by their conserved RING-finger, B-box, and coiled-coil (RBCC) domains, are pivotal regulators of cancer cell signaling and protein homeostasis [19]. While certain members like TRIM21 and TRIM28 have been extensively linked to the tumorigenesis and development of breast cancer [20-24], the functional role of TRIM68 in breast cancer remains poorly understood. In this study, we identified the down-regulation of TRIM68 in breast cancer, and the gain- and loss-of function assays suggested the regulatory role of TRIM68 in migration, invasion and EMT in breast cancer cells. Further experiments revealed that TRIM68 modulated ubiquitination-dependent degradation of Rabex-5, which is a crucial regulator of Rab5 activation.

Our findings not only expand the functional landscape of the TRIM family but also provide a promising therapeutic rationale for targeting the TRIM68/Rabex-5/Rab5-Rac1 axis in metastatic breast cancer.

Methods and materials

Clinical samples

Breast cancer tissues (n=47) and adjacent non-tumor tissues were obtained from patients who received surgical resection at department of breast and thyroid surgery, Xinjiang Medical University Affiliated Tumor Hospital. The protocol was reviewed and approved by the Ethics Committee of the Affiliated Tumor Hospital Xinjiang Medical University (K-2026167), and written informed consents were obtained from all participants. The resected tissues were frozen in liquid nitrogen or paraffin embedded.

Cell culture and transfection

Four malignant breast cancer cells MDA-MB-453, MDA-MB-231, MDA-MB-468 and MCF7 were purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), two normal human breast epithelial cells MCF10A and HMEC, and a human prostate cancer cell LNCaP were obtained from American Type Culture Collection. Cells were cultured in DMEM medium supplemented with 10% FCS and 1% penicillin/streptomycin.

For generating knockdown cells, short hairpin RNAs (shRNA) targeting TRIM68, Rabex-5 or random sequence were cloned into hU6-MCS-CMV-puromycin lentiviral vectors. For TRIM68 overexpression, full length TRIM68 cDNA sequence (NM_018073.8) was cloned into Ubi-MCS-3FLAG-SV40-EGFP-IRES-puromycin lentiviral vectors. All lentiviral vectors were constructed by GeneChem (Shanghai, China). Cells reached 70-80% confluence were transfected with lentiviral vectors at an MOI of 20. The cells were incubated for 48 h and subsequently selected with 5 mg/L puromycin for one week. The knockdown and overexpression efficiency were further examined by qRT-PCR and western blot.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (TaKaRa, Japan). Subsequently, 5 μ g RNA from

each sample were served as template to synthesize complementary DNA using PrimeScript RT kit (Takara, Japan). The qRT-PCR was carried out with SYBR Green qPCR Master Mix (TaKaRa, Japan) on ABI 7500 fast RT-PCR System (Applied Biosystems, USA). Primers used were: TRIM68 (F: 5'-AGGGCCCTGACAACCTCTTT-3', R: 5'-GGGAGCCACAGTCAGTCACATTG-3'), EEA1 (F: 5'-CTTCTAGCCACCAGGCAAGATC-3', R: 5'-CCAA-TGTAGCCTTGGCAGTCTTC-3'), RABEP1 (F: 5'-GC-CACAGTCTCTGAGAACACCAAG-3', R: 5'-GGAAC-TGGTCTCATAGTCACGAA-3'), APPL1 (F: 5'-CATC-CAGAAAGAAACAACACCA-3', R: 5'-CATTAAAGGTA-TCCAGCCTTTTCG-3'), Rabex-5 (F: 5'-ATGTGGATC-AATCGGATCTCCT-3', R: 5'-GCTTTGTGGTACTCTT-CCCTCC-3'), RBSN (F: 5'-CCGACTTCAAAAAACA-CCGAGCTG-3', R: 5'-TGGACAGAAAGGGACATCC-TGGTC-3'), β -actin (F: 5'-TGACGTGGACATCCGC-AAAG-3', R: 5'-CTGGAAGGTGGACAGCGAGG-3'). β -actin was employed as the internal control, and the expression of target genes were calculated using $2^{-\Delta\Delta Ct}$ method [25].

Western blotting

Cells or tissues were first lysed with RIPA buffer containing protease inhibitors (1%), and incubated at 4°C for 30 min. After ultrasonication, samples were centrifuged ultrasonicated. The cell lysates were centrifuged at 12000 rpm for 5 min to remove the debris. The supernatants were quantified using a commercial BCA kit (Beyotime, China) and then subjected to 4% SDS-PAGE for separation. Protein samples were then transferred onto a polyvinylidene fluoride (PVDF) membrane (Pierce Biotechnology, USA). Membranes were incubated with no-fat milk (5%) at room temperature for 1 h. After washing, the membranes were incubated with primary antibodies which were diluted as recommended by the manufacturers at 4°C for 12 h and subsequently washed using TBST. The membranes were incubated with horseradish peroxidase-conjugated secondary antibody for another 1 h at room temperature. After washing, the protein signals were visualized using ECL chemiluminescence reagent. The primary antibodies used in this study were listed: anti-TRIM68 (sc-517118, Santa Cruz Biotechnology), anti-E-cadherin (ab40772, Abcam), anti- β -catenin (ab32572, Abcam), anti-N-cadherin (ab18-203, Abcam), anti- α -SMA (ab223068, Abcam), anti-Rab5 (ab218624, Abcam), anti-Rac1 (ab15-5938, Abcam), anti-EEA1 (ab109110, Abcam), anti-RABEP1 (ab176578, Abcam), anti-APPL1

(ab180140, Abcam), anti-RBSN (HPA044878, Sigma Aldrich), anti-Rabex-5 (ab113480, Abcam), anti- β -actin (ab6276, 1:3000, Abcam).

GST-pull down assay

The active forms (GTP-bound) of Rab5 and Rac1 were examined by GST-pull down assays. Briefly, cell lysates were incubated with GST-RBD coated beads at 4°C for 30 min, and washed with lysis buffer, the abundance of GTP-bound Rab5 and Rac1 were subsequently analyzed by western blot.

Immunohistochemistry

The paraffin-embedded tissues were cut into 4 μ m slices, and then deparaffinized and rehydrated using xylene and gradient ethanol series. The slices were boiled for 5 min and sealed with 3% H₂O₂ for 10 min. After blocked by 5% horse serum, the slices were subsequently incubated with primary TRIM68 antibody at 4°C overnight, and secondary antibodies for another 1 h at 37°C. Finally, the slices were stained with DAB for 10 min at room temperature. Staining was blindly evaluated by two independent pathologists using a semi-quantitative scoring system based on staining intensity (0-3) and the percentage of positive cells (0-4).

Transwell assay

Cells were harvested overnight and cultured in the upper transwell chamber, containing 0.5% FBS DMEM medium, the lower chamber was supplied with 10% FBS DMEM medium. The lower and upper chambers were separated with Matrigel-coated insert. After incubation at 37°C for 24 h, the non-invasive cells (remained on the upper surface of the insert) were removed using cotton swabs, while for the invasive cells (on the lower surface of the insert) were fixed with 4% paraformaldehyde and subsequently stained with 0.05% crystal violet. The migration capacity of cancer cells was conducted in a similar method, the difference is that there is no Matrigel-coated insert placed between lower and upper chambers. For the quantification of cell migration and invasion, four random fields were selected under a 200 $\times$ magnification microscope. The number of cells per field was manually counted, and the quantitative results were expressed as the average count per field.

Ubiquitination analysis

Cells infected with shTRIM68 or scramble shRNA were transfected with HA-tagged ubiquitin and subsequently treated with proteasome inhibitor MG132 (30 μ M) for 6 h. Extracted protein was immunoprecipitated with Rabex-5 antibody, and the separated by SDS-PAGE. The Ub-Rabex-5 was detected by immunoblotting with anti-HA antibody.

Statistical analysis

Data was presented as the mean $\pm$ SD. SPSS 17.0 software (SPSS Inc., IL, USA) were employed for statistical analyses. Differences ($P < 0.05$) among groups were analyzed using student's *t* test or one-way ANOVA test followed by 's post hoc test, specially, the difference between the expression of TRIM68 in cancerous tissues and corresponding para-carcinoma tissues was analyzed using paired *t*-test. The data was presented as the mean $\pm$ standard deviation, * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

Results

Downregulation of TRIM68 in breast cancer

Previous investigations have illustrated the implication of TRIM68 in human tumors, e.g., prostate cancer, osteosarcoma, and colorectal cancer [26-28], while its role in breast cancer is unknown. We first analyzed the expression of TRIM68 in breast cancer using online tools Gene Expression Profiling Interactive Analysis (GEPIA) [29] and the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN) [30] based on TCGA and some other databases. As indicated in **Figure 1A, 1B**, lower TRIM68 expression was observed in breast cancer samples compared with the normal samples. Kaplan-Meier plotter is an online database containing data about gene expression and prognosis in several human tumors, which is available to analyze the correlation between gene expression and survival with flexible variables [31]. We next employed Kaplan-Meier plotter database to explore whether the expression of TRIM68 is associated with the survival of breast cancer patients. We noticed that the expression of TRIM68 is positively correlated with the relapse-free survival of breast cancer patients (**Figure 1C**, $P = 0.000$). Similar

trends were observed in HER2 negative (**Figure 1D**, $P = 0.000$) and luminal A (**Figure 1E**, $P = 0.029$) breast cancer, but not other molecular subtypes (data not shown). The expression of TRIM68 was subsequently determined in 47 pairs of breast cancer tissues and corresponding para-carcinoma tissues using qRT-PCR and IHC, and the results indicated both mRNA and protein were downregulated in tumor tissues (**Figure 1F, 1G**, $P < 0.05$). These results suggested a reduced expression of TRIM68 in breast cancer and lower TRIM68 predict poor prognosis.

Attenuated expression of TRIM68 in breast cancer cells

Knowing the low expression of TRIM68 in breast cancer tissues, we thus examined TRIM68 expression in breast cancer cells. Two normal breast epithelial cells, MCF10A and HMEC, and three malignant breast cancer cells, MDA-MB-231, MDA-MB-468 and MCF7, were selected for TRIM68 expression detection. As indicated in **Figure 2A**, normal cells tended to have higher TRIM68 mRNA level than cancer cells, and strikingly MCF7 had the lowest TRIM68 expression. Likewise, lower TRIM68 protein abundance was found in cancer cells, especially in MCF7 and MDA-MB-468 cells (**Figure 2B**).

TRIM68 modulated the migration and invasion of breast cancer cells

Intriguingly, based on the abovementioned results, TRIM68 seemed to have diverse roles in contrast to other tumors, since its low expression in tumor tissues and cells, and negatively correlated to survival. Consequently, gain- and loss-of-function of TRIM68 were performed in MCF7 and MDA-MB-231 cells, respectively. By using short hairpin RNAs targeting TRIM68 and vectors harboring TRIM68 sequence, we successfully knocked down and overexpressed TRIM68 in two breast cancer cells, as assessed by mRNA and protein levels (**Figure 3A, 3B**). Next, cell migration and invasion capacities were determined in TRIM68 knockdown MDA-MB-231 cells and TRIM68 overexpression MCF7 cells by transwell assays. As shown in **Figure 3C**, suppressed TRIM68 expression increased the migrated number of MDA-MB-231 cells, while elevated expression of TRIM68 blocked the migration of MCF7 cells. Similar trends were observed in cell invasion, TRIM68 knock-

TRIM68 regulates breast cancer

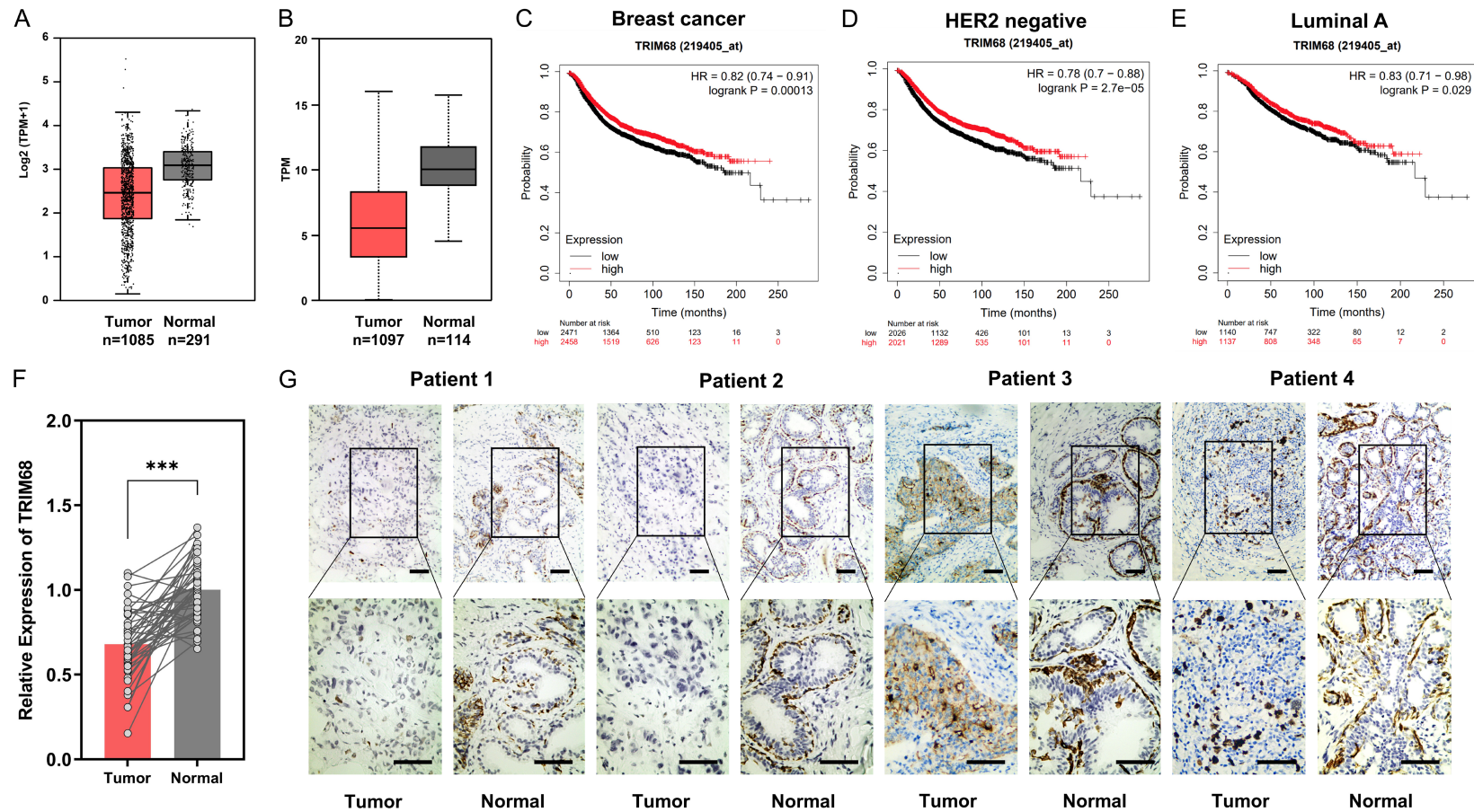


Figure 1. Lower expression of tripartite motif 68 (TRIM68) in breast cancer. The expression of TRIM68 in breast cancer tissues and non-tumor tissues obtained from public databases (A) Gene Expression Profiling Interactive Analysis (GEPIA) and (B) the University of Alabama at Birmingham Cancer Data Analysis Portal (UALCAN). The relapse-free survival of patients with breast cancer (C), HER2 negative breast cancer (D), and luminal A breast cancer (E). The expression of TRIM68 in local clinic breast cancer tissues (n=47) and corresponding para-carcinoma tissues (n=47) detected by qRT-PCR (F) and immunohistochemistry (IHC) (G), scale bars=100 μ m. *** P <0.001.

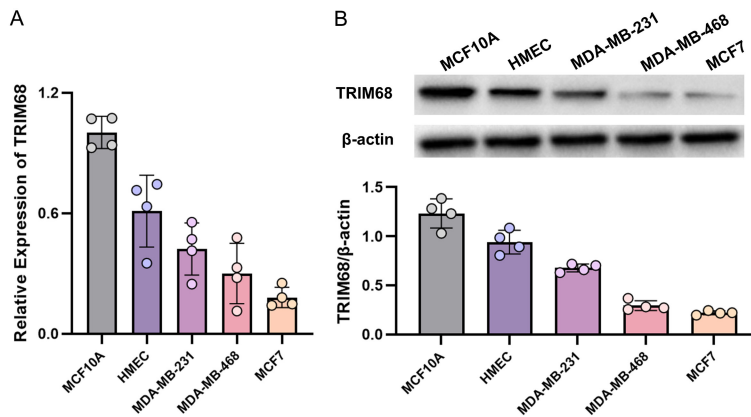


Figure 2. Lower expression of TRIM68 in breast cancer cells. The expression of TRIM68 in two normal breast epithelial cells, MCF10A and HMEC, and three malignant breast cancer cells, MDA-MB-231, MDA-MB-468 and MCF7, was determined by qRT-PCR (A) and western blot (B), $n=4$ biological replicates, data was presented as mean $\pm$ standard deviation.

down promoted, but TRIM68 overexpression impaired cells invading through the Matrigel (**Figure 3D**). Besides, we tested the overexpression of TRIM68 in another breast cancer cell line, MDA-MB-453, and a prostate cancer cells, LNCaP, however, the results seemed to be contradictory. The migration and invasion did not be affected by TRIM68 in MDA-MB-453 cells, while elevated TRIM68 promoted the migration and invasion of LNCaP cells (data not shown).

TRIM68 modulated the epithelial-mesenchymal transition (EMT) and morphology of breast cancer cells

Since the crucial involvement of EMT in cancer cell fates, such as migration, invasion and colonization, we were curious whether the expression of TRIM68 associate with the EMT. Epithelial markers, i.e., E-cadherin and β -catenin, and mesenchymal markers, N-cadherin and α -SMA, were determined in breast cancer cells with TRIM68 knockdown or overexpression. In MDA-MB-231 cells, the depletion of TRIM68 decreased the expression of E-cadherin and β -catenin, whereas enhanced the expression of N-cadherin and α -SMA. In MCF-7 cells, the forced TRIM68 elevated the expression of E-cadherin and β -catenin, but inhibited N-cadherin and α -SMA (**Figure 4A**). Further, the morphology of MDA-MB-231 and MCF-7 cells with TRIM68 alterations were investigated under light microscope, as depicted in **Figure 4B**, MDA-MB-231 cells with normal TRIM68 expression processed cobblestone-shaped morphol-

ogy, while in TRIM68 knock-down cells, more spindle-shaped populations, a classic mesenchymal-like morphology, were observed, especially in those transfected with shTRIM68-1. On the contrary, in MCF-7 cells with forced expression of TRIM68, cobblestone-shaped populations seemed to take the dominance.

Involvement of TRIM68 in Rab5 activation in breast cancer cells

Rab5 is a small GTPases which is widely implicated in the migration and invasion of malignant cells [32]. Besides,

our lab has gained a profound understanding of the role of Rab5 in breast cancer (unpublished data). These motivated us to disclose whether a potential association between TRIM68 and Rab5. First, we detected the activation of Rab5 in TRIM68 knockdown and overexpression breast cancer cells, and the results indicated that TRIM68 knockdown elevated the Rab5-GTP level in MDA-MB-231 while not altering the total Rab5, similar effect was observed in the activation of Rac1, a well-known GTPase activated by Rab5 [33]. On the contrary, forced TRIM68 expression in MCF-7 cells led to a decrease of Rab5-GTP and Rac1-GTP (**Figure 5A**). We further knocked down TRIM68 using shTRIM68-1 (hereafter referred to as shTRIM68) in MCF-7 cells, and the activation of Rab5 and Rac1 were accessed. As expected, TRIM68 depletion in MCF-7 cells promoted the activation of Rab5 and Rac1 (**Figure 5B**). These results suggested the correlation between TRIM68 and Rab5, and TRIM68 negatively regulates the activation of Rab5.

Ubiquitination of Rabex-5 regulated by TRIM68

Since the lack of insight in the relation between TRIM68 and Rab5, we turned to the interactors of Rab5. We first examined the expression of some Rab5 interact proteins obtained from STRING Database (ver. 11.5, <https://string-db.org/>) and BioGRID (ver. 4.4, <https://thebiogrid.org/>), including EEA1, RABEP1, APPL1, Rabex-5, and RBSN. We noticed blocked or forced TRIM68 in MCF-7 cells have no impact on the

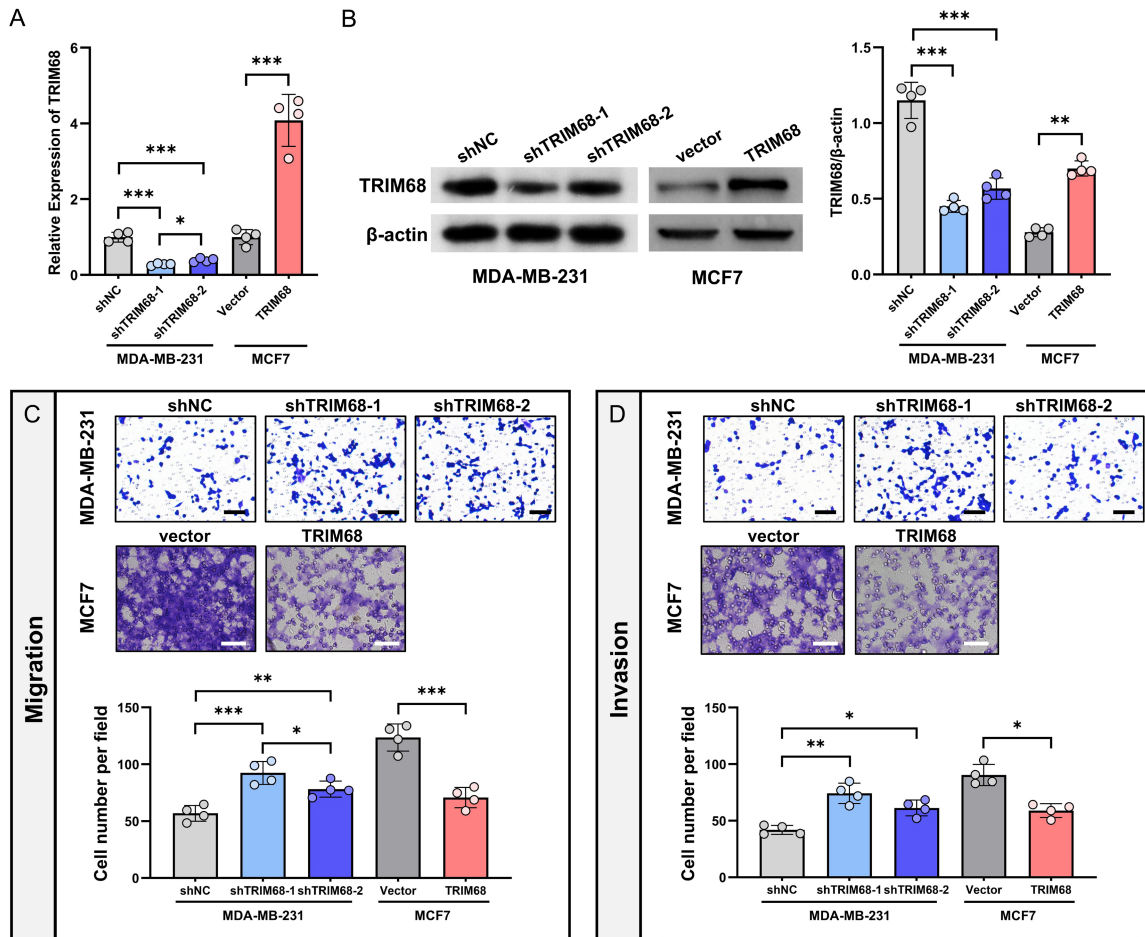


Figure 3. TRIM68 regulated the migration and invasion of breast cancer cells. The knockdown and overexpression efficiencies were determined by qRT-PCR (A) and western blot (B). The migration (C) and invasion (D) capacities of breast cancer cells with indicated transfections were determined by transwell assays, original magnification 200×, scale bars=100 μm. n=4 biological replicates, data was presented as mean ± standard deviation, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mRNA expression of these proteins, whereas, protein level of Rabex-5 was significantly altered, increased with TRIM68 knockdown and decreased with TRIM68 overexpression (**Figure 6A, 6B**). Since TRIM family members are usually involved in various biological process as E3 ubiquitin (E3-Ub) ligases [34], we thus conjectured whether TRIM68 could modulate Rabex-5 by altering its stability. We subsequently subjected the TRIM68-knockdown MCF-7 cells to cycloheximide (CHX), a protein synthesis inhibitor to block the endogenous Rabex-5 synthesis. Rabex-5 in scramble cells was decreased with overtime, while when TRIM68 knocked down, the decrease of Rabex-5 slowed down, assessed by greater portion detected comparing to the scramble (**Figure 6C**). We further conducted ubiquitination assay by transfecting HA-

tagged ubiquitin (HA-Ub) in MCF-7 cells incubated with MG132, the proteasome inhibitor, to figure out whether TRIM68-mediated Rabex-5 instability is ubiquitin-proteasome dependent. As indicated in **Figure 6D**, less HA was immunoprecipitated with Rabex-5 in MCF-7 cells transfected with shTRIM68 compared with the counterparts transfected with scramble shRNA. Besides, lysosome inhibitor chloroquine slightly enhanced the abundance of Rabex-5, while proteasome inhibitor MG132 largely elevated the abundance of Rabex-5 caused by TRIM68 knockdown (**Figure 6E**). To further investigate the specific ubiquitin linkage type involved in TRIM68-mediated Rabex-5 degradation, MCF7 cells were co-transfected with HA-Ub-K63 or HA-Ub-K48 plasmids. The results demonstrated that TRIM68 primarily induced the K48-

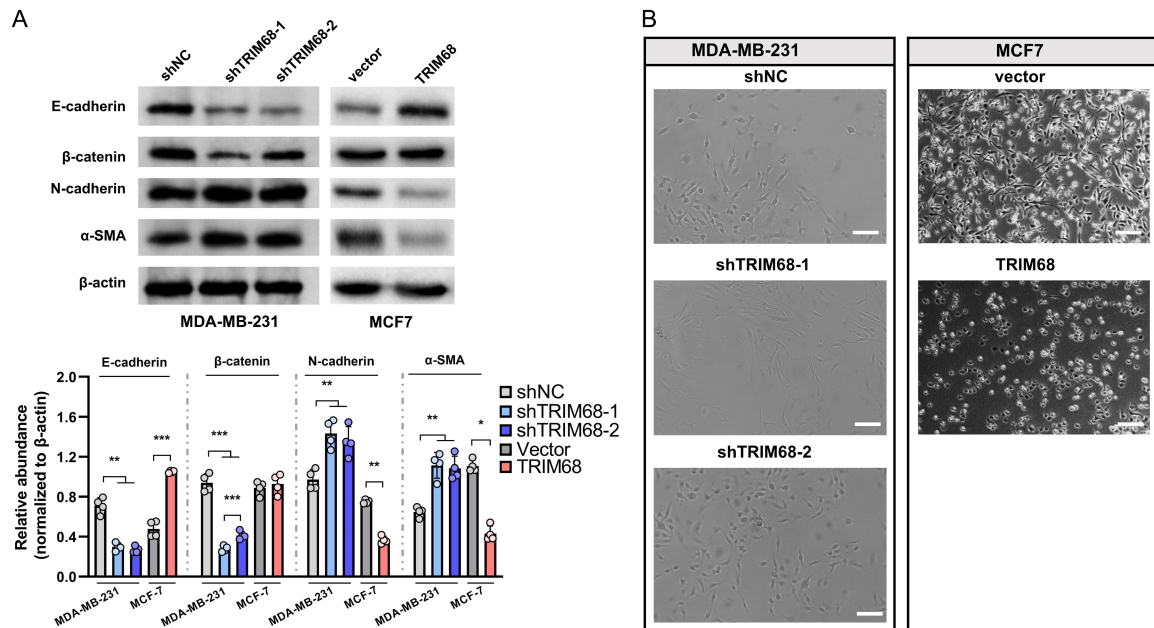


Figure 4. TRIM68 regulated the epithelial-mesenchymal transition (EMT) of breast cancer cells. A. The EMT of breast cancer cells with indicated transfections was determined by western blot analysis of epithelial (E-cadherin, β -catenin) and mesenchymal (N-cadherin, α -SMA) hallmarks. B. Cell morphology was investigated by light microscope, original magnification 200 $\times$, scale bars=100 μ m. n=4 biological replicates, data was presented as mean $\pm$ standard deviation, * P <0.05, ** P <0.01, *** P <0.001.

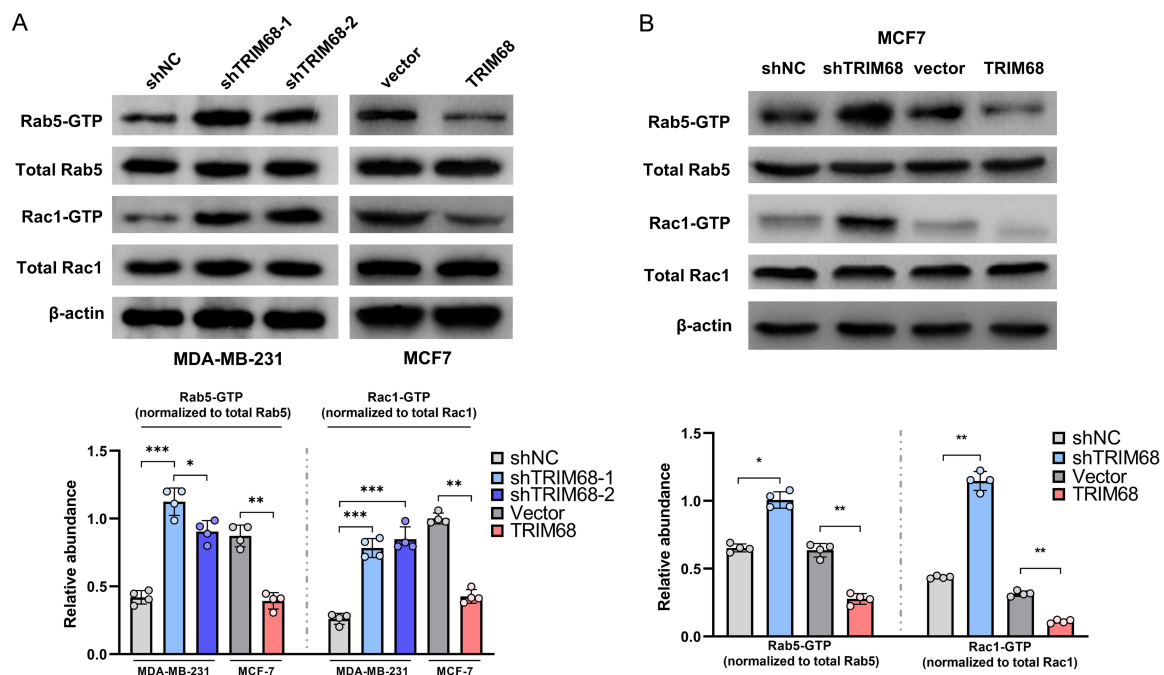


Figure 5. TRIM68 regulated the activation of Rab5-Rac1 signaling. A. The activation of Rab5 (Rab5-GTP) and Rac1 (Rac1-GTP) were determined in TRIM68 knockdown MDA-MB-231 cells and TRIM68 overexpression MCF7 cells by GST-pull down and western blot. B. The activation of Rab5 and Rac1 were determined in MCF7 cells with TRIM68 knockdown or overexpression. n=4 biological replicates, data was presented as mean $\pm$ standard deviation, * P <0.05, ** P <0.01, *** P <0.001.

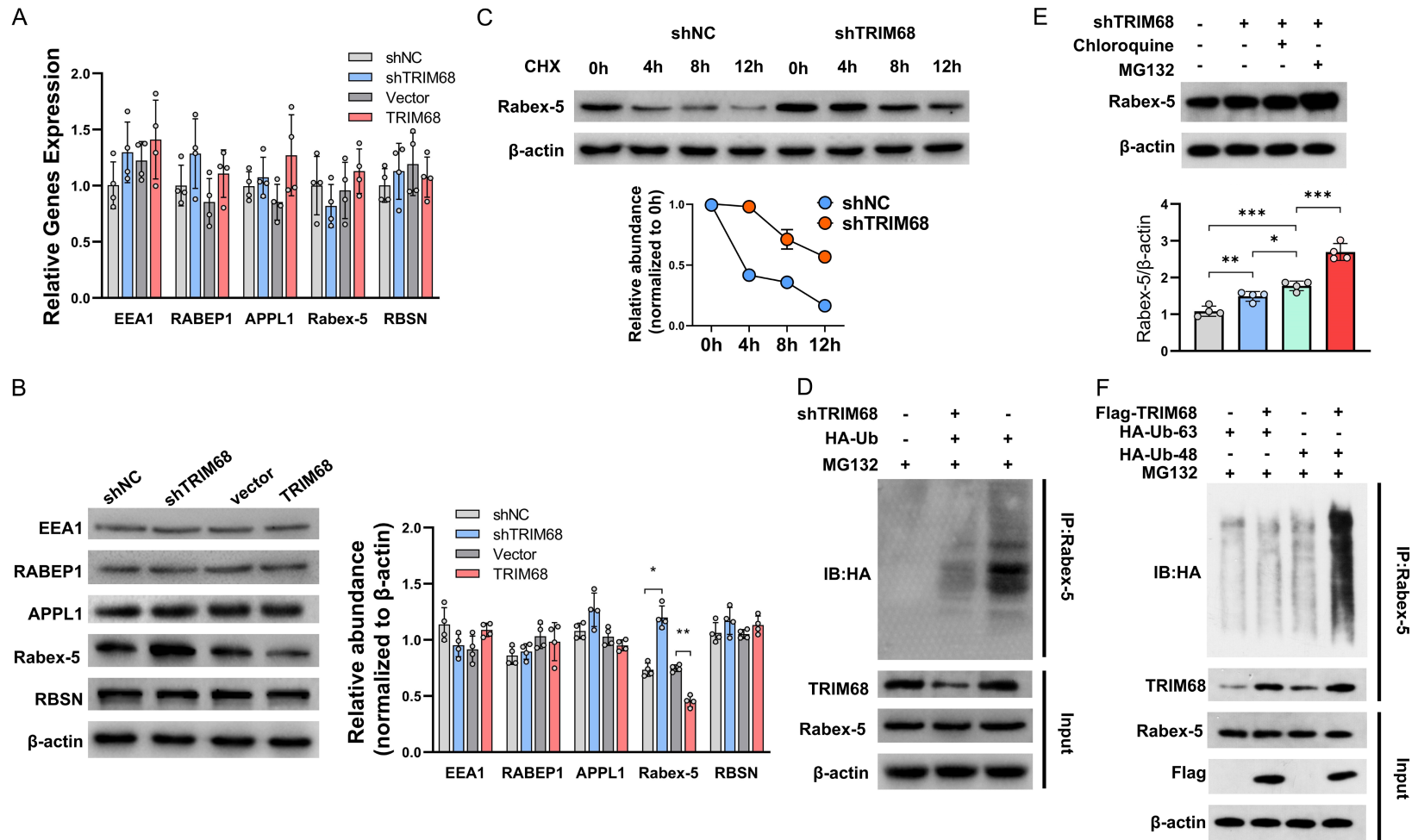


Figure 6. TRIM68 regulated the ubiquitination-dependent degradation of Rabex-5. The mRNA expression (A) and protein abundance (B) of Rab5 interactors (EEA1, RABEP1, APPL1, Rabex-5 and RBSN) in MCF7 cells with TRIM68 knockdown or overexpression. (C) The stability of Rabex-5 protein in MCF7 cells with TRIM68 knockdown treated with protein synthesis inhibitor, cycloheximide (CHX, 25 μ g/mL). (D) The ubiquitination-dependent degradation of Rabex-5 in MCF7 cells with TRIM68 knockdown treated with 20 μ M MG132. (E) TRIM68-knockdown MCF7 cells were treated with chloroquine (50 μ M) or MG132 (20 μ M), and Rabex-5 expression was detected by Western blot analysis. (F) MCF7 cells were co-transfected with Flag-TRIM68, HA-Ub-K63, and HA-Ub-K48 plasmids, and the cellular lysates were pulled down using Rabex-5 antibody by immunoblotting analysis. n=4 biological replicates, data was presented as mean $\pm$ standard deviation, * P <0.05, ** P <0.01.

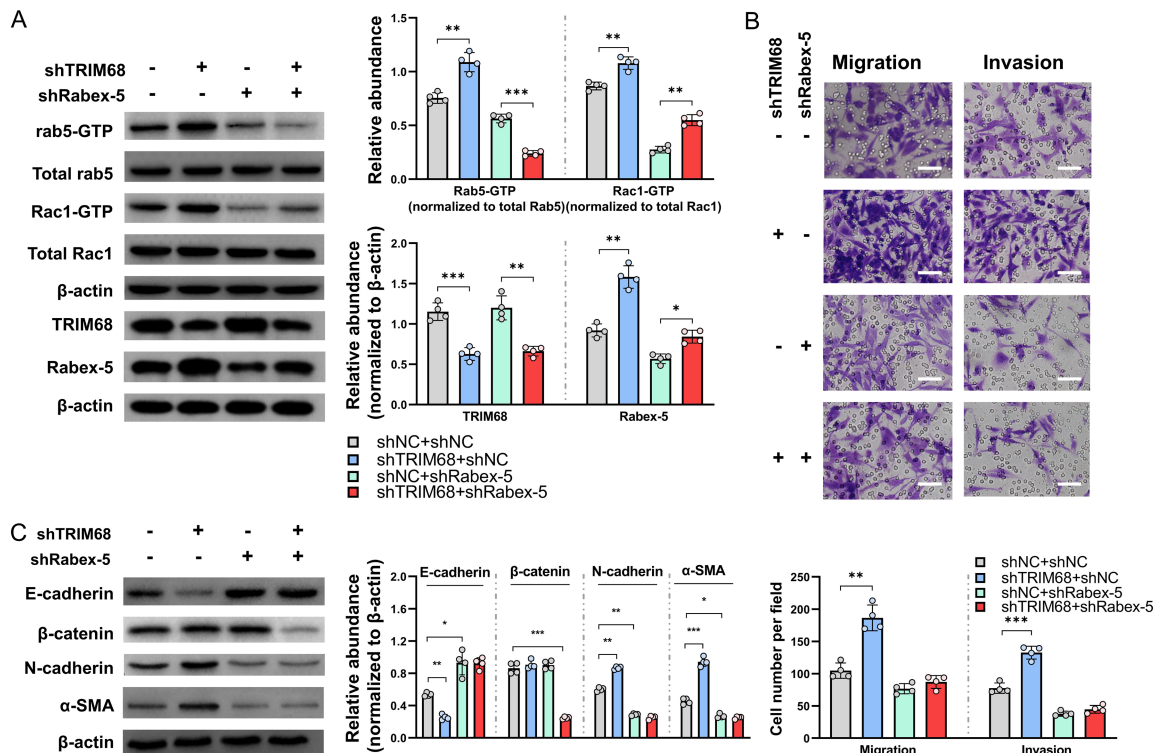


Figure 7. TRIM68 regulated migration, invasion and EMT of breast cancer cells through Rabex-5. (A) The activation of Rab5 and Rac1 in MCF7 cells with knockdown of TRIM68 and Rabex-5. The migration, invasion (B) and EMT (C) in MCF7 cells with knockdown of TRIM68 and Rabex-5, original magnification 200×, scale bars=100 μm. n=4 biological replicates, data was presented as mean ± standard deviation, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

linked ubiquitination of Rabex-5, rather than K63-linked ubiquitination (Figure 6F). These results implied the involvement of TRIM68 in regulating the stability of Rabex-5 protein, and this regulation is K48-linked ubiquitination dependent proteasome degradation.

The participation of Rabex-5 in TRIM68-mediated malignancy of breast cancer cells

TRIM68 determined the fate of Rabex-5 by ubiquitination-dependent degradation according to the abovementioned results, we next carried out the rescue assays by depleting of Rabex-5 in TRIM68 knockdown cells. The activation of Rab5 was firstly examined, TRIM68 knockdown promoted the activation of Rab5, whereas Rabex-5 knockdown suppressed Rab5 activation, and the low level of Rab5-GTP in Rabex-5 depletion cell would not be altered by TRIM68 knockdown. Same trends were seen in the activation of Rac1 (Figure 7A). Furthermore, the enhanced migration and invasion ability in TRIM68 knockdown cells seems completely abolished by Rabex-5 knockdown (Figure 7B).

E-cadherin was decreased by TRIM68 knockdown but increased by Rabex-5 knockdown, and the co-knockdown of TRIM68 and Rabex-5 showed no difference when compared to the sole Rabex-5 knockdown. Despite the attenuated of either TRIM68 or Rabex-5 caused no variation in β-catenin expression, while the knockdown of TRIM68 and Rabex-5 together decreased β-catenin. With regard of two mesenchymal markers, upregulation of these two proteins could be totally abrogated by Rabex-5 knockdown (Figure 7C). Based on these results, we could conclude that TRIM68-mediated migration, invasion and EMT is, at least partially, through Rabex-5.

Discussion

TRIM proteins are expressed ubiquitously in mammalian, and implicated in multi-biological processes, including cell cycle regulation, stemness, development, tumorigenesis, DNA damage repair and so on [19, 35-37]. Some previous investigations have depicted the opposite effects of same TRIM proteins in various can-

cers by distinct mechanism [38, 39], which may imply the flexible role of TRIMs in different context. Such an opposite effect seems not unique [40]. TRIMs have been widely investigated as RING-finger E3 ubiquitin ligases, their complex nature might determine that they could interact with different substrates and thus function differentially.

As a member of TRIM family, TRIM68 is less studied, especially its role in tumorigenesis. Previous study identifies TRIM68 as autoantigen, and could serve as the diagnostic marker for Sjögren's syndrome [41]. Besides, TRIM68 is found to impair the production of IFN- β by acting as a TRK-fused gene destructor, thus involves in viral defense [42]. An early report demonstrates the interaction of TRIM68 and androgen receptor in prostate cancer cells, by which enhances the latter's transcriptional activity, thus facilitates tumor progress [26]. Recently, TRIM68 is identified as oncogene in colorectal cancer cells by performing lose-of-function assays [28], but its underlying basis is not elucidated. Moreover, the ectopic expression of TRIM68 has considerable merit in serving diagnostic marker for osteosarcoma [27]. Xu et al. found a positive correlation between MOAP1 and TRIM68 in lung cancer patients, and further functional experiments revealed that TRIM68 directly interacts with MOAP1 and stabilizes MOAP1 through inducing K63-linked polyubiquitination of MOAP1, thus suppresses the malignant biological properties of lung cancer [43]. While the expression of TRIM68 in breast cancer is less understood, as well as its potential role. In current study, we first determined the expression of TRIM68 in breast cancer, also its role in migration, invasion and EMT in breast cancer cells, and further its underlying molecular basis. We found TRIM68 positively correlated to the survival of breast cancer patients, especially those with HER2 negative and luminal A breast cancer. Lower TRIM68 expression was noted in breast cancer tissues. TRIM68 knockdown facilitated, but overexpression suppressed the migration, invasion and EMT of breast cancer cells, and TRIM68 was associated with the activation of Rab5. Furthermore, Rabex-5 was subsequently identified as the target of TRIM68, in a K48-linked ubiquitination-dependent proteasomal degradation. The ubiquitination-proteasome system (UPS) is a hallmark of post-translational regulation in cancer progression. In this study, we demon-

strated that TRIM68 facilitates the K48-linked polyubiquitination of Rabex-5. It is well-established that K48-linked ubiquitin chains serve as the canonical signal for recognition by the 26S proteasome, leading to substrate degradation [44]. Our observation that the proteasome inhibitor MG132, but not the lysosomal inhibitor chloroquine, largely increases Rabex-5 abundance after TRIM68 knockdown further confirms that this process is proteasome-dependent. This regulatory mode is consistent with other TRIM family members, such as TRIM21 and TRIM25 [45, 46], which also utilize K48-linkage to maintain the homeostasis of key signaling proteins. By specifically targeting the GEF Rabex-5 for degradation, TRIM68 acts as a molecular switch that shuts down the downstream Rab5-Rac1 signaling cascade, thereby inhibiting the invasive phenotype of breast cancer cells.

It seemed the effects of TRIM68 varied dependent on tumor types. We also tested the overexpression of TRIM68 in MDA-MB-453 cells and a prostate cancer cells, while the tumor suppressive role of TRIM68 was not observed in these two cells, it seemed such effects is HER2-negative breast cancer cells specific, while our current study did not intend to uncover the underlying mechanism, which deserved further investigations.

Rabex-5 is a guanine nucleotide exchange factor (GEF) for several Rab members [47], and originally identified to form complex with rabaptin-5, by which for Rabex-5 to gain GEF activity [48]. The GEF activity of Rabex-5 is determined by the helical bundle domain and Vps9 domain located in the middle of the protein [49]. By interacting with Rabex-5, Rab5 transforms the GDP-bound inactive state to GTP-bound active state, to participate early endosome fusion and endocytosis. Previous report has demonstrated higher expression of Rabex-5 in breast cancer, and its crucial role in cell proliferation and mobility by acting as the transcriptional regulator of MMP-9 [50], and its oncogene role has been also noticed in some other cancer types [51, 52]. In this study, we revealed the role of TRIM68 in modulating the activation of Rab5 is by affecting the stability of Rabex-5, and the absence of TRIM68 in breast cancer caused less ubiquitination-dependent degradation of Rabex-5, which following led to the activation of Rab5, and subsequently con-

tributed to the tumorigenesis and progress of breast cancer.

Our findings significantly extend the current understanding of the TRIM family in several ways. First, while many TRIM proteins are known to regulate the stability of transcription factors (e.g., p53 or Snail) [53-55], our work demonstrates that TRIM68 controls the Rab5 signaling by degrading Rabex-5. This suggests that TRIM proteins may have a broader role in regulating cellular “logistics” than previously thought. Second, although some TRIM members act as oncogenes, our data unequivocally positions TRIM68 as a tumor suppressor in breast cancer, similar to the roles reported for TRIM29 [39] and TRIM35 [56], but operating through a distinct Rab5-Rac1 signaling axis. This highlights the functional diversity and tissue-specific roles of the TRIM proteome. In conclusion, our findings underlined the significance of TRIM68 in the migration, invasion and EMT of breast cancer via Rabex-5/Rab5 signaling. TRIM68 was first identified as a tumor suppressor in breast cancer, which might help to expand our new understanding in the biological role of TRIM68, as well as the development of diagnosis and therapeutic strategies for breast cancer.

Despite the mechanistic insights provided by this study, several limitations should be acknowledged. First, our findings are primarily based on *in vitro* cell line models and clinical tissue correlations. While these models have allowed us to rigorously characterize the TRIM68-Rabex-5-Rab5-Rac1 signaling axis, the absence of *in vivo* validation - such as using orthotopic xenograft or metastatic mouse models - remains a limitation. Future studies incorporating animal models will be essential to fully evaluate the physiological impact of TRIM68 on tumor growth and spontaneous metastasis in a complex systemic environment. Additionally, while we identified Rabex-5 as a key substrate of TRIM68, we cannot exclude the possibility that TRIM68 may target other proteins involved in cancer progression, which warrants further global proteomic investigation.

Conclusion

In conclusion, our study identified TRIM68 as a novel negative regulator of breast cancer metastasis. We have delineated a comprehensive signaling cascade wherein TRIM68 func-

tions as an E3 ubiquitin ligase to promote the K48-linked ubiquitination and proteasomal degradation of Rabex-5. This molecular event effectively silences the Rab5-Rac1 signaling axis, leading to the potent inhibition of EMT-associated phenotypic remodeling and invasive potential in breast cancer cells. These findings not only uncover a sophisticated layer of post-translational regulation governing small GTPase activity but also identify the TRIM68-Rabex-5 interface as a viable prognostic biomarker and a promising therapeutic vulnerability for intervention in advanced breast cancer.

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Disclosure of conflict of interest

None.

Abbreviations

ANOVA, Analysis of variance; APPL1, Adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; CHX, Cycloheximide; DMEM, Dulbecco's modified Eagle's medium; DNA, Deoxyribonucleic acid; E3-Ub, E3 ubiquitin; ECL, Enhanced chemiluminescence; EEA1, Early endosome antigen 1; EGF, Epidermal growth factor; EMT, Epithelial-mesenchymal transition; FAK, Focal adhesion kinase; FBS, Fetal bovine serum; GEPIA, Gene Expression Profiling Interactive Analysis; GDP, Guanosine diphosphate; GST, Glutathione S transferase; GTP, Guanosine triphosphate; HER2, Human epidermal growth factor receptor 2; HGF, Hepatocyte growth factor; IHC, Immunohistochemistry; MT1-MMP, Membrane-type 1 matrix metalloprotease; qRT-PCR, Quantitative Real-time PCR; RABEP1, RAB GTPase binding effector protein 1; Rabex-5, RAB guanine nucleotide exchange factor 1; Rac1, Rac family small GTPase 1; RBSN, Rabenosyn; RIPA, Radio Immunoprecipitation Assay; PVDF, Polyvinylidene fluoride; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; shRNA, Short hairpin Ribonucleic Acid; TBST, Tris Buffered Saline with Tween; TCGA, The Cancer Genome Atlas; TRIM68, Tripartite

motif 68; UALCAN, University of Alabama at Birmingham Cancer Data Analysis Portal.

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References

- [1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021; 71: 209-249.
- [2] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024; 74: 229-263.
- [3] Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, Ruddy K, Tsang J and Cardoso F. Breast cancer. *Nat Rev Dis Primers* 2019; 5: 66.
- [4] Park M, Kim D, Ko S, Kim A, Mo K and Yoon H. Breast cancer metastasis: mechanisms and therapeutic implications. *Int J Mol Sci* 2022; 23: 6806.
- [5] Deluche E, Antoine A, Bachelot T, Lardy-Cleaud A, Dieras V, Brain E, Debled M, Jacot W, Mouret-Reynier MA, Goncalves A, Dalenc F, Patouris A, Ferrero JM, Levy C, Lorgis V, Vanlemmens L, Lefeuvre-Plesse C, Mathoulin-Pelissier S, Petit T, Uwer L, Jouannaud C, Leheurteur M, Lacroix-Triki M, Courtinard C, Perol D, Robain M and Delaloge S. Contemporary outcomes of metastatic breast cancer among 22,000 women from the multicentre ESME cohort 2008-2016. *Eur J Cancer* 2020; 129: 60-70.
- [6] Sundquist M, Brudin L and Tejler G. Improved survival in metastatic breast cancer 1985-2016. *Breast* 2017; 31: 46-50.
- [7] Xu S, Cao B, Xuan G, Xu S, An Z, Zhu C, Li L and Tang C. Function and regulation of Rab GTPases in cancers. *Cell Biol Toxicol* 2024; 40: 28.
- [8] Pensalfini A, Jiang Y, Kim S and Nixon RA. Assessing rab5 activation in health and disease. In: editors. *Rab GTPases: Methods and Protocols*. Springer; 2021. pp. 273-294.
- [9] Chen M, Huang X, Li B, Xiao Y, Chen L, Zhu F, Hong S, Tang J, Li S, Min J, Jin W, Zhang Y, Yang L, Li Y, Zhang S and Hong L. ArfGAP3 protects mitochondrial function and promotes autophagy through Rab5a-mediated signals in ageing skeletal muscle. *J Cachexia Sarcopenia Muscle* 2025; 16: e13725.
- [10] Zhang Z, Chen J, Ma R, Xu C, Lu Y, Zhou J, Xia K and Lu P. Tight junction component Occludin binds to FIP5 to regulate endosome trafficking and mitotic spindle function. *Adv Sci (Weinh)* 2024; 11: 2308822.
- [11] Zehrbach NM, Dubois F and Turner CE. Paxillin regulates Rab5-mediated vesicle motility through modulating microtubule acetylation. *Mol Biol Cell* 2023; 34: ar65.
- [12] Yuan Z and Wei W. RAB5A promotes the formation of filopodia in pancreatic cancer cells via the activation of cdc42 and β 1-integrin. *Biochem Biophys Res Commun* 2021; 535: 54-59.
- [13] Stamatovic SM, Sladojevic N, Keep RF and Anđelkovic AV. Relocalization of junctional adhesion molecule A during inflammatory stimulation of brain endothelial cells. *Mol Cell Biol* 2012; 32: 3414-3427.
- [14] Mendoza P, Ortiz R, Díaz J, Quest AF, Leyton L, Stupack D and Torres VA. Rab5 activation promotes focal adhesion disassembly, migration and invasiveness in tumor cells. *J Cell Sci* 2013; 126: 3835-3847.
- [15] Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG and Zerial M. APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment. *Cell* 2004; 116: 445-456.
- [16] Cao GJ, Wang D, Zeng ZP, Wang GX, Hu CJ and Xing ZF. Direct interaction between Rab5a and Rab4a enhanced epidermal growth factor-stimulated proliferation of gastric cancer cells. *World J Gastrointest Oncol* 2021; 13: 1492-1505.
- [17] Silva P, Soto N, Díaz J, Mendoza P, Díaz N, Quest AF and Torres VA. Down-regulation of Rab5 decreases characteristics associated with maintenance of cell transformation. *Biochem Biophys Res Commun* 2015; 464: 642-646.
- [18] Frittoli E, Palamidessi A, Marighetti P, Confalonieri S, Bianchi F, Malinverno C, Mazzarol G, Viale G, Martin-Padura I, Garré M, Parazzoli D, Mattei V, Cortellino S, Bertalot G, Di Fiore PP and Scita G. A RAB5/RAB4 recycling circuitry induces a proteolytic invasive program and promotes tumor dissemination. *J Cell Biol* 2014; 206: 307-328.
- [19] Huang N, Sun X, Li P, Liu X, Zhang X, Chen Q and Xin H. TRIM family contribute to tumorigenesis, cancer development, and drug resistance. *Exp Hematol Oncol* 2022; 11: 75.
- [20] Chen X, Ma C, Li Y, Liang Y, Chen T, Han D, Luo D, Zhang N, Zhao W, Wang L, Chen B, Guo H and Yang Q. Trim21-mediated CCT2 ubiquitina-

- tion suppresses malignant progression and promotes CD4⁺ T cell activation in breast cancer. *Cell Death Dis* 2024; 15: 542.
- [21] Zhang R, Shen Y, Zhang Q, Feng X, Liu X, Huo X, Sun J and Hao J. TRIM21-mediated Sohlh2 ubiquitination suppresses M2 macrophage polarization and progression of triple-negative breast cancer. *Cell Death Dis* 2023; 14: 850.
- [22] Chen H, Zhou Y, Zhang Y, Fan Y, Zheng L and Guo Q. TRIM21-mediated ubiquitination of SIX2 attenuates breast cancer stemness via LGSN suppression. *Oncogene* 2025; 44: 4173-4189.
- [23] Xue C, Meng H, Niu W, Li M, Wei J, Chen S, Zheng L, Duan Y, Deng H, Tang F, Fan S, Tan M, Xiong W and Zhou M. TRIM28 promotes tumor growth and metastasis in breast cancer by targeting the BRD7 protein for ubiquitination and degradation. *Cell Oncol (Dordr)* 2024; 47: 1973-1993.
- [24] Fu L, Ma B, Zhang L, Xu H, Chen W, Wu D, Gao F and Huo Y. ER α coregulator TRIM28 promotes breast cancer progression by activating the AKT/GSK3 β pathway. *Environ Toxicol* 2024; 39: 5162-5172.
- [25] Adams G. A beginner's guide to RT-PCR, qPCR and RT-qPCR. *Biochem (Lond)* 2020; 42: 48-53.
- [26] Miyajima N, Maruyama S, Bohgaki M, Kano S, Shigemura M, Shinohara N, Nonomura K and Hatakeyama S. TRIM68 regulates ligand-dependent transcription of androgen receptor in prostate cancer cells. *Cancer Res* 2008; 68: 3486-3494.
- [27] Jiang J, Liu D, Xu G, Liang T, Yu C, Liao S, Chen L, Huang S, Sun X, Yi M, Zhang Z, Lu Z, Wang Z, Chen J, Chen T, Li H, Yao Y, Chen W, Guo H, Liu C and Zhan X. TRIM68, PIKFYVE, and DYNLL2: the Possible novel autophagy- and immunity-associated gene biomarkers for osteosarcoma prognosis. *Front Oncol* 2021; 11: 643104.
- [28] Tan Z, Liu X, Yu E, Wang H, Tang L, Wang H and Fu C. Lentivirus-mediated RNA interference of tripartite motif 68 inhibits the proliferation of colorectal cancer cell lines SW1116 and HCT116 in vitro. *Oncol Lett* 2017; 13: 2649-2655.
- [29] Li C, Tang Z, Zhang W, Ye Z and Liu F. GEPIA2021: integrating multiple deconvolution-based analysis into GEPIA. *Nucleic Acids Res* 2021; 49: W242-W246.
- [30] Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, Netto GJ, Qin ZS, Kumar S, Manne U, Creighton CJ and Varambally S. UALCAN: an update to the integrated cancer data analysis platform. *Neoplasia* 2022; 25: 18-27.
- [31] Lánczky A and Györfy B. Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. *J Med Internet Res* 2021; 23: e27633.
- [32] Yuan W and Song C. The emerging role of Rab5 in membrane receptor trafficking and signaling pathways. *Biochem Res Int* 2020; 2020: 4186308.
- [33] Jikko Y, Deguchi E, Matsuda K, Hino N, Tsukiji S, Matsuda M and Terai K. Front-biased activation of the Ras-Rab5-Rac1 loop coordinates collective cell migration. *J Cell Sci* 2025; 138: jcs263779.
- [34] Meroni G and Desagher S. Cellular function of TRIM E3 ubiquitin ligases in health and disease. *Cells* 2022; 11: 250.
- [35] Hatakeyama S. TRIM family proteins: roles in autophagy, immunity, and carcinogenesis. *Trends Biochem Sci* 2017; 42: 297-311.
- [36] Jaworska AM, Wlodarczyk NA, Mackiewicz A and Czerwinska P. The role of TRIM family proteins in the regulation of cancer stem cell self-renewal. *Stem Cells* 2020; 38: 165-173.
- [37] Petrera F and Meroni G. TRIM proteins in development. *TRIM/RBCC Proteins* 2013; 131-141.
- [38] Xu W, Chen B, Ke D and Chen X. TRIM29 mediates lung squamous cell carcinoma cell metastasis by regulating autophagic degradation of E-cadherin. *Aging (Albany NY)* 2020; 12: 13488.
- [39] Wu Q, Nandi D and Sharma D. TRIM-endous functional network of tripartite motif 29 (TRIM29) in cancer progression and beyond. *Cancer Metastasis Rev* 2024; 44: 16.
- [40] Li X, Zhou F, Niu K, Wang Y, Shi Y, Li Y, Gao X, Zhao W, Chen T and Zhang Y. Emerging discoveries on the role of TRIM14: from diseases to immune regulation. *Cell Death Discov* 2024; 10: 513.
- [41] Jawed B, Kanwal R, Zakir SK, Gaudio F, Esposito JE, Ishaqui AA, Martinotti S, Botteghi M and Toniato E. TRIM proteins as emerging regulators of immune pathways: potential therapeutic targets in immune-related disorders. *Front Immunol* 2026; 17: 1764253.
- [42] Wynne C, Lazzari E, Smith S, McCarthy EM, Gabhann JN, Kallal LE, Higgs R, Cryan SA, Biron CA and Jefferies CA. TRIM68 negatively regulates IFN- β production by degrading TRK fused gene, a novel driver of IFN- β downstream of anti-viral detection systems. *PLoS One* 2014; 9: e101503.
- [43] Xu X, Yang M, Liu X, Gong A, Guo Q, Xu W and Qian H. Tripartite motif-containing 68-stabilized modulator of apoptosis-1 retards the proliferation and metastasis of lung cancer. *Biochem Biophys Res Commun* 2023; 648: 11-20.
- [44] Liu F, Chen J, Li K, Li H, Zhu Y, Zhai Y, Lu B, Fan Y, Liu Z, Chen X, Jia X, Dong Z and Liu K. Ubiq-

- ubitination and deubiquitination in cancer: from mechanisms to novel therapeutic approaches. *Mol Cancer* 2024; 23: 148.
- [45] Han T, Wen X, Guo Y, Zhao X, Zhang J, Chen Y and Xu F. E3 ubiquitin ligase TRIM21-mediated K48-linked ubiquitination of ALDH2 rs671 mutant promotes adverse cardiac remodeling. *JCI Insight* 2026; 11: e197555.
- [46] Mei P, Xie F, Pan J, Wang S, Gao W, Ge R, Gao B, Gao S, Chen X, Wang Y, Wu J, Ding C and Li J. E3 ligase TRIM25 ubiquitinates RIP3 to inhibit TNF induced cell necrosis. *Cell Death Differ* 2021; 28: 2888-2899.
- [47] Zhang Z, Zhang T, Wang S, Gong Z, Tang C, Chen J and Ding J. Molecular mechanism for Rabex-5 GEF activation by Rabaptin-5. *Elife* 2014; 3: e02687.
- [48] Parkinson G, Roboti P, Zhang L, Taylor S and Woodman P. His domain protein tyrosine phosphatase and Rabaptin-5 couple endo-lysosomal sorting of EGFR with endosomal maturation. *J Cell Sci* 2021; 134: jcs259192.
- [49] Nakashima S, Matsui T and Fukuda M. Vps9d1 regulates tubular endosome formation through specific activation of Rab22A. *J Cell Sci* 2023; 136: jcs260522.
- [50] Zhang X, Min J, Wang Y, Li Y, Li H, Liu Q, Liang X, Mu P and Li H. RABEX-5 plays an oncogenic role in breast cancer by activating MMP-9 pathway. *J Exp Clin Cancer Res* 2013; 32: 52.
- [51] Wang S, Lu A, Chen X, Wei L and Ding J. RABEX-5 is upregulated and plays an oncogenic role in gastric cancer development by activating the VEGF signaling pathway. *PLoS One* 2014; 9: e113891.
- [52] Zhang F, Zhang M, Hu G, Cai Q and Xu T. Elevated RABEX-5 protein expression predicts poor prognosis in combined small cell lung cancer. *Tumour Biol* 2015; 36: 8287-8293.
- [53] Wang X, Zhang Y, Pei X, Guo G, Xue B, Duan X and Dou D. TRIM3 inhibits P53 signaling in breast cancer cells. *Cancer Cell Int* 2020; 20: 559.
- [54] Xia T, Meng L, Xu G, Sun H and Chen H. TRIM33 promotes glycolysis through regulating P53 K48-linked ubiquitination to promote esophageal squamous cell carcinoma growth. *Cell Death Dis* 2024; 15: 740.
- [55] Ma X, Ma X, Qiu Y, Zhu L, Lin Y, You Y, Ma D, Qin Z, Sun C, Zhao Y, Sun Y and Han L. TRIM-50 suppressed hepatocarcinoma progression through directly targeting SNAIL for ubiquitous degradation. *Cell Death Dis* 2018; 9: 608.
- [56] Jing X, Li F, Zhou J, Zhang J, Cao L, Guo C, Li Q, Peng H, Jiang Q, Wang X, Chen Y, Ding J, Tong D, Zhao Z and Huang C. TRIM35, a novel DNA-binding protein, epigenetically modifies H3 to promote HSPA6 transcription and suppress breast cancer progression. *Cell Death Discov* 2025; 11: 479.